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Recognition sequence of a highly conserved DNA binding protein RBP-Jκ

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ABSTRACT

DNA binding specificity of the RBP-Jκ protein was extensively examined. The mouse RBP-Jκ protein was originally isolated as a nuclear protein binding to the Jκ type V(D)J recombination signal sequence which consisted of the conserved heptamer (CACTGTG) and nonamer (GGTTTTTGT) sequences separated by a 23-base pair spacer. Electrophoretic mobility shift assay using DNA probes with mutations in various parts of the Jκ recombination signal sequence showed that the RBP-Jκ protein recognized the sequence outside the recombination signal in addition to the heptamer but did not recognize the nonamer sequence and the spacer length at all. Database search identified the best naturally occurring binding motif (CACTGTGGGAA-CGG) for the RBP-Jκ protein in the promoter region of the m8 gene in the Enhancer of split gene cluster of *Drosophila*. The binding assay with a series of m8 motif mutants indicated that the protein recognized mostly the GTGGGAA sequence and also interacted weakly with ACT and CG sequences flanking this hepta-nucleotide. Oligonucleotides binding to the RBP-Jκ protein were enriched from a pool of synthetic oligo-nucleotides containing 20-base random sequences by the repeated electrophoretic mobility shift assay. The enriched oligomer shared a common sequence of CGTGGGAA. All these data indicate that the RBP-Jκ protein recognizes a unique core sequence of CGTG-GGAA and does not bind to the V(D)J recombination signal without the flanking sequence.

INTRODUCTION

The vast diversity in specificities of the antigen recognition is primarily generated by somatic recombinatorial assembly of multiple germline DNA segments called variable (V), diversity (D), and joining (J) segments in the immunoglobulin and T cell receptor genes (1–3). Each segment of V, D, and J is flanked by a recombination signal sequence which consists of a palindromic heptanucleotide sequence and an AT-rich nonameric sequence separated by a spacer of either 12 or 23 base pairs. The heptamer and nonamer sequences are highly conserved during evolution whereas the spacer sequence is not (4–6). Several lines of evidence indicate that the V(D)J recombination processes are directed by the recognition of the recombination signals (7–9).

In an attempt to identify proteins involved in V(D)J recombination, we isolated, from nuclear extract of a mouse preB cell line, a nuclear protein named RBP-Jκ which specifically recognized the recombination signal with a 23-base pair spacer (Jκ type signal) (10). Its cDNA and genomic clones have been isolated (11, 12). The deduced amino acid sequence of mouse RBP-Jκ revealed the presence of a 40 amino acid motif in the middle of the protein which shared homology with the catalytically active site common to a group of site-specific recombinases known as the integrase family (11, 13, 14). The binding specificity and the homology to the integrase family suggested that the RBP-Jκ protein might be involved in V(D)J recombination.

The RBP-Jκ gene has been shown to be conserved highly during evolution from *Drosophila* to human (15, 16). The *Drosophila* RBP-Jκ protein showed 75% identity to the mouse counterpart (15). The human and mouse RBP-Jκ proteins were 98% identical (16). More recently, the *Drosophila* RBP-Jκ gene was shown to be identical to Suppressor of Hairless [Su(H)], a gene involved in regulation of the peripheral nervous system development (17, 18). Heterozygous loss-of-function mutations of *Drosophila* Hairless (H) gene show two distinct phenotypes on the bristle sensilla of the adult fly; bristle loss phenotype and double socket phenotype (19, 20). Heterozygous loss-of-function mutations of RBP-Jκ suppress and gain-of-function mutations enhance the H phenotypes (18, 21). H and RBP-Jκ may function in an antagonistic manner at two distinct developmental stages; at the stage of singling out of sensory mother cells and later at the stage of cell fate determination of a precursor cell into trichogen and tormogen cells. Loss-of-function mutations of the H and RBP-Jκ genes are both recessive lethal in late larval to early pupal stages.

The function of RBP-Jκ in mouse is not clear. Expression of the RBP-Jκ gene in mouse is ubiquitous and almost constant in various adult mouse tissues and in various culture cells (22) in

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contrast to the restricted expression of the *Drosophila* RBP-Jκ gene in the peripheral nervous system at the embryonal, larval and pupal stages (18). Understanding the function of the RBP-Jκ gene requires identification of genes regulated by the RBP-Jκ gene. In order to clarify the target genes for the RBP-Jκ protein, we have to know the precise DNA binding specificity. Here, we describe the detailed analysis of DNA binding specificity of purified mouse RBP-Jκ protein.

MATERIALS AND METHODS

Biological materials

The RBP-Jκ protein was purified from a mouse preB cell line, 38B9, as described by Hamaguchi *et al.* (10). Fractions of the second DNA affinity column chromatography were used in this study and referred to as the purified RBP-Jκ fractions. The purities (around 50%) and the protein concentrations (approx. 0.5−2 ng of the RBP-Jκ protein/μl) of the purified preparations were estimated by the SDS−polyacrylamide gel electrophoresis followed by silver staining (10). Restriction enzymes, the Klenow fragment of *Escherichia coli* DNA polymerase I, T4 DNA ligase, T4 polynucleotide kinase, *Thermus aquaticus* DNA polymerase (AmpliTaq, Perkin-Elmer Cetus) were purchased from Takara Shuzo Co. Ltd (Kyoto, Japan); radiolabelled nucleotides were from Amersham Corp.

DNA probes

pJH299 plasmid containing the Jκ wild type and the Vκ wild type recombination signals was a generous gift from Dr M.Gellert (NIH) and its sequence was described (8). The DNA fragment containing one of the recombination signals was isolated by digestion of pJH299 with *Bam*HI (for JκRS[wild]) or with *Sal*I (for VκRS[wild]) and cloned into the pUC19 vector. JκRS[−11] and JκRS[−11*Xba*I] were chemically synthesized using a DNA synthesizer (model 380B, Applied Biosystems) and cloned in the pUC19 vector. JκRS[77] was derived from a V(D)J recombination product formed *in vivo* in a transgenic mouse containing a V(D)J recombination substrate (9). To make JκRS[7] and JκRS[9] probes, an *Eco*RI/*Hin*dIII fragment containing JκRS[wild] was digested at the *Sca*I site within the spacer. The resulting small DNA fragments were cloned separately into the *Eco*RI/*Hin*cII (JκRS[9]) and *Sma*I/*Hin*dIII (JκRS[7]) sites of pUC19. Sequence and direction of each recombination signal cloned in pUC19 were confirmed by sequencing using Sequenase kits (United States Biochemical Corp.). The recombination signal probes were excised from pUC19 by digestion with *Eco*RI and *Hin*dIII, and purified by agarose gel electrophoresis. The purified probes were labelled with α-^{32}P-dATP and the Klenow fragment as described by Hamaguchi *et al.* (10). Sequences of recombination signal probes used are listed in Fig. 1a.

In some experiments, oligonucleotides were used directly without cloning into pUC19. Single-strand oligonucleotides (50 ng) were labelled at the 5′ ends by incubation for 45 min at 37°C in reaction mixtures (10 μl) containing 50 mM Tris−HCl (pH 7.5), 10 mM $MgCl_2$, 10 mM 2-mercaptoethanol, 0.93 MBq γ-^{32}P-ATP and 5 units of T4 polynucleotide kinase. After the incubation, the reaction mixtures containing complementary oligonucleotides were mixed, heated for 3 min at 95°C, then for 5 min at 72°C and slowly cooled to 25°C to anneal the strands. The mixture was diluted with H_2O to 100 μl and used for binding assays.

Electrophoretic mobility shift assay

Electrophoretic mobility shift assay (EMSA) was carried out as described by Hamaguchi *et al.* (10) using 1−5 ng of labelled DNA probe and 1 μl of the purified RBP-Jκ fraction (approx. 0.5−2 ng of the RBP-Jκ protein). To determine the binding activity of the probes quantitatively, the bands corresponding to the free and protein-bound probe were excised from the gel and radioactivity in the bands was measured by a scintillation counter (model 460, Packard). The binding activity was calculated as percentage of the protein-bound radioactivity in the total radioactivity.

Enrichment of binding oligonucleotides

Oligonucleotides binding to the purified RBP-Jκ protein were enriched from a pool of random oligonucleotides by a modified method of Blackwell and Weintraub (23). Synthetic oligonucleotides (50 ng) of 60 bases (BF20) containing 20-base random sequences at the middle (its structure is shown in Fig. 3a) were labelled with γ-^{32}P-ATP and T4 kinase as described above. After the labelling reaction, 25 ng of the HBP3R primer (5′-GTCAGAATTCTTCAGTGACTGC-3′) was added to the mixture. The mixture was heated for 2 min at 95°C and for 5 min at 75°C, and then allowed to cool slowly to 25°C over a period of 30 min to anneal the primer with BF20. The annealed products were incubated with the Klenow fragment to synthesize the complementary strand in a mixture (25 μl) containing 10 mM

a

DNA probe	Sequence		
	nonamer	spacer	heptamer
JkRS(wild)	GGTTTTTGT	ACAGCCAGACAGTGGAGTACTAC	CACTGTG GGATCC
VkRS(wild)	GGTTTTTGT	TCCAGTCTGTAG	CACTGTG GTCGAC
JkRS(-11)	GGTTTTTGT	ACAGCC----------TACTAC	CACTGTG GGATCC
JkRS(-11Xba)	GGTTTTTGT	ACAGCC----------TACTAC	CACTGTG TCTAGA
JkRS(77)	GGTTTTTGT	ACAGCCAGACAGTGGAGTACTAC	CACTGTG CACAGTG-12spacer-ACATAAACC
JkRS(7)	---------	---------------CCCACTAC	CACTGTG GGATCC
JkRS(9)	GGTTTTTGT	ACAGCCAGACAGTGGAGT-----	------- ------

b

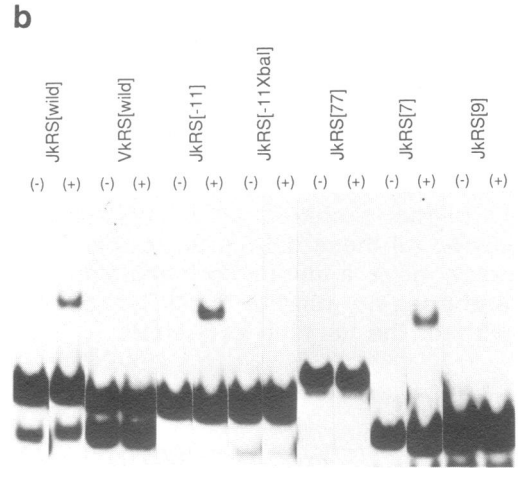

Figure 1. Binding of mutant recombination signal probes to mouse RBP-Jκ protein. Panel **a**, nucleotide sequences of DNA probes used. Only the sequence of the inserted recombination signal is shown. The probes contain the polylinker sequences derived from the pUC19 vector on both sides of the inserts. Double underlines or hyphens show the nucleotides changed or deleted, respectively. Panels **b**, EMSA of the mutant probes. The binding reaction was carried out using 1 ng of DNA probe with (right lane, shown with +) or without (left lane, shown with −) the addition of 1 μl of the purified RBP-Jκ fraction.

Tris−HCl (pH 7.5), 10 mM $MgCl_2$, 1 mM dithiothreitol, 50 mM NaCl, 0.5 mM each of deoxynucleotide triphosphates and 5 units of the enzyme for 45 min at 20°C. The double-stranded BF20 probe (4.5 ng) was incubated with 2 μl of the purified RBP-Jκ fraction and the protein-bound DNA was separated by EMSA. The protein-bound DNA was recovered from the gel using Whatman DE81 paper. One hundredth of the recovered DNA was amplified by the polymerase chain reaction (PCR) (25 cycles at 94°C for 1 min, at 45°C for 2 min, and then at 72°C for 2 min) using the HBP3R and HBP4 primers (5′-GATGAAGCTT-CCTGGACAAT-3′) in a mixture (50 μl) containing 67 mM Tris−HCl (pH 8.8), 6.7 mM $MgCl_2$, 16.6 mM dimethyl sulfoxide, 0.1 mM each of deoxynucleotide triphosphates, 200 ng each of the primers, and 1.25 units of *T.aquaticus* DNA polymerase. The amplified products recovered by ethanol precipitation were heat denatured, annealed with the HBP3R and HBP4 primers, and incubated with the Klenow fragment as described above in the presence of α-^{32}P-dCTP for labelling and to make the DNA completely double-stranded. The double-stranded DNA was purified by agarose gel electrophoresis under the EMSA conditions and used for another cycle of the EMSA enrichment. The enrichment was repeated 4 times in total. The DNA recovered from the final enrichment was amplified by PCR, digested with *Eco*RI and *Hin*dIII, and cloned into the Bluescript KS(+) plasmid vector. When BF10 probe (see Fig. 3a for structure) was used for enrichment, *Eco*RI-F and SK primers were used in place of HBP3R and HBP4 primers, respectively. The sequences of the primers are; *Eco*RI-F, 5′-AATTCGAGG-ATCCGGGTACCATGG-3′ and SK, 5′-TCTAGAACTA-GTGGATC-3′.

RESULTS

Binding of mutated recombination signal probes

In our previous paper (10) we showed that the purified mouse RBP-Jκ protein specifically bound to the Jκ type recombination signal with a 23-base pair spacer but did not bind to the Vκ type signal with a 12-base pair spacer (also see Fig. 1b). This binding specificity had been one of the characteristics suggestive of the involvement of the RBP-Jκ protein in V(D)J recombination. To re-examine the binding specificity, we deleted 11 nucleotides within the spacer of the wild type Jκ recombination signal probe (JκRS[wild]). Contrary to our expectation, the deletion probe (JκRS[−11]) could bind to the RBP-Jκ protein as equally well as the JκRS[wild] probe (Fig. 1b). The JκRS[−11] probe was a Vκ type probe with respect to the spacer length. We then examined the recombined signal sequence (JκRS[77]) which had both the Jκ type and Vκ type signals joined tail-to-tail at the precise ends of two heptamers through *in vivo* V(D)J recombination. This probe did not bind to the RBP-Jκ protein, although it contained the complete sequence of JκRS[wild]. We therefore suspected the involvement of the sequence (GGATCC) outside the heptamer of the JκRS[−11] probe and replaced it with a different sequence (TCTAGA, JκRS[−11 *Xba*I]). This replacement abolished the binding to the RBP-Jκ protein.

We then truncated the JκRS[wild] probe within the spacer. A probe (JκRS[7]) containing a part of the spacer, the heptamer, and the sequence following the heptamer could bind to the RBP-Jκ protein as equally well as the JκRS[wild] probe (Fig. 1b). On the othe hand, a probe (JκRS[9]) containing the nonamer and most of the spacer did not bind to the RBP-Jκ protein. All these

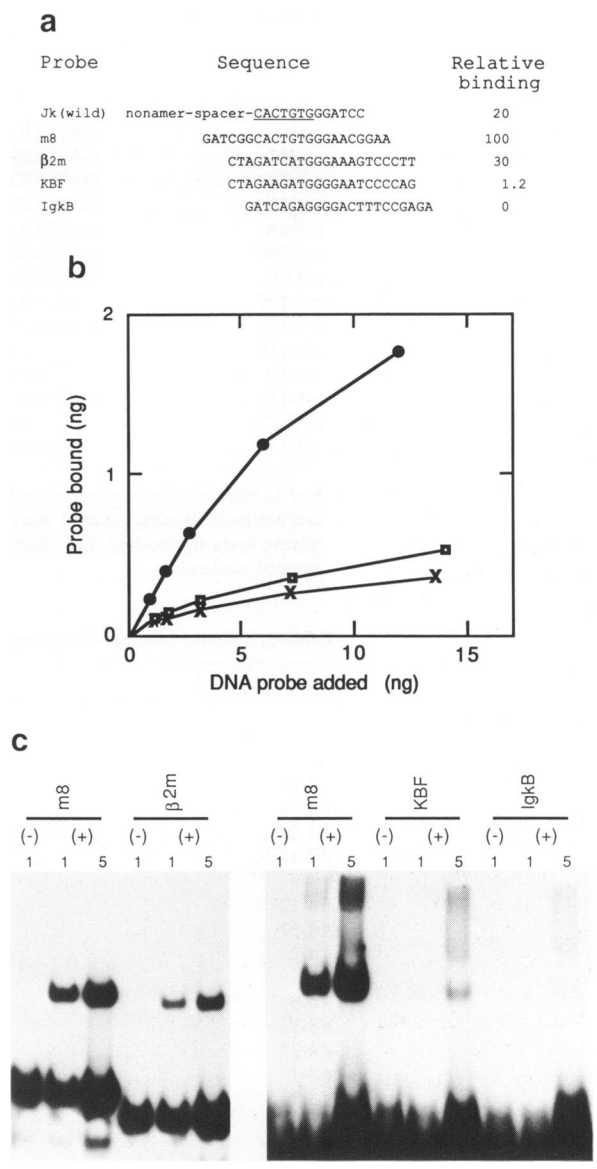

Figure 2. Binding of naturally occurring DNA sequences to mouse RBP-Jκ protein. Panel **a**, sequences of DNA probes containing an upstream sequence of the m8 gene of *Drosophila* Enhancer of split (m8), and NF-κB recognition sequences derived from mouse β_2-microglobulin gene (β2m), mouse MHC class I H-2k^b gene (KBF), and mouse immunoglobulin κ chain gene (IgκB). The underline shows the heptamer sequence in the JκRS[wild] probe. The relative binding activity for each probe was determined from the data shown in b and c (binding of m8 probe = 100%). Panel **b**, binding of m8, JκRS[−11], and JκRS[wild] probes to the RBP-Jκ protein as a function of probe concentration. The binding reaction was carried out with 1 μl of the purified RBP-Jκ fraction and various amounts of probe indicated. After electrophoresis, radioactivities in the gel regions corresponding to the free and protein bound probe were measured by a scintillation counter. (●), binding of m8; (□), binding of JκRS[−11]; (×), binding of JκRS[wild]. Panel **c**, EMSA of the m8 and β2m (left 6 lanes) and m8, KBF, and IgκB probes (right 9 lanes). In the left 6 lanes, m8 and β2m DNA probes used were restriction fragments derived from pUC19 plasmids containing the 36-base pair m8 sequence and the 22-base pair β2m sequence. In the right 9 lanes, DNA probes used were 22-base pair synthetic oligonucleotides. The right autoradiogram showing the binding of m8, KBF, and IgκB was obtained after a long exposure to show the weak binding of IgκB. The binding reaction was carried out using 1 or 5 ng of a labelled DNA probe (lanes marked with 1 or 5) with (+) or without (−) 1 μl of the purified RBP-Jκ fraction. The proportion of protein-bound probe in the total was determined as described above and the relative binding activity was shown in Panel a.

Table 1. Binding of m8 mutant probes to the RBP-Jκ protein

DNA probe	Sequence (1 5 10 15)	Relative binding
m8	GATCGGCACTGTGGGAACGGAA	100
m8-2T	GATCGGCTCTGTGGGAACGGAA	66
m8-5A	GATCGGCACTATGGGAACGGAA	23
m8-5T	GATCGGCACTTTGGGAACGGAA	0
m8-6A	GATCGGCACTGAGGGAACGGAA	0
m8-78C	GATCGGCACTGTCCGAACGGAA	0
m8-9C	GATCGGCACTGTGGCAACGGAA	0
m8-10T	GATCGGCACTGTGGGTACGGAA	2
m8-10G	GATCGGCACTGTGGGGACGGAA	2.5
m8-11C	GATCGGCACTGTGGGACCGGAA	16
m8-11G	GATCGGCACTGTGGGAGCGGAA	72
m8-12G	GATCGGCACTGTGGGAAGGGAA	32
m8-12T	GATCGGCACTGTGGGAATGGAA	58
m8-13C	GATCGGCACTGTGGGAACCGAA	88

End-labelled synthetic oligonucleotides (1 ng) were used for EMSA as described in Materials and Methods. Binding activity was determined as described in Fig. 2 and shown as per cent relative to the m8 binding. The underline shows the heptamer motif and double underlines show mutated nucleotides.

Table 2. Relative binding of oligonucleotides enriched by binding to the RBP-Jκ protein

DNA probe	Sequence (1 5 10)	Relative binding
m8	CACTGTGGGAACGG	100
#4-24	CTGCGTGGGAACTA	350
#4-35	t AGCGTGGGAATAT	340
#4-29	ctgcGTTGGGAATCGTGTGAACTT	330
#4-14	ACGCGTGGGAAACT	310
#4-8	AACCGTAAGAACCG	230
#4-12	a a t CGTGGGAAAAC	220
#4-32	AACCGTGAGAAAAT	210
#4-2	TCACGTGGGAATTT	200
#4-19	TGGCGTGGGA a t t g	200
#4-9	c t g c GTGGGAGAAT	200
#3-12	TACCGTGAGAACAG	200
#4-25	GTCCGTGTGAACCT	160
#4-15	AACCGTGTGAAAGC	150
#3-14	t GTCGTGGGTACTC	80
#4-13	ATTGGTGGGAACCG	60
#4-22W	CACTGTGGATAATC	40
#4-3	t AGCGTGAAAACTA	0

EMSA was carried out as described in Materials and Methods using 1 ng of DNA fragment recovered from plasmid by *ClaI* and *XbaI* digestion. The binding activity of each probe is shown as per cent relative to the m8 binding.

data indicate that the RBP-Jκ protein does not recognize the nonamer sequence or the spacer length but does recognize the heptamer (CACTGTG) and the sequence which follows the heptamer and starts with GGATCC. This GGATCC sequence is absent in the Vκ wild type probes used in our experiments (ref. 10 and Fig. 1a).

The DNase I foot printing analysis described in our previous paper (10) demonstrated that, in addition to the GTG sequence in the heptamer, at least two nucleotides outside the heptamer showed different DNase I sensitivities before and after the binding to the RBP-Jκ protein. This finding agrees well with the present results.

Naturally occurring RBP-Jκ binding sequences

We searched for naturally occurring candidates of the RBP-Jκ binding sequence among databases using the CACTGTGGGATCC sequence as reference. Since the *Drosophila* RBP-Jκ gene has been shown to be involved in the peripheral nervous system development (17, 18), the search was focused on genes functioning in the development of the peripheral nervous system in *Drosophila*. One gene in the cluster of Enhancer of split [E(spl)] (24, 25) was picked up by this search. This gene, m8 of E(spl), contained a sequence of CACTGTGGGAACG at around 610 bp upstream of the transcription initiation site (25). A synthetic oligonucleotide probe containing this sequence was examined for binding to the RBP-Jκ protein. As shown in Fig. 2b, the m8 probe bound to the RBP-Jκ protein approximately 5 times better than the JκRS[wild] or JκRS[−11] probe at all probe concentrations examined. The sequence preceding the heptamer motif of the m8 probe is completely different from the spacer sequence of the JκRS[wild] probe, indicating that the RBP-Jκ protein did not recognize the sequence preceding the heptamer.

KBF2 is a mouse nuclear protein that has been identified as a protein binding to the enhancers of the H-2K^b and

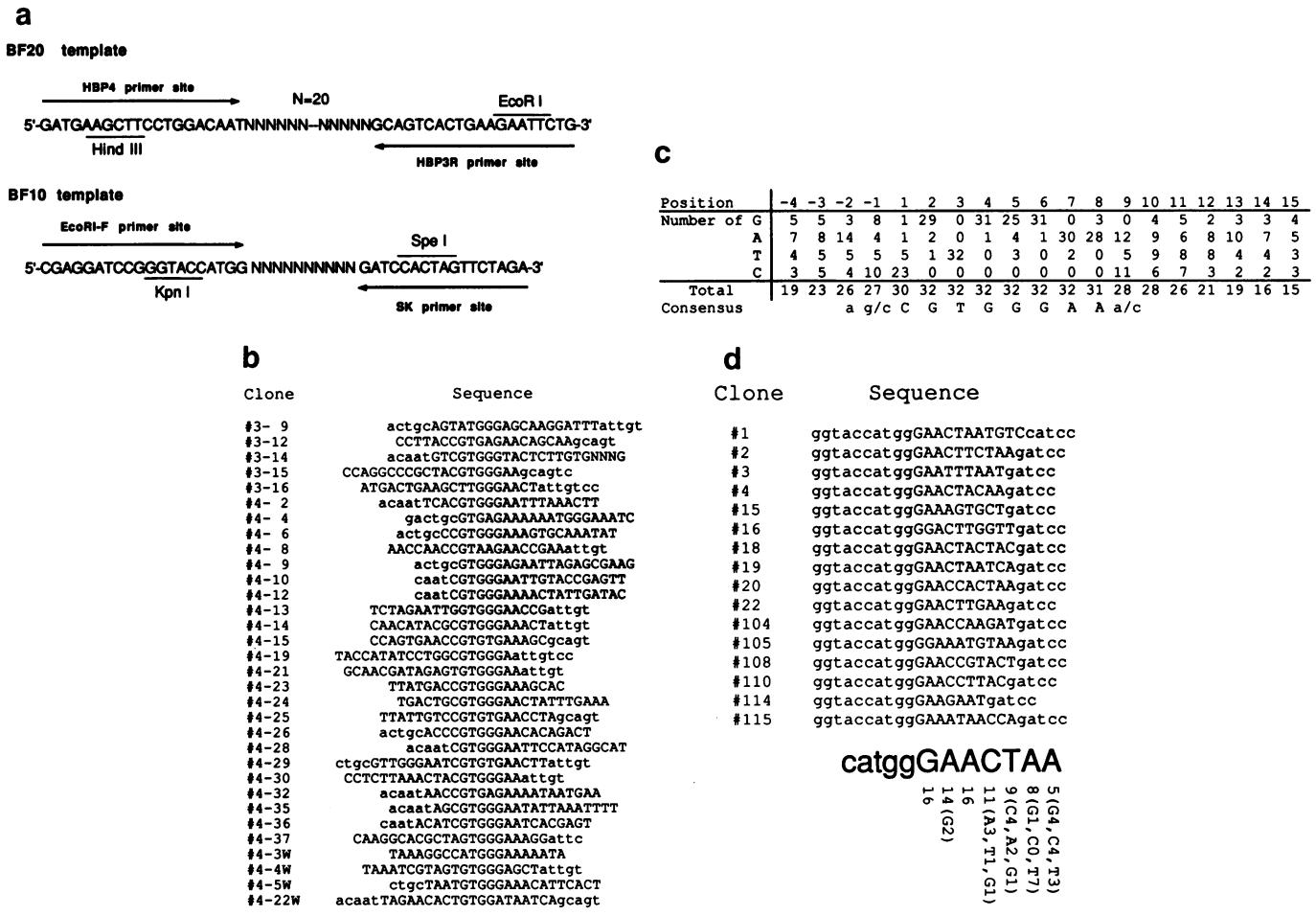

Figure 3. Enrichment of oligonucleotides binding to the RBP-Jκ protein. Panel **a**; structure of the BF10 and BF20 probes and primers used for PCR. Panel **b**; sequences of oligonucleotides enriched from the BF20 probe. Capitals show nucleotides appeared in the random sequence region and small letters show those in the primer regions. The clone names started with #3- or #4- represent oligonucleotides obtained after 3 or 4 cycles of EMSA, respectively. The #4-3 clone which did not bind to the RBP-Jκ (see Table 2) was omitted in this figure. Panel **c**; frequency of base appearance in the random region. Nucleotides in the primer regions are omitted in this table. The position of the first C in the consensus sequence was designated as 1. Panel **d**; sequences of oligonucleotides enriched from the BF10 probe. Capitals show nucleotides appeared in the random sequence region and small letters show those in the primer region. The predicted consensus sequence and the frequency of base occurrence are shown at the bottom.

β_2-microglobulin genes (26). Unexpectedly, however, the human KBF2 protein was shown to be closely related to the human RBP-Jκ protein (16, and A.Israël, personal communication). The enhancers of the mouse H-2K^b and β_2-microglobulin genes contain NF-κB sites that share some similarity to the RBP-Jκ recognition sequence. Three or four Gs followed by one or two As and then by a pyrimidine stretch are found in NF-κB sites (27). The mouse KBF2 protein has been shown to interact with the cluster of Gs in the NF-κB sites (26). We examined three mouse NF-κB site probes for the binding to the RBP-Jκ protein (Fig. 2a). An NF-κB site probe (IgκB) derived from the intron enhancer region of the mouse immunoglobulin κ chain gene (26, 28) did not bind to the RBP-Jκ protein (Fig. 2c). Another NF-κB site probe (KBF) from the H-2K^b gene (26, 29) bound to the RBP-Jκ protein very poorly. The other NF-κB probe (β2m) from the mouse β_2-microglobulin gene (26, 30) bound to the RBP-Jκ protein only one-third as much as the m8 probe but still 1.5-fold more than the JκRS[wild] probe. This palindromic β2m probe contained a sequence of ATGGGAATC on one strand; a sequence very similar to cactGTGGGAACgg in the m8 probe.

Binding of m8 mutant probes

We introduced a series of point mutations into the m8 probe and examined the binding to the RBP-Jκ protein (Table 1). The binding decreased markedly when mutation was introduced between the 5th and 10th nucleotide from the beginning of the heptamer motif in the m8 probe. Replacement of the second A to T (m8-5T), the 11th A to G (m8-11G), and the 12th C to T (m8-12T) decreased the binding slightly but significantly. The transversion replacement of the 11th and the 12th bases had stronger effects on the binding than the transition replacement. This result together with the result of the mutated recombination signal probes indicates that the core binding sequence for the RBP-Jκ protein is GTGGGAA and suggests that two or three nucleotides on both sides of the core sequence also may have some interaction with the RBP-Jκ protein.

Identification of consensus binding sequence for the RBP-Jκ protein

To identify the best recognition sequence of the RBP-Jκ protein, we enriched oligonucleotides bound to the RBP-Jκ protein from a pool of random oligomers (Fig. 3). After enrichment through

three or four cycles of EMSA using the BF20 oligomer (Fig. 3a), oligonucleotides obtained were cloned into a plasmid vector and 32 clones were sequenced. Fig. 3c summarizes frequencies of bases after alignment of the sequences for the maximum match (Fig. 3b). An eight-nucleotide sequence of CGTGGGAA appeared most significantly conserved and therefore is the core consensus recognition sequence for the RBP-Jκ protein. An $A^G/_C$ dinucleotide sequence in front of this core motif was weakly conserved. The BF10 oligomer contained a part of RBP-Jκ recognition sequence (CATGG) in front of 10 bases of random nucleotide. After 4 cycles of EMSA, the binding oligomers enriched from the BF10 probe showed high frequencies of $CT^A/_T$ following the TGGGAA motif (Fig. 3d).

Seventeen oligonucleotides were chosen from the clones obtained in the experiment with BF20 and examined for their relative binding activities to the RBP-Jκ protein (Table 2). Among them, clones containing the exact core binding sequence (#4-24, #4-35, #4-14, #4-12, #4-2, #4-19) showed 2- to 3.5-fold more binding than the m8 probe. Transition replacements at positions 4, 5 and 8 seemed tolerable (#4-8, #4-32, #4-9, #3-12). But replacement of positions 5 and 7 to T (#4-25, #4-15, #3-14, #4-22W) and of position 1 to G (#4-13) decreased the binding considerably . Clone #4-3 had a change at positions 5 and 6 to A and did not bind to the RBP-Jκ protein. Clone #4-29 showed high binding to the RBP-Jκ protein, probably because this clone contained two tandem consensus sequences, although diverged considerably. These data indicate that the consensus sequence of the RBP-Jκ protein is $a^g/_cCGTGGGAAct^a/_t$ and that, among these 13 nucleotides, the middle 8-nucleotide sequence is the core recognition motif.

DISCUSSION

The mouse RBP-Jκ protein was originally isolated as a DNA binding protein specific to the Jκ type signal of V(D)J recombination (10). But the present study with the mutated Jκ type signal probes in this paper has revealed that the RBP-Jκ protein recognizes neither the spacer length nor the nonamer sequence but requires a sequence outside the heptamer along with the heptamer itself for binding. The binding sequences enriched from random oligonucleotides revealed an unique consensus consisting of $a^g/_cCGTGGGAAct^a/_t$ which only partly overlapped with the heptamer (CACTGTG) of the recombination signal. The spacer sequence of V(D)J recombination signals is not conserved at all (4−6). The coding sequence flanking the heptamer of the antigen receptor genes usually does not have the sequence of GGAA or the complementary TTCC. For example, most members in all families of the human immunoglobulin heavy-chain V segments have a coding sequence of (G/A)AGA(G/C)A followed by a recombination signal sequence. Therefore, the sequences around most, if not all, recombination signals do not contain the consensus binding sequence of the RBP-Jκ protein. In view of the complete absence of RBP-Jκ binding to m8-9C which has a single replacement at position 9 corresponding to the second nucleotide in the coding sequence of V segment, it is unlikely that the RBP-Jκ protein *per se* binds to the V(D)J recombination signal sequences of antigen receptor genes with significant affinity. It might be possible, however, that the RBP-Jκ protein modified covalently or modified by interaction with other proteins could recognize the recombination signals.

Some naturally occurring sequences can bind to the RBP-Jκ protein better than the Jκ recombination signal sequence. Binding of the RBP-Jκ protein to a sequence derived from the putative promoter region of the m8 gene of *Drosophila* E(spl) seems relevant to the physiological role of the RBP-Jκ protein. We and another group identified Su(H) as being mutated alleles of *Drosophila* RBP-Jκ gene (17, 18). Hairless (H) and Su(H) antagonistically control the cell fate determination at two distinct stages in the peripheral nervous system development probably through regulating the expression of a group of neurogenic genes. The observation that, in the embryo, H suppresses the neural hyperplasia resulting from mutations of various neurogenic genes except for E(spl) has led to the suggestion that E(spl) may be the major target of the H function and hence of the RBP-Jκ function (32, 33). Presence of an RBP-Jκ binding motif in the promoter region of m8 strongly supports the genetical data. In view of many regulatory proteins in differentiation being transcription factors, it seems probable that H and Su(H) are also transcription factors. Study is underway to demonstrate transactivation of the m8 promoter activity by *Drosophila* RBP-Jκ.

Interestingly, the RBP-Jκ binding motif and the 'A' sequence (27) of some NF-κB binding sites share a common sequence of GGGAA. Besides NF-κB, two proteins (KBF1 and KBF2) have been reported to bind to the NF-κB sites of the mouse MHC class I and β_2-microglobulin genes. KBF1 turned out to be homodimer of the p50 subunit of NF-κB (34, 35). Recently, human KBF2 was shown to be closely related, if not identical, to RBP-Jκ (A.Israël, personal communication). We demonstrated that the mouse RBP-Jκ protein bound to the NF-κB site in the mouse β_2-microglobulin gene but not to the NF-κB sites of the mouse MHC class I ($H\text{-}2K^b$) and immunoglobulin κ chain intron enhancers. It is not yet known whether KBF2(or RBP-Jκ) actually regulates the expression of β_2-microglobulin *in vivo* (26). But it may be a fascinating possibility that RBP-Jκ collaborates with NF-κB or its related proteins in the expression of a set of NF-κB regulated genes.

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