The Ku Antigen-Recombination Signal-binding Protein Jκ Complex Binds to the Nuclear Factor-κB p50 Promoter and Acts as a Positive Regulator of p50 Expression in Human Gastric Cancer Cells*

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Received for publication, August 5, 2003, and in revised form, October 16, 2003 Published, JBC Papers in Press, October 21, 2003, DOI 10.1074/jbc.M308609200

The p50 subunit of NF-κB is a transcription factor that regulates the expression of a variety of genes. Previously, we showed that the expression of Ku antigen, a DNA repair protein, is mediated by NF-κB in gastric cancer AGS cells (Lim, J. W., Kim, H., and Kim, K. H. (2002) J. Biol. Chem. 277, 46093–46100). In this study, we report that the inhibition of Ku activity reduced both p50 expression and nuclear NF-κB activity in AGS cells. A co-immunoprecipitation experiment demonstrated that Ku antigen interacted with recombination signal-binding protein Jκ (RBP-Jκ), a DNA-binding protein. Ku antigen, RBP-Jκ, and p50 were found to bind to the DNA region containing the κB element in the p50 promoter. Supershift and competition experiments demonstrated that Ku antigen and RBP-Jκ bound sequence-specifically to downstream elements of κB at GCTTC and TGGGGG. mRNA expression and de novo synthesis of p50 were inhibited in cells transfected with the mutant gene expression constructs for IkBa, Ku80, and RBP-Jκ. A reporter assay demonstrated that p50 transcription was positively mediated by NF-κB, Ku antigen, and RBP-Jκ and that the binding elements for these proteins were required for optimal p50 expression. The interaction of Ku antigen with RBP-Jκ and NF-κB p50 may act as a positive regulator of p50 expression in gastric cancer AGS cells.

NF-κB is a transcription factor that regulates a wide variety of the genes that respond to immune or inflammatory signals (1). NF-κB is a member of the Rel family, which includes p50 (NF-κB1), p52 (NF-κB2), RelA (p65), and c-Rel and RelB (2). In resting cells, NF-κB is localized in the cytoplasm as a hetero- or homodimer, which is noncovalently associated with cytoplasmic inhibitory proteins, including IκBa. Upon stimulation by a variety of pathogenic inducers such as viruses, mitogens, bacteria, and inflammatory cytokines, IκBa is degraded in the cytoplasm, allowing the NF-κB complex to migrate into the nucleus and to bind to a DNA recognition site in the regulatory regions of the target genes (3). Some stimuli, including phorbol ester, tumor necrosis factor, and platelet-activating factor, induce p50 mRNA expression (4, 5). p50 expression in leukemia cells and in lung and skin cancer tissues is higher than that in their normal counterparts, suggesting that its expression may be related to tumor or cancer development (6–9). It has been reported that p50 induction is partially mediated by members of the NF-κB family by binding the κB element in the p50 promoter (10, 11). However, comparatively little is known about the factors and signal transduction pathways that contribute to the regulation of p50 expression.

Recombination signal-binding protein Jκ (RBP-Jκ)1 is a DNA-binding protein that participates in the control of both cytokine and NF-κB p52 protein expression (12–16). Several genes known to be repressed or activated by RBP-Jκ possess a κB element containing overlapping NF-κB and RBP-Jκ-binding elements in their promoter regions (12–16). Therefore, there is a possibility of interplay between NF-κB and RBP-Jκ for binding to the κB element in the p50 promoter. Ku antigen, a heterodimer of Ku70 and Ku80, is the regulatory DNA-binding region of DNA-dependent protein kinase, which has been implicated in several nuclear processes, including DNA double strand break repair and V(D)J recombination (17–19). Ku antigen possesses a strong affinity for DNA ends and for peculiar DNA structures such as nicks, gaps, and hairpins (20–23) in a sequence-specific manner (24–29). Putative Ku antigen-specific binding elements have been located in variety of genes such as c-myc, collagen III, and human immunodeficiency virus type 1 (24–29), and Ku antigen has been suggested to be involved in the positive or negative regulation of these genes. Moreover, Um et al. (30) showed that the overexpression of Ku antigen increases nuclear NF-κB activity in Rat-1 fibroblasts. Therefore, NF-κB subunit expression and NF-κB activity might be regulated by the Ku antigen expression level in certain cells.

Here, we show that Ku antigen regulates the expression of the NF-κB p50 subunit and interacts with RBP-Jκ. Our results also show that Ku antigen, RBP-Jκ, and NF-κB p50 are associated with the DNA region that contains the κB element in the p50 promoter and that these proteins positively regulate p50 expression. Thus, we propose that the interaction of Ku antigen with RBP-Jκ and NF-κB p50 acts as a positive regulator of p50 expression in gastric cancer AGS cells.

EXPERIMENTAL PROCEDURES

Cell Culture and Stable Transfection—Human gastric cancer AGS cells (adenocarcinoma, CRL1739, American Type Culture Collection, Manassas, VA) were cultured in RPMI 1640 medium supplemented with 10% fetal bovine serum. Ku dominant-negative cells (KuDN-2 and KuDN-7) and pcN-3 cells, as described previously (31), were derived

* This work was supported by grants from the Korean Ministry of Health and Welfare (to H. K.) and from the Brain Korea 21 Project for Medical Science, Yonsei University College of Medicine, Seoul, Korea. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

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1 The abbreviations used are: RBP-Jκ, recombination signal-binding protein Jκ; RT, reverse transcription; EMSA, electrophoretic mobility shift assay.
from AGS cells stably transfected with a Ku80 mutant gene containing a C-terminal Ku80 fragment (amino acids 427–732) and with a control pcDNA3 expression vector (Invitrogen). KuDN-2, KuDN-7, and pcN-3 cells were cultured in medium containing 200 μg/ml G418. Cytoplasmic, nuclear, and whole cell extracts were prepared as described (31). Protein concentrations were determined using the Bradford assay (Bio-Rad).

Western Blot Analysis—Western blot analysis was performed as previously described (31). Whole cell extract (50 μg of protein/lane) was subjected to 8% SDS-PAGE and transferred to nitrocellulose membrane (Bio-Rad). The membranes were then incubated for 1 h at room temperature in blocking buffer (10 mM Tris-HCl, pH 7.5, 0.1 M NaCl, 0.1% Tween 20, 0.05% sodium azide) and then incubated in the primary antibody for 1 h at room temperature. The primary antibodies used for this analysis were anti-p50 (1:1000; sc-7968), anti-p50 antibody (Roche Applied Science) for 48 h. Wild-type RBP-Jk and mutant RBP-Jk were prepared as described above under “Plasmid Construction.” The anti-p50 mutant gene is an IseB gene mutated at serines 32 and 36 to inhibit NF-kB activation (31), and the Ku80 mutant gene contains a C-terminal Ku80 fragment (amino acids 427–732). pcDNA cells were transiently transfected with a control pcDNA3 expression vector. Whole cell, nuclear, and cytoplasmic extracts were isolated and used in the experiment on the de novo synthesis of p50 and in EMSA for NF-kB activity. For luciferase assay, cells were cotransfected with the Renilla luciferase expression plasmid pRL-SV40 (50 ng of DNA/well) as a control to determine transfection efficiency and interassay variability. Whole cell extracts prepared from the cells were assessed for firefly and Renilla luciferase activities using the Dual-Luciferase Reporter assay system (Promega) according to the manufacturer’s instructions. Firefly luciferase activity was normalized to Renilla luciferase activity in whole cell extracts.

Do de Novo Synthesis and Immunoprecipitation—Wild-type cells or cells transiently transfected with each construct (pcDNA; wild-type RBP-Jk; or mutant genes for RBP-Jk, IseB, and Ku80) were preincubated in methionine- and cysteine-free RPMI 1640 medium containing 2% fetal bovine serum for 30 min and then pulsed with [35S]methionine (120 μCi/ml) for 5 h. The cells were lysed in radioimmunoprecipitation assay buffer and precleared with IgG. Wild-type pcDNA cells were then incubated with anti-p50 antibody and protein G-agarose overnight at 4 °C. The protein G-antibody-antigen complex was washed three times with ice-cold radioimmunoprecipitation assay buffer. The proteins were fractionated by 8% SDS-PAGE, and the dried gel was exposed to x-ray film at −80 °C. The level of p50 synthesized de novo in pcDNA cells was considered to be 100%.

RESULTS

Ku-dependent DNA Binding Activity Is Associated with p50 Expression and Nuclear NF-kB Activity—The C-terminal Ku80 fragment (amino acids 427–732) is known to interact with Ku70, but the heterodimer formed by Ku70 and the C-terminal Ku80 fragment was found to be unable to bind double-stranded DNA, which reduces Ku-dependent DNA binding activity (36–39). Our previous study showed that AGS cells stably transfected with the Ku80 mutant gene containing the C-terminal Ku80 fragment (KuDN-2 and KuDN-7 cells) have lower Ku-dependent DNA binding activity than cells transfected with a control pcDNA3 vector (pcN-3 cells) (31). Here, we show that the protein levels of p50/p105 in KuDN-2 and KuDN-7 cells were lower than those in pcN-3 and wild-type cells, whereas the p65 and RBP-Jk protein levels in these cells remained unchanged (Fig. 1A). The protein levels of p50 and p105 were detected simultaneously using anti-p50 antibody. The p50 mRNA levels were lower in KuDN-2 and KuDN-7 cells than in pcN-3 and wild-type cells (Fig. 1B), whereas p50 and β-actin mRNA levels were not detected in the reverse transcription negative control. To determine NF-kB activity in these cells, we performed EMSA using a commercial NF-kB oligonucleotide containing a consensus NF-kB-binding element. Nuclear NF-kB activities in KuDN-2 and KuDN-7 cells were lower than those in pcN-3 and wild-type cells (Fig. 2, fifth through eighth lanes). A supershift experiment using the nuclear extract showed that the NF-kB band was supershifted by anti-p50 (but not anti-p65) antibody (Fig. 2, tenth and eleventh lanes). These results suggest that the major NF-kB band in the nuclear extract is a p50 homodimer and that Ku antigen activity may
be associated with p50 expression and nuclear NF-κB activity in AGS cells. In cytoplasmic extracts, a significant reduction in the NF-κB band (indicated by an arrow) was shown in KuDN-2 and KuDN-7 cells versus wild-type and pcN-3 cells (Fig. 2, first through fourth lanes). The NF-κB band was identified as a p50 homodimer. The upper band of a p50 homodimer was attributed to a p65/p50 heterodimer, which has previously been reported to bind to the NF-κB oligonucleotide (10, 11). The highest band was attributed to an unknown protein bound to the NF-κB oligonucleotide, which requires further investigation.

*Ku Antigen, RBP-Jκ, and NF-κB p50 Bind to the κB Element in the p50 Promoter.—*The κB element in the human NF-κB p50 promoter is critical for p50 expression in cells in response to tumor necrosis factor and phorbol ester. Moreover, deletion of this element leads to a decrease in basal promoter activity in unstimulated cells (10, 40). Thus, we examined whether κB element binding activity in the p50 promoter is associated with Ku antigen activity using wild-type AGS cells, Ku dominant-negative cells (KuDN-2 and KuDN-7), and pcN-3 cells. κB element binding activities in the p50 promoter in both cytoplasmic and nuclear extracts of KuDN-2 and KuDN-7 cells were lower than those in cytoplasmic and nuclear extracts of pcN-3 and wild-type cells (Fig. 3, left panel) as monitored by EMSA using the 32P-labeled p50 oligonucleotide probe. For the supershift experiment, nuclear extracts were preincubated with 30 min with the indicated antibody (specific for p50, p65, RBP-Jκ, Ku70, or Ku80) and subjected to EMSA. Control represents the p50 activity in the nuclear extract preincubated without antibody. The arrow indicates a p50 band.

*Ku Antigen Interacts with RBP-Jκ—*RBP-Jκ is known to mediate gene expression by physically associating with several proteins, e.g. SMART (silencing mediator of retinoïd and thyroid hormone receptors), histone deacetylase-1, and SHARP (SMRT/histone deacetylase-1-associated repressor protein) (41, 42). We investigated the possibility of p50 expression induction due to direct binding between RBP-Jκ and Ku antigen using a co-immunoprecipitation study. Whole cell extracts were immunoprecipitated with anti-Ku70 or anti-Ku80 antibody, and then immunoprecipitates were subjected to Western blotting with anti-RBP-Jκ antibody (Fig. 4). RBP-Jκ was detected in the anti-Ku70 and anti-Ku80 immunoprecipitates, but the amount of RBP-Jκ that co-immunoprecipitated with Ku70 and Ku80 was lower in KuDN-2 and KuDN-7 cells than in pcN-3 and wild-type cells. Using a different approach, Ku70 and Ku80
were detected in anti-RBP-Jx immunoprecipitates, and the amounts co-immunoprecipitated by RBP-Jx were found to be lower in KuDN-2 and KuDN-7 cells than in pcN-3 and wild-type cells. These results suggest that Ku antigen directly interacts with RBP-Jx and that the C-terminal Ku80 fragment produced by the Ku80 mutant gene inhibits Ku antigen/RBP-Jx association.

**p50 Promoter Region Contains Sequences That Interact with the Ku Antigen-RBP-Jx Complex and NF-xB p50—To further investigate whether Ku antigen, RBP-Jx, and NF-xB p50 bind to the xB element in the p50 promoter, three oligonucleotides containing the binding elements for Ku antigen, RBP-Jx, and NF-xB were constructed and used as competitors (Fig. 5). The Ig-xB and m8 oligonucleotides contain the consensus binding elements for NF-xB and RBP-Jx, respectively (43, 44). The Ku antigen oligonucleotide was used to determine Ku-dependent DNA binding activity because Ku antigen specifically binds this oligonucleotide (31, 34). EMSA of nuclear extracts using the 32P-labeled p50 oligonucleotide as a probe showed that the p50 band was completely competed by the unlabeled Ig-xB, m8, and Ku antigen oligonucleotides (Fig. 6), suggesting that Ku antigen, RBP-Jx, and NF-xB p50 were all involved in the complex DNA-protein interaction. Thus, we introduced a site-specific mutation into the p50 oligonucleotide to locate the potential binding elements for these proteins. The NF-xB-binding element site of p50 is known to be GGGGGGGTCCCT (10). The mutated bases are represented by lowercase letters.

**Fig. 4. Interaction of Ku antigen with RBP-Jx in AGS cells transfected with the Ku80 mutant gene.** Whole cell extracts were prepared from wild-type cells and from cells stably transfected with the control vector (pcN-3 cells) or the Ku80 mutant gene (KuDN-2 and KuDN-7 cells) and were immunoprecipitated (IP) with the indicated antibody (specific for RBP-Jx, Ku70, or Ku80). The immunoprecipitates were analyzed with the indicated polyclonal antibody (specific for RBP-Jx, Ku70, or Ku80) by Western blot analysis (WB).

**Fig. 5. Sequences of oligonucleotides used as probes and/or competitors.** Upper, the Ig-xB and m8 oligonucleotides are consensus NF-xB- and RBP-Jx-binding elements, respectively. The Ku antigen oligonucleotide is the Ku antigen-binding element used as the Ku probe. Lower, the p50 promoter region (located at positions −80 to −50) contains NF-xB- and putative RBP-Jx-binding elements, RBP-Jx(1) and RBP-Jx(2), indicated by the boxes. The mutated bases are represented by lowercase letters.

(47). Therefore, RBP-Jx-binding sequences are not strict, and various RBP-Jx binding could occur. We found that RBP-Jx DNA binding activity was reduced by inhibiting Ku antigen DNA binding activity, suggesting that the association between Ku and RBP-Jx may contribute to RBP-Jx binding to this poor consensus sequence in the p50 promoter. Additionally, NF-xB binding to the NF-xB-binding element (GGGGGGGGTCCCT) as indicated by supershifts was induced by anti-p50 antibody in EMSA using the p50mt1 and p50mt2 oligonucleotides as probes, but not when the p50mt3 and p50mt4 oligonucleotides were used (Fig. 7), which is consistent with previously reported results (10). Anti-p65 antibody did not supershift the p50 band in EMSA when mutant oligonucleotide probes were used, suggesting that NF-xB in the nuclear protein of AGS cells exists as a p50 homodimer. Therefore, the NF-xB p50 homodimer and RBP-Jx bind to the xB element in the p50 promoter. However, the p50 band was competitively shifted with the Ku antigen oligonucleotide and was supershifted by anti-Ku70 and anti-Ku80 antibodies in supershift experiments using the p50mt1, p50mt2, p50mt3, and p50mt4 oligonucleotide probes (Figs. 6 and 7). These results suggest that Ku antigen may not bind to the NF-xB- and RBP-Jx-binding elements, but to another DNA region in the p50 promoter. To determine the Ku antigen-binding element, we introduced various nucleotide changes into the 5′-flanking sequence of the xB element (TGGGGG) in the p50 promoter, which overlaps with the p50-binding element. RBP-Jx is known to bind to an essential core consensus DNA sequence, TGGGAA (46). However, the RBP-Jx-binding element RBP-Jx(1) in the p50 promoter has a poor consensus sequence, TGGGGG. Recently, the promoter regions in a number of other genes were found to contain slightly modified RBP-Jx-binding elements.
xB- and RBP-Jk-binding elements and the mutated Ku antigen-binding element in the p50 oligonucleotide, competed with the p50 band, suggesting that Ku antigen does not bind to the p50 promoter without NF-xB and RBP-Jk binding to this region.

Ku Antigen, RBP-Jk, and NF-xB p50 Positively Regulate p50 Expression and Promoter Activity—To investigate whether RBP-Jk regulates p50 expression, AGS cells were transiently transfected with constructs coding for wild-type RBP-Jk or its dominant-negative mutant gene (mutant RBP-Jk), which does not show RBP-Jk activity, but which nevertheless binds to DNA (48). We also transfected the cells with constructs coding for the Ku80 and IxBa mutant genes, which were reported to inhibit NF-xB activation and Ku DNA binding activity (49). After transfection, de novo protein synthesis and the mRNA expression of p50 were determined. To examine the level of p50 synthesized de novo, cells were transfected with these constructs and then subjected to pulse labeling with [35S]methionine. Levels of labeled p50 protein in whole cell extracts were quantified by immunoprecipitating with anti-p50 antibody, SDS-PAGE, and autoradiography. Transfection with mutant genes for IxBa, RBP-Jk, and Ku80 decreased the de novo synthesis of p50 (Fig. 8A). Additionally, p50 mRNA levels were reduced in the cells transfected with these constructs (Fig. 8B). These results suggest that NF-xB, RBP-Jk, and Ku antigen positively regulate p50 expression. However, p50 expression was reduced in cells overexpressing wild-type RBP-Jk even though this reduction in the level of synthesis and the mRNA level of p50 were less than those in cells transfected with mutant genes for IxBa, RBP-Jk, and Ku80 (Fig. 8, A and B). As shown in Figs. 1, 4, and 8, wild-type gastric cancer AGS cells expressed high levels of NF-xB (p65 and p50), RBP-Jk, Ku70, and Ku80. Therefore, the uncontrolled expression of RBP-Jk may disrupt the proper stoichiometry of NF-xB, RBP-Jk, and Ku antigen, resulting in reduced p50 expression. Further study should be performed on p50 expression in untransformed gastric epithelial cells containing low levels of constitutive NF-xB, RBP-Jk, and Ku antigen. Transfection with mutant genes for IxBa, RBP-Jk, and Ku80 significantly decreased p50-specific DNA binding activity versus cells transfected with the control pcDNA3 vector (Fig. 9). To determine whether these proteins also regulate p50 transcription, the pHNwt luciferase construct containing the wild-type p50 promoter was transiently cotransfected with constructs expressing wild-type RBP-Jk and the mutant genes for IxBa, RBP-Jk, and Ku80. The coexpression of the constructs expressing mutant genes for IxBa, RBP-Jk, and Ku80 significantly decreased p50 transcription. Similar to reduced p50 expression in cells overexpressing wild-type RBP-Jk shown in Fig. 8, the coexpression of the construct expressing wild-type RBP-Jk also repressed pHNwt luciferase activity. Repression of p50 transcription by coexpression with the construct expressing wild-type RBP-Jk may be explained by possible interference with complex formation between NF-xB, Ku antigen, and RBP-Jk in gastric cancer AGS cells containing high levels of constitutive RBP-Jk. To investigate the functional role of each DNA-binding element in p50 gene transcription, luciferase reporter constructs containing mutations within these binding elements were constructed and transfected into AGS cells. The pHNmt1, pHNmt4, and pHNmt3 probes. Control represents the p50 activity in the nuclear extract preincubated without antibody. The arrow indicates a p50 band.
elements for RBP-Jκ, NF-κB and RBP-Jκ, respectively. The pHNmt3 luciferase reporter construct contained mutated DNA-binding elements for both NF-κB and RBP-Jκ. However, the pHNmt2 reporter construct did not contain mutated DNA-binding elements for these proteins. pHNwt and pHNmt2, carrying NF-κB, RBP-Jκ, and Ku antigen-binding elements, were transcribed (Fig. 10B). In contrast, the pHNmt1, pHNmt3, pHNmt4, and pHNmt13 reporter constructs were less transcribed than the pHNwt reporter construct (Fig. 10B). These results demonstrate that NF-κB-, RBP-Jκ-, and Ku antigen-binding elements are required for full p50 expression.

DISCUSSION

NF-κB activity is known to be induced by the overexpression of Ku antigen in Rat-1 fibroblasts (30), suggesting that the expression of NF-κB subunits may be related to Ku antigen activity. In this study, the inhibition of Ku antigen activity by transfection with a C-terminal Ku80 expression gene was found to suppress the expression of p50 (but not of p65) in gastric cancer AGS cells. Transfection of the C-terminal Ku80 expression gene was also found to reduce nuclear NF-κB activity. We then investigated whether RBP-Jκ is involved in p50 expression because p50 expression is mediated by the Ku antigen-RBP-Jκ complex. We treated AGS cells with a C-terminal Ku80 expression gene and determined the inhibition of Ku antigen activity by transfection with a C-terminal Ku80 expression gene. The results were normalized with respect to Renilla luciferase activity. The data are shown as the mean ± S.D. of four separate experiments.

FIG. 8. De novo synthesis and mRNA levels of p50 in AGS cells transiently transfected with wild-type RBP-Jκ or mutant genes for IκB, RBP-Jκ, and Ku80. A, wild-type cells or cells transiently transfected with constructs (i.e. control pcDNA vector; wild-type [wt] RBP-Jκ or mutant genes [mt] for IκB, RBP-Jκ, and Ku80) were pulse-labeled with [35S]methionine, and p50 was immunoprecipitated with anti-p50 antibody. The autoradiograph shown is representative of four separate experiments. The level of p50 synthesized de novo in pcDNA cells was considered to be 100%. B, total RNAs were extracted from the cells, and p50 mRNA levels were determined by RT-PCR analysis. The RT-PCR p50 products were analyzed on ethidium bromide-stained gels and quantitated versus the β-actin housekeeping gene. For the reverse transcriptase negative control (RT (−)), reverse transcriptase was not treated to the reaction mixture, following the procedure described for the other samples of wild-type cells or of cells transiently transfected with wild-type RBP-Jκ or mutant genes for RBP-Jκ, IκBα, and Ku80. The level of pcDNA was considered to be 100%. Each bar represents the mean ± S.D. of four separate experiments.

FIG. 9. Binding activity of p50 in AGS cells transiently transfected with wild-type RBP-Jκ or mutant genes for IκB, RBP-Jκ, and Ku80. Cytoplasmic and nuclear extracts were prepared from wild-type cells or from cells transiently transfected with the constructs (i.e. control pcDNA vector; wild-type [wt] RBP-Jκ or mutant genes [mt] for IκBα, RBP-Jκ, and Ku80). The binding activity of p50 was determined using a 32P-labeled p50 oligonucleotide probe. The arrow indicates a p50 band.

FIG. 10. Luciferase activity of AGS cells transiently transfected with mutated binding elements in the p50 promoter. A, AGS cells were transiently transfected with pRL-SV40 as a control vector, the pHNwt luciferase construct, and the expression constructs (i.e. wild-type [wt] RBP-Jκ or mutant genes [mt] for IκBα, RBP-Jκ, and Ku80). B, cells were transiently transfected with pRL-SV40 as a control vector and the luciferase construct of the p50 promoter unmutated (pHNwt) or mutated (pHNmt) by oligonucleotide-directed in vitro mutagenesis using the mutant oligonucleotide. Mutant pHN plasmids (viz. pHNmt1, pHNmt2, pHNmt3, pHNmt4, and pHNmt13) were generated by oligonucleotide-directed in vitro mutagenesis using the p50mt1, p50mt2, p50mt3, p50mt4, and p50mt13 oligonucleotides, respectively. Luciferase activity was measured after 48 h, and the results were normalized with respect to Renilla luciferase activity. The data are shown as the mean ± S.D. of four separate experiments.
a novel role of Ku antigen as a RBP-Jκ-interacting protein. Furthermore, RBP-Jκ was found to bind to the 5′-flanking sequence of the Kβ element (TGGGGA) in the p50 promoter, which overlaps with the p50-binding site. RBP-Jκ has been shown previously to bind to an essential core consensus DNA sequence, TGGGAA (46). However, the p50 promoter has a poor consensus sequence (i.e., TGGGGA) to which RBP-Jκ nevertheless binds as determined by supershift experiments. Promoter regions in a number of other genes also contain slightly modified RBP-Jκ-binding sites. For example, RBP-Jκ binds to a poor consensus sequence (GCTGAGAT) in the cyclin D1 promoter (47). Therefore, the RBP-Jκ-binding sequence is not strict, and variant RBP-Jκ binding could occur. In this study, RBP-Jκ DNA binding activity was reduced by inhibition of Ku antigen activity. This result suggests that association between Ku antigen and RBP-Jκ contributes to RBP-Jκ binding to this poor consensus sequence.

In this study, we found that Ku antigen bound specifically downstream of the Kβ element in the p50 promoter. A competition experiment with mutant oligonucleotides identified the Ku antigen-binding sequence, GGGTC. The promoter. A competition experiment with mutant oligonucleotides identified the Ku antigen-binding sequence, GGGTC. Therefore, the stoichiometric expression of p50 might be associated with both the cellular and nuclear up-regulation of cell growth and proliferation (31, 45, 49).

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