H-2RIIBP, a member of the nuclear hormone receptor superfamily that binds to both the regulatory element of major histocompatibility class I genes and the estrogen response element

(H-2 genes/zinc-finger protein/trans-acting factor/steroid hormone receptor)

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ABSTRACT Transcription of major histocompatibility complex (MHC) class I genes is regulated by the conserved MHC class I regulatory element (CRE). The CRE has two factor-binding sites, region I and region II, both of which elicit enhancer function. By screening a mouse λ gt11 library with the CRE as a probe, we isolated a cDNA clone that encodes a protein capable of binding to region II of the CRE. This protein, H-2RIIBP (H-2 region II binding protein), bound to the native region II sequence, but not to other MHC cis-acting sequences or to mutant region II sequences, similar to the naturally occurring region II factor in mouse cells. The deduced amino acid sequence of H-2RIIBP revealed two putative zinc fingers homologous to the DNA-binding domain of steroid/thyroid hormone receptors. Although sequence similarity in other regions was minimal, H-2RIIBP has apparent modular domains characteristic of the nuclear hormone receptors. Further analyses showed that both H-2RIIBP and the natural region II factor bind to the estrogen response element (ERE) of the vitellogenin A2 gene. The ERE is composed of a palindrome, and half of this palindrome resembles the region II binding site of the MHC CRE. These results indicate that H-2RIIBP (i) is a member of the superfamily of nuclear hormone receptors and (ii) may regulate not only MHC class I genes but also genes containing the ERE and related sequences. Sequences homologous to the H-2RIIBP gene are widely conserved in the animal kingdom. H-2RIIBP mRNA is expressed in many mouse tissues, in agreement with the distribution of the natural region II factor.

Major histocompatibility complex (MHC) class I genes are expressed in a tissue-specific and developmentally dependent fashion and are inducible by interferons. Their transcription is controlled by two conserved upstream cis-acting sequences, the MHC class I regulatory element (CRE) and the interferon consensus sequence (ICS) (refs. 1–3; Fig. 1a). These elements elicit positive and negative regulation as well as interferon-mediated induction of the MHC class I genes. In the MHC CRE there are at least three sequences, regions I–III, that bind nuclear factors (4). Region I, composed of a palindrome, binds Kbf1 (5) and NF- κ B and H2TF1 (6) and has an enhancer activity in fibroblasts. The upstream region II binds another factor and also exerts an enhancer activity (7). Region I binding activity is expressed in tissues that express MHC class I genes at high levels, while the region II factor is detected in various tissues irrespective of the MHC class I gene expression (7). To isolate genes encoding trans-acting factors for the MHC CRE, we screened a phage expression library by a method that relies on the detection of

FIG. 1. (a) MHC CRE sequence. The CRE contains three factor-binding sequences, regions I, II, and III (RI, RII, RIII), and partially overlaps the ICS (1–4). (b) Specificity analyses by filter binding assays. H-2RIIBP fusion protein transferred onto nitrocellulose filters was tested for binding to ^{32} P-labeled concatenated oligomers (see Table 1 for sequences). (Left) Wild-type (WT RII) and mutant (M1–M3) regions II. (Right) AP-1 and hormone response elements. ERE, estrogen response element; GRE, glucocorticoid response element; PRE, progesterone response element; TRE, thyroid hormone (T_3) response element.

in situ DNA–protein interactions (6, 8). We report here the isolation of a cDNA clone encoding a protein capable of binding to region II of the MHC class I genes.[§] This protein, H-2RIIBP, has structures characteristic of the nuclear hormone receptor superfamily (9, 10).

MATERIALS AND METHODS

Library Screening. A λ gt11 library from mouse liver was screened by the method of Vinson *et al.* (8) with modifica-

Abbreviations: MHC, major histocompatibility complex; CRE, class I regulatory element; ICS, interferon consensus sequence; ERE, estrogen response element; GRE, glucocorticoid response element; PRE, progesterone response element; TRE, thyroid hormone response element.

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[§]The sequence reported in this paper has been deposited in the GenBank data base (accession no. M26804).

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tions. Phage plaques were adsorbed onto nitrocellulose filters presoaked with isopropyl β -D-thiogalactoside. Filters were subjected to a denaturation/renaturation cycle in 6 M guanidine hydrochloride, pretreated with binding buffer (40 mM KCl/20 mM Hepes, pH 7.9/3 mM MgCl₂/1 mM dithiothreitol) containing 5% skim milk, and then incubated with radioactive CRE and ICS oligonucleotide probes ($\approx 10^6$ cpm/ml) in binding buffer for 2 hr at 4°C, washed, and autoradiographed. Oligonucleotides used as probes were phosphorylated with [γ -³²P]ATP by T4 polynucleotide kinase to a specific activity of $0.5\text{--}1 \times 10^7$ cpm/pmol and concatenated with T4 ligase.

Expression of H-2RIIBP in the pBS Vector and Gel Mobility Shift Assays. An *Eco*RI–*Acc*I fragment of the λ gt11 H-2RIIBP clone was subcloned into the *Sma*I site of the pBS(+/-) vector (Stratagene). *Escherichia coli* HB101 cells harboring the plasmid were grown in LB medium with the inducer isopropyl β -D-thiogalactoside for 90 min. Cell pellets were lysed by 6 M guanidine hydrochloride in 50 mM NaCl/10 mM Tris-HCl, pH 7.6/1 mM EDTA/5% glycerol. Supernatants were dialyzed first in 1.5 M guanidine hydrochloride in the same buffer as above supplemented with 10 μ M ZnSO₄ and 0.5 mM phenylmethanesulfonyl fluoride and then in the same buffer without guanidine hydrochloride. For gel mobility shift assays, bacterial extracts (4.5 μ g of protein) were incubated with ³²P-labeled probes (5000 cpm, ≈ 0.5 ng per lane) together with 200 ng of poly(dI-dC) (Pharmacia) in 100 mM NaCl/10 mM Tris-HCl, pH 7.6/1 mM EDTA/5% glycerol and electrophoresed. The natural region II binding factor was detected in gel mobility shift assays with 5 μ g of nuclear extract (11) prepared from the murine T-cell line LH8, using ³²P-labeled region II probe (4, 7) in the presence of 2 μ g of poly(dI-dC).

DNA Sequencing and Blot Hybridization. The entire *Eco*RI insert of the H-2RIIBP clone was inserted into M13mp19 and sequenced in both directions by the dideoxy chain-termination method. Genomic DNA (10 μ g) was subjected to Southern blot hybridization using a labeled *Eco*RI fragment of H-2RIIBP. The filters were washed twice with $2\times$ SSC/0.1% SDS at room temperature and twice with $1\times$ SSC/0.1% SDS at 65°C. (SSC is 0.15 M NaCl/0.015 M sodium citrate, pH 7.) RNA blot hybridization was performed with RNA (5 μ g per lane) passed once through an oligo(dT)-cellulose column. RNA blots were washed under the same conditions as above.

RESULTS

Isolation of a cDNA Clone That Encodes a Region II Binding Protein. We screened a λ gt11 mouse liver library with ³²P-labeled CRE and ICS probes by the method of Vinson *et al.* (8). Among $\approx 10^6$ recombinant phage screened, one cDNA

FIG. 2. Gel mobility shift assays. (a) Lysates of bacteria containing the H-2RIIBP coding sequence in pBS (three left panels) were tested with ³²P-labeled CRE, region II (RII), ICS, region I (RI), or ERE probe (indicated at the top). Unlabeled competitor oligomers (bottom) were added at about 100 times molar excess. Lysates of bacterial carrying pBS without insert (two right panels) were used as a control. (b) LH8 T-cell nuclear extracts (4, 7) were tested with a region II probe. Competitor oligomers were added at about 10, 100, and 1000 times molar excess (lanes 1, 2, and 3, respectively). Lane -, no competitor.

clone was isolated which expressed a β -galactosidase fusion protein that bound to region II, but not to region I, region III, or the ICS, and was designated H-2RIIBP. Binding of H-2RIIBP was tested with mutant region II sequences (Table 1) in a filter binding assay (Fig. 1b). The binding site of the naturally occurring region II factor was previously determined by methylation interference tests (4). Mutants M1 and M2 had single 2-base-pair (bp) substitutions within the region II binding site. The mutation in M3 was outside of the binding

Table 1. Oligonucleotides used in this study

Site	Sequence	Ref(s).
MHC CRE region II		
Wild type	-207 AGGCGGTGAGGTCAGGGTGGGGAA -183	
Mutant M1	AGGCGGTGATTTTCAGGGTGGGGAA	
Mutant M2	AGGCGGTGAGGTCAGTTGTGGGGAA	
Mutant M3	AGGCGGTGAGGTCAGGGGTGTTGAA	
AP-1 recognition sequences		
Simian virus (AP-1a)	127 ATCTCAATTAGTCAGCAACCATAGT 103	12
Human metallothionein II _A (AP-1b)	-115 GGAGCCGCAAGTCACTCAGCGCGGG -91	13
Human collagenase (AP-1c)	-78 AAAGCATGAGTCAGACACCTCTGGC -54	13
Hormone response elements		
Vitellogenin A2 ERE	-337 AAGTCAGGTCACAGTGACCTGATCA -313	14, 15
Uteroglobin PRE	-2398 TTTAGTGAACATCAAGTACTTGGCT -2374	16
Tyrosine aminotransferase GRE	-2514 TCTGCTGTACAGGATGTTCTAGCTA -2490	17, 18
Growth hormone TRE	-183 AAGATCAGGGACGTGACCGCAGGAG -159	19, 20

All oligomers had a 5' GCC/CGG overhang used for ligation. Dots on wild-type region II sequence indicate sites of methylation interference seen with natural region II factor.

site. H-2RIIBP bound efficiently to the native region II oligomer, less efficiently to M2 and M3, and failed to bind to M1 (Fig. 1*b*). This pattern was similar to that found with the natural region II factor in nuclear extracts of most cells (4). Because region II resembles the AP-1 sites and purified AP-1 is reported to bind to region II (21), we tested AP-1 sites (Table 1) in the simian virus 40 genome (12) and the human metallothionein II_A and collagenase genes (13) for binding to H-2RIIBP. Consistent with our previous observations with the natural region II factor (7), H-2RIIBP did not bind to any of the AP-1 sites (Fig. 1*b*). To confirm binding of H-2RIIBP to region II of the CRE, the H-2RIIBP coding-region fragment was subcloned into pBS to express a fusion protein

containing only a short stretch of β -galactosidase, and the protein was tested for binding in gel mobility shift assays (Fig. 2*a*). With the CRE probe containing both region I and region II, H-2RIIBP lysates generated two retarded bands (arrowheads) that were eliminated by excess unlabeled region II oligomer, but not by region I or mutant M1 oligomer. The region II probe also yielded two bands that were eliminated by excess region II competitor, but not by region I competitor. As expected, H-2RIIBP lysates did not generate detectable retarded bands with labeled region I or ICS probes (Fig. 2*a*). Control cell lysates containing pBS with no insert did not yield detectable retarded bands with any of the probes tested. These results establish that H-2RIIBP binds to region II of the

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GluPheProArgSerProAspSerSerSerProAsnProLeuSerGlnGlyIleArgProSerSerProProGlyProProLeuThrPro
1 GAATTCCTCCCGAAGCCAGACAGCTCCTCCCAATCCCTTTCTCAGGGGATCCGTCGGTCTTCTCTCTGGCCACCTTTACCCCT

SerAlaProProProProMetProProProProLeuGlySerProPheProValIleSerSerSerMetGlySerProGlyLeuProPro
91 TCAGCACTCCACCTCCATGCCACCCCGCCACTGGGCTCCCTTCCAGTCATCAGTTCTCCATGGGGTCCCTGGTCTGCCCTCT

ProAlaProProGlyPheSerGlyProValSerSerProGlnIleAsnSerThrValSerLeuProGlyGlyGlySerGlyProProGlu
181 CCGGCTCCCCAGGATTCTCCGGGCTGTACGACGCCCTCAGATCACTCCACAGTGTGCTCCCTGGGGTGGGTCTGGCCCCCTGAA

AspValLysProProValLeuGlyValArgGlyLeuHisCysProProProProGlyGlyProGlyAlaGlyLysArgLeuCysAlaIle
271 GATGTGAAGCCACCGGTCTTAGGGTCCGGGCTGCAGTGTCCACCCCTCCAGTGGTCTGGGGTGGCAACCGCTCTGTGCAATC

CysGlyAspArgSerSerGlyLysHisTyrGlyValTyrSerCysGluGlyCysLysGlyPhePheLysArgThrIleArgLysAspLeu
361 TCGGGGACCGAAGCTCAGGCAAGCACTATGGGTTTACAGTGCAGGGCTGCAAGGTTCTTCAAGCGCACCATTCGGAAGGACCTG

ThrTyrSerCysArgAspAsnLysAspCysThrValAspLysArgGlnArgAsnArgCysGlnTyrCysArgTyrGlnLysCysLeuAla
451 ACCTACTCGTGTCTGATAACAAAGACTGTACAGTGGACAAGCGCCAGCGAATCGCTGTCTAGTACTGTCTATCAGAAGTGCCTGGCC

ThrGlyMetLysArgGluAlaValGlnGluGluArgGlnArgGlyLysAspLysAspGlyAspGlyAlaGlyGlyAlaProGlu
541 ACTGCGATCAAAAGGAGGCGGTTCAGGAGGAGCGTCAACGGGGAAGGACAAAGACGGGATGGAGATGGGGTGGGGAGCCCTGAG

GluMetProValAspArgIleLeuGluAlaGluLeuAlaValGluGlnLysSerAspGlnGlyValGluGlyProGlyAlaThrGlyGly
631 GAGATGCCTGTGGACAGGATCTTGAGGAGAGCTTGTCTGTGGAGCAGAAGAGTGACCAAGCGTTGAGGGTCTGGGGCCACCGGGGT

GlyGlySerSerProAsnAspProValThrAsnIleCysGlnAlaAlaAspLysGlnLeuPheThrLeuValGluTrpAlaLysArgIle
721 GGTGGCAGCAGCCCAATGACCCAGTACTAACATCTGCCAGGAGCTGACAAACAGCTGTTCACTCTGTTGAGTGGCAAAGAGGATC

ProHisPheSerSerLeuProLeuAspAspGlnValIleLeuLeuArgAlaGlyTrpAsnGluLeuLeuIleAlaSerPheSerHisArg
811 CCGCACTTCTCTCTCTACCTCTGGACGATCAGGTACATGCTCGGGCAGGCTGGAACGAGCTCCTCATTGCGTCTTCTCCATCGG

SerIleAspValArgAspGlyIleLeuLeuAlaThrGlyLeuHisValHisArgAsnSerAlaHisSerAlaGlyValGlyAlaIlePhe
901 TCCATTGATGTCCGAGATGGCATCTCTTGGCCACGGGTCTTCATGTGCACAGAACTCAGCCATTCCGACGGGTGGGAGCCATCTTT

AspArgValLeuThrGluLeuValSerLysMetArgAspMetArgMetAspLysThrGluLeuGlyCysLeuArgAlaIleIleLeuPhe
991 GATCGGGTGTGACAGAGCTAGTGTCCAAATGCGTGACATGAGGATGGACAAGACAGAGCTTGGCTGCCTCGGGCAATCATATGTTT

AsnProAspAlaLysGlyLeuSerAsnProGlyGluValGluIleLeuArgGluLysValTyrAlaSerLeuGluThrTyrCysLysGln
1081 AATCCAGACGCCAAGGGCTCTCCAACCTGGAGAGGTGAGATCCTTCGGGAGAAGGTGTACGCTCACTGGAGACCTATTGCAAGCAG

LysTyrProGluGlnGlnGlyArgPheAlaLysLeuLeuLeuArgLeuProAlaLeuArgSerIleGlyLeuLysCysLeuGluHisLeu
1171 AAGTACCCTGAGCAGCAGGGCGGTTTGCCAAAGCTGTGTTACGTCTTCTGCCCCTCCGCTCCATCGGCCTCAAGTGTCTGGAGCACCTG

PhePhePheLysLeuIleGlyAspThrProIleAspThrPheLeuMetGluMetLeuGluAlaProHisGlnLeuAlaEnd
1261 TTCTTCTCAAGCTCATTGGCGACCCCCATTGACACCTTCTCATGGAGATGCTTGAGGCTCCCCACCACTAGCTTACGAGCCAGATG

1351 CACACCGAGTGTCACTGAGGAGACTTGAAGCTGGGAGGGGAGAGCCATGGGACAGGTGCAGAGCAGGAGGGGACTTGCCACGCTG

1441 CCAGGATCTGGCAACACTTAGCAGGGTTCGCTTGGTCTCCAAGTCGAAGGGGACCCAGATCCCTGTGAGGACTTATGTCTACCTTCA

1531 GTGGCCTTGAGTCTCTGAATTTGTGCGGGTCTCCCATGGTGCAGGTGATTCTTCATCTGGCTCCCGACACAAAGCACTGCCCTGCTTC

1621 CTCTCATTTGGCCTCACTCCCTTCTGAAGAGTGAACAGAGCTCCCCAGAAAGGGTGTGTGGGGCAGGCCCCCAAGCTGATGATC

1711 ATGGGAGCAGGCTCTGACAGCTTTATCTCTCAGACTTGACAGATGGGGCAGAGGAGGACCTGCCTCTGTCTCTGTACGCCCAT

1801 TTCCACAGTCCCTCTGCAGTCAGACTGAAGAATAAAGGGGTAGTGAAGGGCTGCTGGAGGTGGAGAACCCATTGCTCTTTTAATTTCT

1891 CTGTGAGGAGAGACTGGGAGTTAGACTCAAAGAAGTACTGTACATCCCCAGGTGACTTAAATGTCAGGGCTGGAGATGGCATGTGGGCA

1981 AGGAGGCCCTCAGTGGGCTGTCCAAAGCTCCCTGGGCTCTGCTCGGGTGGCCCTACAGCTCTTCCCTAGTCTTAAGCACAGCTAGG

2071 CTGGGAGCAAGTGGGACATTGATGGGGTGGCCAGCTGCAGAGTTGGGTGCTGGGCTGCATGGTTTTTGCCTGGACCTCTTTTGGG

2161 GTTCCCTCCCATCTTTCACTTGACATAAAGTTGCTTTCCAGTT (A) n

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FIG. 3. DNA and deduced amino acid sequence of the H-2RIIBP clone. The first methionine is underlined. The conserved zinc-finger domain is boxed with solid lines. Two moderately conserved stretches in the putative ligand-binding domain are boxed with dashed lines. The canonical glycosylation site (Asn-Ser-Thr) is underlined with a dashed line. Putative polyadenylation sites are underlined with solid lines.

FIG. 4. Comparison of zinc-finger domains of H-2RIIBP, murine *nur/77* (22), human retinoic acid receptor (hRAR) (23), human estrogen receptor (hER) (14, 15, 24), human c-erbA (25), v-erbA (26), rabbit progesterone receptor (rbPR) (27), murine glucocorticoid receptor (mGR) (28), rat vitamin D receptor (rVDR) (29), and *Drosophila* *knirps* (30) and *knrl* (31). Amino acids conserved in all proteins listed are boxed. Cysteines are indicated by solid circles. Amino acids specific for the estrogen/thyroid hormone receptor group are indicated by triangles.

MHC CRE. Binding of H-2RIIBP to the ERE, presented in Figs. 1 and 2, is described below.

Structure of H-2RIIBP. The entire 2246-bp *EcoRI* fragment of H-2RIIBP was sequenced (Fig. 3), showing a long open reading frame of 1338 bp extending from the fusion point to the TGA termination codon at position 1339. This is followed by a 3' untranslated region of 866 bp. Judging from the mRNA size (Fig. 5b), this cDNA insert is likely to be close to full-length. In addition to a modified polyadenylation site at position 2185, another potential polyadenylation site is found at position 1832. The first methionine codon is found at nucleotide position 109. This methionine codon is preceded by a contiguous stretch of 36 codons, and it is not clear whether this ATG is the initiation codon. RNA synthesized *in vitro* from the H-2RIIBP insert cloned in pBS was translated in a reticulocyte system to yield a product of ≈ 50 kDa as judged by SDS/PAGE (data not shown), in agreement with the predicted size of the protein. The most notable feature of the deduced amino acid sequence is a domain of two putative zinc fingers (9, 10, 22–32) (boxed in Fig. 3). These are typical of Cys₄–Cys₅ zinc fingers, conserved in steroid/thyroid hormone receptors (Fig. 4) and known to be responsible for binding to target DNA sequences. The highest amino acid identity (68%) was found between H-2RIIBP and the protein encoded by the murine *nur/77* gene, a gene induced by serum and growth factors (22). Sequence homology with the other proteins listed is substantial, decreasing only slightly when H-2RIIBP is compared to the products of the *Drosophila* genes *knirps* (30) and *knrl* (31) (Fig. 4). The N-terminal portion of H-2RIIBP (115 amino acids) is proline-rich (33%; refs. 33 and 34) and contains a canonical glycosylation site. This region may correspond to the transcription activation domain of hormone receptors (9, 10). The C-terminal portion of H-2RIIBP (263 amino acids) may correspond to the hinge and ligand-binding domain of nuclear hormone receptors. Although the overall amino acid sequence of this region is quite distinct, two short stretches (boxed with dashed lines in Fig. 3) display some similarity with these receptors. Based on these similarities, H-2RIIBP most likely belongs to the large superfamily of nuclear hormone receptors.

Binding of H-2RIIBP to the ERE. Possible binding of H-2RIIBP to various hormone response elements was next examined. These elements (Table 1), often composed of a palindrome, bind to respective hormone receptors (9, 10, 14–20, 24) and affect transcription of the target genes. We tested response elements for estrogen (14, 15, 24), progesterone (16), glucocorticoid (17, 18), and thyroid (19) hormone receptors (Table 1). As seen in filter binding assays (Fig. 1b), H-2RIIBP expressed from λ phage bound strongly to the ERE of the vitellogenin A2 gene, but not to the other response elements tested. Binding of H-2RIIBP to the ERE was verified in gel mobility shift assays with the fusion protein expressed in the pBS vector (Fig. 2a). Unlabeled ERE oligonucleotides competed efficiently with both the CRE and region II probes. Labeled ERE oligomer produced retarded bands when tested with H-2RIIBP lysates, but not

with control pBS lysates, which produced only a nonspecific band (Fig. 2a). Specific retarded bands generated by the ERE was eliminated by competition with excess ERE and region II oligomers (data not shown). These results show that H-2RIIBP is capable of binding both the MHC CRE and the ERE. We also examined binding of the natural region II factor to the hormone response elements. Results of competition gel shift assays carried out with mouse cell nuclear extracts are shown in Fig. 2b. The labeled region II probe generated a retarded band (arrowhead). As expected, unlabeled region II oligomer was an efficient competitor. The ERE oligonucleotide also competed for the band, although slightly less efficiently than the region oligomer (Fig. 2b). The PRE and GRE oligomers did not cause significant competition. The TRE oligomer at the highest concentration showed a weak competition (10, 20). These results are consistent with the data seen with H-2RIIBP and show that both the natural and cloned region II factors are capable of the binding to the ERE. It is noteworthy that the region II binding site of the CRE resembles a half palindrome of the ERE, suggesting a structural relatedness between region II and the ERE (Table 1).

DNA and RNA Hybridization Analyses. Southern blot hybridization was performed with an H-2RIIBP probe under moderately stringent conditions (Fig. 5a). A single band of >23 kb was seen in mouse DNA digested with *Xba* I, suggesting that there are few copies of the H-2RIIBP gene in the mouse genome. Strong bands were detected in human, chicken, *Xenopus*, and *Drosophila* DNA, while yeast DNA did not show detectable hybridization. These results suggest that sequences homologous to H-2RIIBP occur widely in the animal kingdom. RNA blot hybridization revealed a single band of ≈ 2.4 kb in all four mouse tissues tested (Fig. 5b).

FIG. 5. DNA and RNA hybridization analyses. (a) *Xba* I or *EcoRI*-digested genomic DNA from BALB/c mice, human Namalwa B cells, *Xenopus laevis*, chicken, *Drosophila melanogaster*, and yeast was hybridized with ³²P-labeled H-2RIIBP probe. (b) RNA from adult BALB/c tissues was hybridized with the same probe. Moderately stringent conditions were used for both analyses. kb, Kilobases.

These data are in agreement with the broad tissue distribution of the natural region II factor (7).

DISCUSSION

In this paper we describe a cDNA clone coding for a zinc-finger protein that binds to region II of the CRE. Region II elicits an enhancer activity in MHC class I gene transcription (4, 7). In gel mobility shift and filter binding assays H-2RIIBP fusion proteins in both λ and pBS clones bind specifically to region II of the MHC CRE. The deduced amino acid sequence of H-2RIIBP shows that it has zinc fingers of the Cys₄-Cys₅ type, conserved in the superfamily of nuclear hormone receptors. Among the known members of the superfamily, the zinc fingers of the murine protein encoded by *nur/77*, an immediate early gene induced by growth factors (22), exhibit the highest homology to H-2RIIBP. However, the levels of amino acid identity in the zinc-finger region are also high when H-2RIIBP is compared with other hormone receptors (Fig. 4). It has been suggested (10) that the Cys₄-Cys₅ zinc fingers may be classified into two subgroups, based on variant amino acids (triangles in Fig. 4). This classification would place H-2RIIBP in the estrogen/thyroid hormone/retinoic acid receptor group, rather than in the glucocorticoid group. That H-2RIIBP and the estrogen receptor are both capable of binding to the ERE suggests that these subgroup-specific amino acids are involved in conferring target specificity. In addition, H-2RIIBP has a distinct modular structure, characteristic of this superfamily, although in the N- and C-terminal regions, sequence similarity with other members of the superfamily is very low. As with other "orphan receptors" (9), a specific ligand for H-2RIIBP is not known at present. As shown in Figs. 1 and 2, H-2RIIBP binds to the ERE. Binding of H-2RIIBP to the ERE was confirmed with the protein expressed in a baculovirus vector (B.-Z.L., S.H., and K.O., unpublished work). The ERE contains a perfect palindrome, TCAGGTCACAGTGACCTGA. Region II includes a sequence, TGAGGTCA, corresponding to a half-palindrome of the ERE, suggesting a structural kinship between region II and the ERE. It is possible that hormone response elements do not always require palindromic symmetry, as supported by a report on the ERE of the chicken ovalbumin gene (35). Not only the cloned H-2RIIBP but also the natural region II factor bind to the ERE (and to the TRE weakly) (Fig. 2b). Taken together, our data strongly suggest, but do not prove, that H-2RIIBP is the region II factor detected in mammalian cell extracts. Consistent with this suggestion, preliminary data indicate that overexpression of H-2RIIBP in cultured cells results in enhanced transcription of a chloramphenicol acetyltransferase reporter gene driven by the MHC CRE (B.-Z.L. and K.O., unpublished work). These results raise the possibility that H-2RIIBP is involved in regulating not only MHC class I genes but some hormone response genes. The fact that *Drosophila* does not have MHC class I genes, and yet has an H-2RIIBP homologue, is consistent with this broader role for H-2RIIBP.

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