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A central region of Ku80 mediates interaction with Ku70 *in vivo*

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ABSTRACT

Ku, the DNA binding component of DNA-dependent protein kinase (DNA-PK), is a heterodimer composed of 70 and 86 kDa subunits, known as Ku70 and Ku80 respectively . Defects in DNA-PK subunits have been shown to result in a reduced capacity to repair DNA double-strand breaks. Assembly of the Ku heterodimer is required to obtain DNA end binding activity and association of the DNA-PK catalytic subunit. The regions of the Ku subunits responsible for heterodimerization have not been clearly defined *in vivo*. A previous study has suggested that the C-terminus of Ku80 is required for interaction with Ku70. Here we examine Ku subunit interaction using N- and C-terminal Ku80 deletions in a GAL4-based two-hybrid system and an independent mammalian *in vivo* system. Our two-hybrid study suggests that the central region of Ku80, not its C-terminus, is capable of mediating interaction with Ku70. To determine if this region mediates interaction with Ku70 in mammalian cells we transfected xrs-6 cells, which lack endogenous Ku80, with epitope-tagged Ku80 deletions carrying a nuclear localization signal. Immunoprecipitation from transfected cell extracts revealed that the central domain identified by the GAL4 two-hybrid studies stabilizes and co-immunoprecipitates with endogenous xrs-6 Ku70. The central interaction domain maps to the internally deleted regions of Ku80 in the mutant cell lines XR-V9B and XR-V15B. These findings indicate that the internally deleted Ku80 mutations carried in these cell lines are incapable of heterodimerization with Ku70.

INTRODUCTION

The DNA-dependent protein kinase is a trimeric complex composed of the Ku autoantigen and an ~460 kDa catalytic subunit (DNA-PKcs) (1–5). The Ku component of the kinase is a DNA binding heterodimer composed of 70 and 86 kDa subunits. Once bound to DNA, Ku is capable of binding to DNA-PKcs, which in turn results in kinase activation (3,4,6,7). Although Ku is capable of both sequence-independent and sequence-specific DNA binding, it appears that sequence-independent binding requires DNA ends or single- to double-strand transitions (i.e. nicks and gaps) (8–12). This characteristic implicated DNA-PK in DNA repair or DNA damage signaling pathways. Later examination of cell lines defective for DNA double-strand break (DSB) repair revealed that Ku or DNA-PKcs were, in fact, absent or reduced in ionizing radiation-sensitive cell lines falling into the XRCC7 and XRCC5 complementation groups (13–23). Taken together with the finding that DNA-PK phosphorylates a number of transcription factors and DNA binding proteins *in vitro*, including the C-terminal domain of RNA pol II, it is possible that DNA-PK plays a role in DNA damage repair, transcriptional regulation and/or DNA damage signal transduction (6,24,25). Recent microscopic analyses of DNA-PK and Ku suggest that the kinase may also play a structural role in repair by holding the ends of broken DNA together during the end joining process (26).

Although the DNA binding and biochemical properties of Ku have been the subject of extensive study, little is known about the structure and assembly of the Ku heterodimer. Sequence analysis of Ku70 and Ku80 cDNAs has revealed the presence of putative leucine zipper motifs in the predicted amino acid sequence of both of these proteins (27,28). Though the biological role of this motif is unknown, it has been shown to be dispensable for Ku heterodimerization as determined by yeast two-hybrid studies (29). These same studies have also suggested that the C-terminus of Ku80 is required for association with Ku70.

To more precisely define the regions of the Ku70 and Ku80 polypeptides involved in dimerization we examined the ability of truncated Ku70 and Ku80 molecules to interact *in vivo* using a GAL4-based yeast two-hybrid system. The results of quantitative GAL4-based two-hybrid analysis suggest that a central region of Ku80 mediates Ku70 interaction, in contrast to the LexA-based two-hybrid analysis previously presented by Wu and Lieber (29), in which the C-terminus of Ku80 was implicated in Ku70 interaction. To resolve this discrepancy we have turned to an alternative *in vivo* system based on immunoprecipitation from transformed xrs-6 cells, which lack endogenous Ku80. The results of this independent assay support the findings of the GAL4-based two-hybrid study presented here and confirm the involvement of the central region of Ku80 in assembly of the Ku heterodimer.

MATERIALS AND METHODS

Cloning of full-length human Ku70 and Ku80 cDNA

Full-length human Ku70 and Ku80 (22) cDNAs were obtained by screening a human λZAP II cDNA library (Stratagene, La Jolla,

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CA) using Ku80 and Ku70 cDNA fragments amplified by PCR (28). The cDNA phage clone was converted to a plasmid pBK-CMV clone by *in vivo* splicing with helper phage following the manufacturer's instructions (Stratagene). The cDNA clones were subsequently confirmed by restriction digestion and sequencing.

Yeast two-hybrid system

Yeast two-hybrid studies were carried out using the GAL4-based system (30–32) as obtained from Clontech Laboratories Inc. (Palo Alto, CA). The yeast strain SFY526 (MAT**a** *ura3-52 his3-200 ade2-101 lys2-801 trp1-901 leu2-3,112* can^r *gal4-542 gal80-538 URA3*::*GAL1-lacZ*) was used in all assays (33). Qualitative *lacZ* assays were performed on filters placed on the appropriate SD drop-out medium. Quantitative assays were performed using ONPG substrate as follows. Overnight cultures of individual yeast colonies were grown in SD drop-out liquid medium lacking leucine and tryptophan. Prior to assay, overnight cultures were diluted 1:5 into fresh rich medium (YPD) and grown for 4 h at 30°C. OD_{600} measurements were made and 1.5 ml culture were pelleted, washed in Z buffer (16.1 g/l $Na_2HPO_4.7H_2O$, 5.5 g/l $NaH_2PO_4.H_2O$, 0.75 g/l KCl, 0.246 g/l $MgSO_4.7H_2O$, pH 7.0) and resuspended in 300 µl Z buffer. Aliquots of 100 µl were frozen in liquid nitrogen, thawed at 37°C and brought to 800 µl with Z buffer containing 0.27% β-mercaptoethanol. To this was added 160 µl 4 mg/ml ONPG substrate in Z buffer and reactions were allowed to proceed at 30°C. Reactions were stopped by addition of 400 µl 1 M Na_2CO_3. Cellular debris was pelleted by centrifugation and the OD_{420} of the reaction was measured. Reaction units were calculated using $1000 \times OD_{420}/(t \times V \times OD_{600})$, where t is the reaction time in minutes and V is the concentration factor (calculated by dividing the volume of culture pelleted by the volume of buffer used for resuspension). Reactions were performed in triplicate using three independent colonies for each co-transfection assayed.

Construction of two-hybrid plasmids

Plasmids encoding N-terminal GAL4 activation and DNA binding domain fusions were constructed using pGAD424 and pGBT9 respectively (Clontech Laboratories Inc.). C-Terminal fusions of the GAL4 domains were encoded by pTBG2 (DNA binding domain) and pDAG4 (activation domain). The pTBG2 vector was constructed from pGBT9 as previously described (34). The pDAG4 vector was constructed by digestion of pGAD424 with *Hin*dIII to release the nuclear localization signal (NLS), GAL4 activation domain (GAL4-AD) and the multiple cloning site (MCS). The remaining vector sequences were ligated to a PCR-generated insert containing the MCS and the sequences between the NLS and the *Hin*dIII site along with a GCA AAG ATG translation start site and a *Kpn*I site at the 5′-end. The 3′-end of the insert contains the *Hin*dIII site before the T_{ADH1} element. Positive clones were identified by PCR screening and confirmed by DNA sequence analysis. The resulting vector lacked the GAL4-AD and the NLS. This vector was digested with *Sal*I and *Pst*I and ligated to a DNA fragment generated by PCR containing the NLS, the GAL4-AD and a stop codon flanked by *Sal*I and *Nhe*I sites at the 5′-end and a *Pst*I site at the 3′-end to generate pDAG4 (Fig. 1).

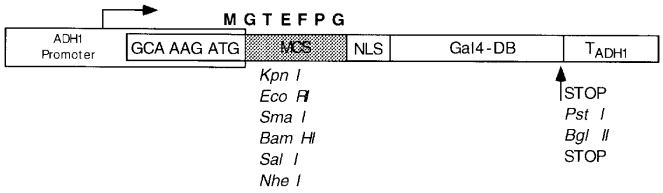

Figure 1. The pGAD424 vector was modified to generate pDAG4, a vector designed to generate fusion proteins with the GAL4 activation domain (GAL4-AD) on the C-terminus. pDAG4-based Ku70 and Ku80 plasmids were constructed by cloning into the *Bam*HI and *Sal*I sites of the vector. Use of the *Bam*HI and *Sal*I sites results in N-terminal addition of the seven amino acids shown above the multiple cloning site (MCS) and addition of the GAL4-AD and a nuclear localization sequence (NLS) to the C-terminus.

Construction of full-length and truncated Ku70 and Ku80

Human Ku70 and Ku80 cDNAs were used for construction of all full-length and truncated GAL4-AD and GAL4 DNA binding domain (GAL4-BD) fusions respectively. Low cycle PCR was used as previously described (35) to construct full-length and truncated versions of Ku70 and Ku80 cDNA and to add *Bam*HI and *Sal*I sites to the 5′- and 3′-ends of the sequences respectively. Inserts subcloned into pDAG or pTBG required removal of the endogenous stop codon to allow read-through to the C-terminally located GAL4 domain. Primers were designed for truncated versions of Ku70 and Ku80 using the approach as for full-length constructs.

Mammalian expression vector

For expression of epitope-tagged human Ku80 cDNA deletions in mammalian cell line xrs-6 we constructed a tagging vector based on the pMAMneo plasmid (Clonetech Inc). The following sequence, containing start and stop codons, was cloned into the *Nhe*I sites of the original pMAMneo vector: ATGGACTACAAGGACGACG-ATGACAAGGTACCGCGGTCGACTCGAGAA**TACCCCTA-CGACGTGCCCGACTACGCC**CGTCGAGAA*CCACCAAA-GAAGAAGCGTAAGGTT*TAA. 5′-**TACCCCTACGACGTGC-CCGACTACGCC**-3′ encodes the hemagglutinin epitope YPYDVPDYA. 5′-*CCACCAAAGAAGAAGCGTAAGGTT*-3′ codes for SV40 T antigen nuclear localization signal PPKKKRKV. The resulting vector is referred to here as pHA-NLS. The *Sac*II site (underlined) was used as the cloning site for truncated versions of Ku80. Ku80 cDNA was PCR amplified using oligonucleotides containing the *Sac*II sequence, digested with *Sac*II and ligated into the pHA-NLS vector.

pHA-NLS-based constructs were transfected into xrs-6 cells by calcium phosphate precipitation using a commercially available kit (Gibco BRL). After 48 h transfected cells were selected using G418 to obtain stably expressing clones. The neomycin gene on the pHA-NLS vector confers G418 resistance and the surviving single colonies were picked and screened with HA-11 antibody by Western blot (data not shown). Positive clones were used in immunoprecipitation studies.

Cell extract preparation and immunoblots

For Western analysis nuclear protein extract preparations were performed as described previously (22). Protein extracts were

subjected to SDS–PAGE. The proteins were transferred to nitrocellulose membranes and probed with HA-11 antibody (BAbCo, CA) and anti-murine Ku70 antibody (Santa Cruz Biotechnologies, CA), followed by detection with peroxidase-conjugated secondary antibody, and visualized by chemiluminescence detection using ECL reagents (Amersham).

Immunoprecipitation

One million cells were lysed using 2 ml RIPA buffer with 1× PBS, 1% NP40, 0.5% sodium deoxycholate, 0.1% SDS, 10 µg/ml PMSF, 30 µg/ml aprotinin and 1 mM sodium orthovanadate. After centrifugation at 3000 r.p.m. at 4°C for 5 min 1 ml supernatant was pre-cleared with 1 µg normal rabbit IgG and 20 µl protein G–agarose and pelleted by centrifugation at 4°C at 1500 r.p.m. for 5 min. The pre-cleared extract was incubated with 0.1 µg HA-11 polyclonal antibody (BAbCo, CA) and 20 µl protein G–agarose overnight. Immunoprecipitates were collected by pelleting at 1500 r.p.m. at 4°C and washed three times with RIPA buffer. The pellet was then resuspended in loading buffer, boiled for 3 min and subjected to SDS–PAGE. For detection of HA-11 immunoprecipitates on Western blots, monoclonal HA-11 was used, to avoid cross-reaction with the pull-down polyclonal antibody by the secondary antibodies.

RESULTS

Full-length Ku70/Ku80 interaction

To determine if the GAL4-based yeast two-hybrid system would prove applicable to the study Ku heterodimerization we cloned full-length versions of Ku70 and Ku80 into the pGAD424 and pGBT9 vectors (Clonetech Inc.). These plasmids carry the coding sequence for the GAL4 activation (pGAD424) or DNA binding (pGBT9) domain 5′ of a polylinker such that encoded fusion proteins carried the GAL4 domain on the N-terminus. Yeast strain SFY526 was transformed with pairs of the GAL4–Ku fusion encoding plasmids or control plasmids lacking inserts. Qualitative β-galactosidase filter assays indicated that the fusion proteins failed to interact under these conditions (Fig. 2).

We reasoned that the GAL4 domains could inhibit Ku dimerization by steric interference or interference with a critical folding conformation. If this were the case it is possible that fusions to the C-terminus of the Ku proteins would prove less disruptive. To address this question we constructed versions of the pGAD and pGBT vectors that allowed cloning 5′ of the GAL4 coding sequence. The resulting vectors are referred to here as pDAG4 (activation domain) and pTBG2 (binding domain). Again, using full-length Ku70, Ku80 and appropriate controls we examined the ability of all pairwise combinations to activate transcription of the *lacZ* reporter gene in SFY526. Interaction between Ku70 and Ku80 provides a robust two-hybrid interaction only when the GAL4 activation domain is fused to the C-terminus of Ku70 and the GAL4 DNA binding domain is fused to the N-terminus of Ku80 (Fig. 2). Other combinations failed to activate the reporter gene.

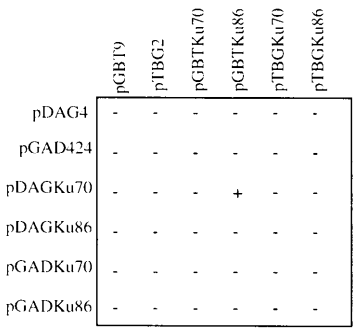

Figure 2. Full-length versions of Ku70 and Ku80 were assayed for interaction in the yeast two-hybrid system using all combinations of available vectors. pGAD424 and pGBT9 lacking inserts were used to control for activation of the reporter gene in the absence of a relevant two-hybrid partner. Reporter gene transcription was activated only when the GAL4-AD was fused to the C-terminus of Ku70 and the GAL4-BD was fused to the N-terminus of Ku80.

Ku70 and Ku80 deletions

Having established a GAL4-based two-hybrid system compatible with the Ku70/Ku80 interaction we sought to map the domains of the subunits responsible for heterodimerization. Deletions of Ku80 were constructed by PCR and cloned into the pGBT9 vector. Pairwise combinations of the Ku80 deletions and full-length pDAGKu70 along with controls were assayed by both qualitative and quantitative β-galactosidase assay. The results are summarized in Figure 3A. Ku80:1–460, which lacks 272 amino acids from the C-terminus, was capable of interaction with full-length Ku70. Ku80:221–732 interacts with Ku70 and produces a signal strength similar to that of Ku80:1–460. The strongest interaction with Ku70, as judged by quantitative β-galactosidase assay, was provided by the Ku80:221–554 construct. Deletions which removed N- or C-terminal amino acids from this central domain resulted in marked decreases in β-galactosidase signal strength. The N- and C-terminal constructs, Ku80:1–220 and Ku:461–732, failed to generate positive two-hybrid interactions. Consistent with a previous report (29), the putative leucine zipper motif (28) of the Ku80 subunit is incapable of conferring interaction in the GAL4-based two-hybrid system (e.g. Ku80:1–220) and is not required for association with Ku70 (e.g. Ku80:221–554). Indeed, quantitative assays indicated that removal of the putative leucine zipper motif in Ku80:221–732 resulted in an increase in β-galactosidase activity compared with full-length Ku80 cDNA.

Using the same approach as for Ku80, we generated deletions of the Ku70 cDNA. These deletions were cloned into the pDAG4 vector and assayed for activation of the *lacZ* reporter gene. In the assay employed here, deletions in the Ku70 coding sequence reduced or abolished interaction with full-length Ku80. The shortest truncations examined removed 90 amino acids from either the C- or N-terminus. In the case of the short C-terminal truncation, interaction with Ku80 was still detectable, although reduced. However, we were unable to detect interactions between Ku80 and the 90 amino acid N-terminal truncation of Ku70 (Fig. 3B).

Immunoprecipitation of epitope-tagged Ku80 deletions in xrs-6 cells

The effects of GAL4 fusions on Ku subunit interactions and the possible discrepancies between our GAL4 two-hybrid results and the LexA two-hybrid results published by Wu and Lieber led us to investigate an alternative method for determining which regions of Ku80 were involved in Ku70 association. Ku70

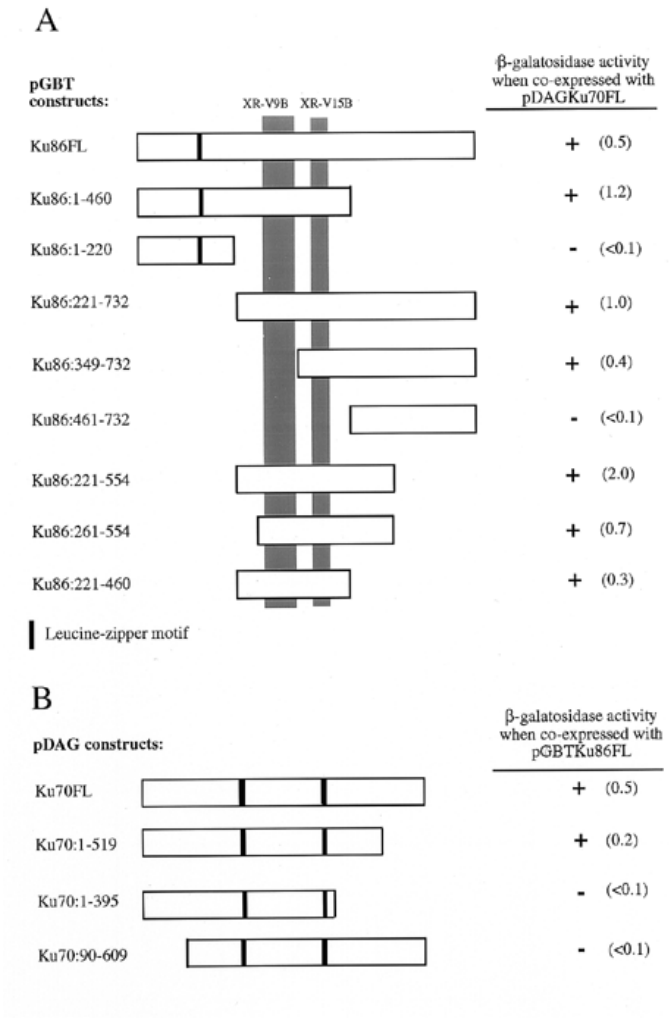

Figure 3. Truncations of Ku70 and Ku80, in pDAG4 and pGBT9 respectively, were used to map the regions required for Ku subunit interaction. Co-transformation of pDAG4- and pGBT9-based constructs were carried out as shown; in all cases negative controls consisting of Ku-based inserts and partner vectors without insert were performed in parallel. Both qualitative and quantitative assessment of reporter gene activation was used. Negative controls with activity above 0.1 U were not observed. Yeast strain SFY526 was co-transfected with truncated versions of the Ku80 cDNA in pGBT9 and the full length Ku70 cDNA in the pDAG vector (**A**) or conversely with truncated versions of the Ku70 cDNA in pDAG and the full length Ku80 cDNA in pGBT9 (**B**).

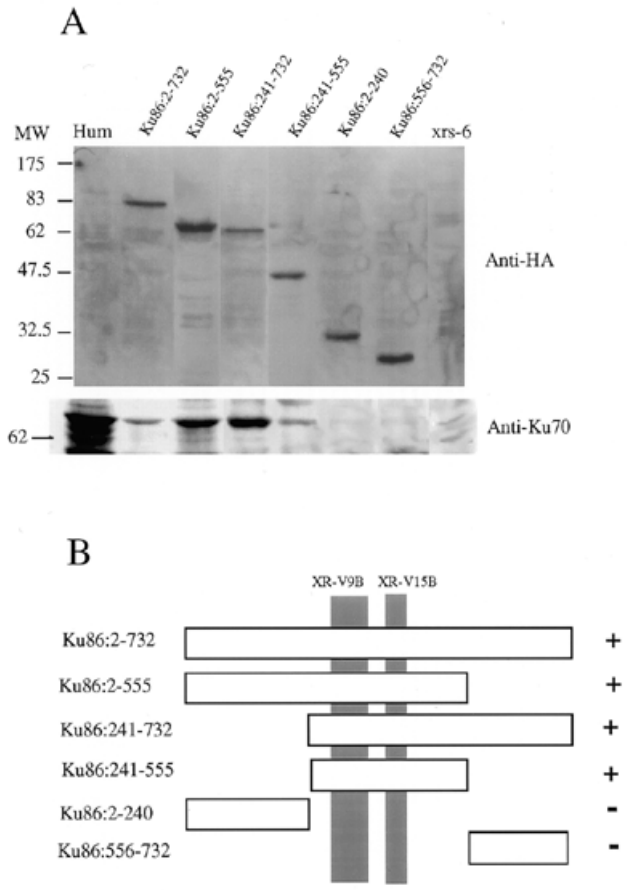

Figure 4. (**A**) Extracts from stable transformants of xrs-6 cells expressing the indicated Ku80 deletions were prepared and subjected to immunoprecipitation using the anti-HA epitope tag antibody. Immunoprecipitations were subjected to SDS–PAGE and immunoblots probed with anti-HA (top) or anti-Ku70 antibody (lower panel). The first lane (Hum) contains total HeLa cell extract, the last lane (xrs-6) contains untransformed xrs-6 cell extract. (**B**) Schematic representation of the Ku80 fragments used in the xrs-6 transfection/immunoprecipitation study shown in (A). The shaded boxes indicate the regions deleted in XR-V9B and XR-V15B.

accumulation is substantially reduced or absent in xrs-6 cells in the absence of the Ku80 subunit (14,19,36). However, exogenous Ku80 expression rescues Ku70 to near wild-type levels (36). HA epitope-tagged versions of Ku80 carrying a N-terminally fused SV40 T antigen nuclear localization sequence were transfected into the Ku80-deficient cell line xrs-6. G418-resistant colonies were isolated and screened for transgene expression by Western blot. Extracts prepared from transfected xrs-6 cells were subjected to immunoprecipitation using a polyclonal anti-HA tag antibody. Western blots of the resulting precipitates were probed with either a monoclonal anti-HA tag antibody or an anti-murine Ku70 antibody (Fig. 4A). The presence of Ku70 reactivity in immunoprecipitates indicates that the exogenous Ku80 fragment is capable of physical association with Ku70. Immunoblots of crude extract from transfected cell lines revealed that the presence of heterodimerization-competent Ku80 fragments resulted in stabilization of endogenous Ku70 (data not shown).

The immunoprecipitation results support the data obtained from our GAL4-based two-hybrid studies (Fig. 4A and B). The C-terminal truncation Ku80:2–555 and the N-terminal truncation Ku80:241–732 both stabilized and co-immunoprecipitated with endogenous Ku70. Immunoprecipitates from extracts of xrs-6 cells expressing Ku80:2–240 or Ku80:556–732 both contained no detectable Ku70, indicating that these polypeptides are unable to form stable complexes with Ku70. However, the Ku80 fragment Ku80:241–555, which contains the central domain identified by the GAL4 two-hybrid studies, stabilized and co-immunoprecipitated with endogenous Ku70.

DISCUSSION

Understanding the interactions between DNA-PK subunits is an important step toward understanding assembly of the DNA-PK holoenzyme and its involvement in DSB repair and V(D)J

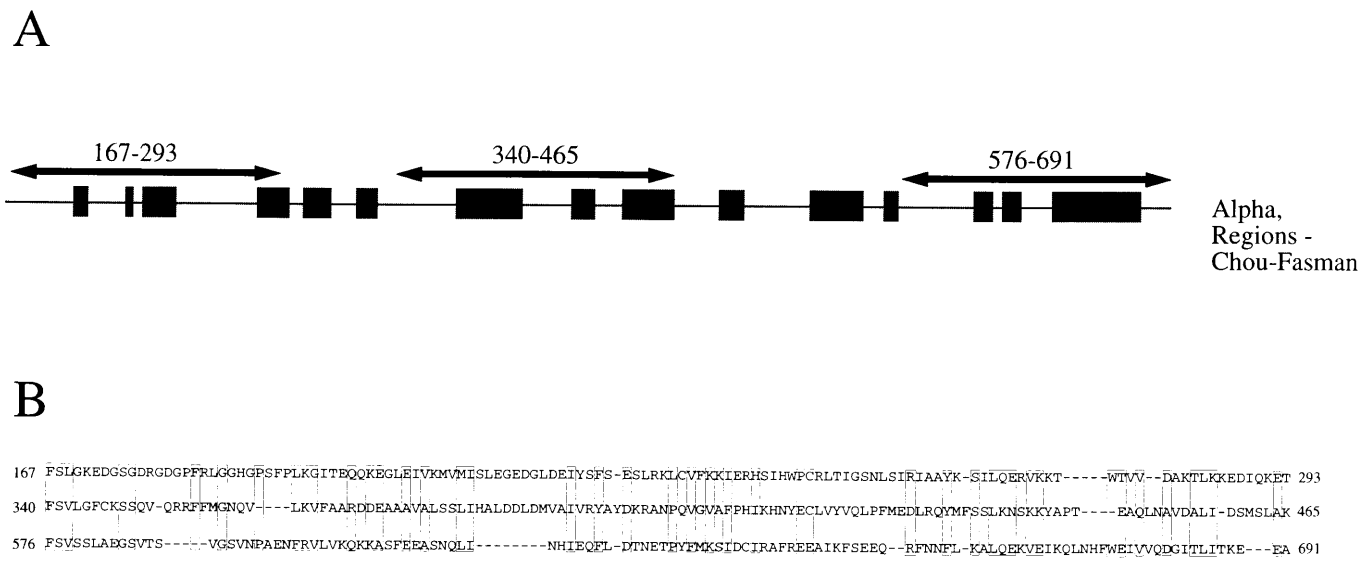

Figure 5. (**A**) Chou–Fasman analysis of residues 167–691 of Ku80. Several stretches of α-helix are predicted in the region found to share homology with dystrophin. (**B**) Division of the Ku80 sequence into three repeats based on both the alignment with dystrophin and the predicted secondary structure. The Clustal algorithm using the PAM250 residue weight table was used to align the repeats, which share an average of 11.5% similarity. Boxed residues are identical in two or more of the aligned sequences. All sequence analysis was performed using the DNAStar software package (DNAStar Inc.)

recombination. This is especially critical information for understanding the phenotype of DSB repair mutants with defects in DNA-PK subunits. Though previous work has demonstrated that the putative leucine zipper regions of the Ku subunits are dispensable for subunit association, these studies relied largely on N-terminal deletions and did not identify a minimal region required for Ku heterodimerization (29).

We have employed a GAL4-based version of the yeast two-hybrid system to examine the interaction of Ku subunits. Fusions of either the GAL4 DNA binding domain or the activation domain to the Ku70 N-terminus completely abolished the ability to obtain two-hybrid interactions. Fusion of the GAL4 activation domain to the C-terminus, however, resulted in an interaction-competent species when co-expressed with Ku80 carrying an N-terminally fused DNA binding domain. This combination of fusions was used for deletion analysis to map the region of Ku80 involved in Ku70 association. Both Ku70 and Ku80 deletion studies in the GAL4-based system disagree with those previously reported using a LexA-based system (29). Deletions of Ku70 >90 amino acids from either terminus failed to interact with full-length Ku80 in the GAL4-based system. In the LexA system, however, large N-terminal deletions of Ku70 were tolerated and a 171 amino acid C-terminal fragment was implicated in mediating association with Ku80. Comparison of Ku80 deletion studies reveals that a C-terminal fragment of Ku80 (449–732) interacts in the LexA system, though a similar fragment (Ku80:461–732) failed to interact in the GAL4 system. In addition, the proline-rich region of Ku80 (amino acids 478–519), suggested by Wu and Lieber (1996) to be involved in interaction with Ku70, is dispensable for interaction in the GAL4-based assay. Considering the ability of C-terminal Ku70 fragments to interact with full-length Ku80 in the LexA system and the discrepancies between LexA and GAL4 Ku80 deletion results, it was necessary to confirm the two-hybrid findings using an alternative assay.

To resolve conflicts between GAL4- and LexA-based two-hybrid studies we employed an independent assay which requires that Ku80 deletions not only interact with Ku70 but stabilize the otherwise labile 70 kDa subunit *in vivo*. We employed hamster cell line xrs-6 to provide a Ku80-deficient background. Ku80 cDNA fragments carrying an N-terminally fused HA epitope and the SV40 T antigen nuclear localization sequence in a mammalian expression vector were transformed into xrs-6 cells. Stable transformants were used for immunoprecipitation with an anti-HA tag antibody. Studies using this assay support the involvement of a central Ku80 region in Ku70 association and stabilization. Interestingly, a previous study which examined Ku80 deletions in xrs-6 cells found that deletions >7 amino acids from the N-terminus resulted in a failure to obtain Ku70 stabilization, despite accumulation of the exogenous Ku80 truncations (36). The Ku80 deletions used in the xrs-6 immunoprecipitation study presented here all carried a nuclear localization sequence from SV40 T antigen on their N-terminus. Thus it is likely that the inability of the Ku80 deletions examined by Singleton *et al.* (1997) to rescue Ku70 in xrs-6 cells was a result of these deletions not undergoing proper nuclear import. Our data suggest that short N-terminal deletions do not result in loss of an interaction domain or misfolding of the polypeptide.

The central domain identified here as mediating Ku70 interaction by two independent assays corresponds closely with regions deleted in the Ku80 mutant cells XR-V9B and XR-V15B (37). Previous reports have demonstrated that internal deletions in the Ku80 gene result in a loss of DNA end binding activity, reduced Ku70 accumulation, reduced DSB repair and V(D)J recombination defects in these mutant cell lines (38,39). The involvement of Ku80 amino acids 261–554 in Ku70 association may explain the phenotype of XR-V9B and XR-V15B. Others have reported that the Ku80 of XR-V9B suffers an internal in-frame deletion resulting in the loss of amino acids 267–350 (39). This region corresponds to the N-terminal portion of the interaction domain

identified here. Similarly, the internal deletion in XR-V15B Ku80 maps to amino acids 372–417, a deletion corresponding to the middle of the interaction domain. The fact that these deletions map to a region which mediates formation of the Ku heterodimer is consistent with the phenotype of these mutant cell lines. In particular, the failure of XR-V9B and XR-V15B to accumulate Ku70 to normal levels supports the contention that association between the mutant Ku80 in these cells is compromised.

The region of the Ku80 polypeptide responsible for mediating interaction with Ku70 shares homology with the spectrin repeats of the dystrophin protein (unpublished observations). Spectrin repeats have been implicated in mediating protein–protein associations (40). Secondary structure prediction algorithms reveal triplets of α-helical stretches within some repeats of spectrin and related proteins (41). Chou–Fasman analysis indicates that the central region of Ku80 is likely to have similar α-helical stretches (Fig. 5A). The distribution of predicted α-helical content in Ku80 and the homology between Ku80 and dystrophin were used to align three Ku80 sub-regions using the Clustal algorithm (Fig. 5B). The average 11.5% similarity of the Ku80 segments is similar to that observed for the repeats of spectrin, which fall in the 9–25% similarity range (41). BLITZ searches using Ku70 and DNA-PKcs sequences revealed the presence of homologies to spectrin repeat proteins similar to those found for Ku80 (unpublished observations). The role of the Ku80 central region in Ku heterodimerization suggests that the regions of spectrin homology in DNA-PK subunits are likely to be involved in holoenzyme assembly or in mediating interaction with other DNA repair proteins.

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