cells negative for Tac antigen (the α chain of IL-2R) encoded by chromosome 10 (*22*).

The chromosomal localization of the gene encoding the HTLV receptor gene will be useful for further elucidation of the receptor by immunological and genetic techniques.

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A DNA Binding Protein Regulated by IL-4 and by Differentiation in B Cells

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The class II (Ia) major histocompatibility complex antigens are a family of integral membrane proteins whose expression is limited to certain cell types, predominantly B lymphocytes, macrophages, and thymic epithelial cells. In B cells, Ia expression is both developmentally regulated and responsive to external stimuli. The differentiation of early B stem cells to mature B lymphocytes is accompanied by the appearance of cell surface Ia antigens; the transition to plasma cells results in loss of class II gene expression. In Ia-expressing B cells, the T cell–derived lymphokine interleukin-4 (IL-4) increases such expression by an as yet undefined mechanism. Chloramphenicol acetyltransferase gene expression was cis-activated by a region of the Ia $A\alpha^k$ gene in a B lymphoma line, but not in a myeloma line. A nuclear protein that bound to two sites within this region, upstream from previously described transcription elements, was found in normal spleen cells. This binding activity was also found in spleen extracts from athymic mice, which lack T lymphocytes, and in Ia-positive B lymphocyte tumor cell lines, demonstrating that it is a B cell protein. Further analysis showed the activity to be undetectable in an Ia-negative pre-B cell line and in three plasmacytoma cell lines that are Ia negative. IL-4 treatment of normal and athymic mouse spleen cells greatly increased the binding of this nuclear protein to these two sites, concomitant with increased MHC class II gene transcription. Thus, B cells contain a sequence-specific DNA-binding activity whose level is influenced both by IL-4 and by differentiation signals.

THE PRESENCE AND THE LEVEL OF cell surface class II major histocompatibility complex (MHC) gene products contribute to the intensity of an immune response [reviewed in (*1*)]. Tissue-specific and inducible control of transcription affect the level of class II MHC (Ia) gene expression (*2*). Because DNA regions upstream from several other class II MHC genes were active in cis (*3*), we studied a region upstream from the murine MHC Aα gene. A 1.3-kb Sau 3AI restriction fragment was cloned upstream from the SV40 promoter of pA10CAT (*4*). This construct (Fig. 1B) directed the synthesis of 70-fold more chloramphenicol acetyltransferase than the control vector, pA10CAT, when each was transfected (*5*) into the class II–positive B lymphoma M12.A2 (Fig. 1A). In the myeloma cell line XAg653, this DNA region provided no cis-activating effect (Fig. 1A). Transcriptional motifs, called X and Y, are conserved in all class II MHC genes (*6*) and are present at the downstream end of the Aα Sau 3AI fragment. Similar assays showed that the Aα X and Y sequences conferred little cis-activating effect in M12.A2 (*7*). Thus, the DNA region upstream from Aα (Fig. 1A) was active in cis in a B cell line but not in a myeloma cell line, and sequences other than the X-Y region appeared to contribute to cis-activation.

The activation of transcription by specific DNA sequences is often mediated by transcription factors that bind to these sequences. Therefore, we assayed nuclear extracts (*8*) by mobility shift analyses (*9*) with radiolabeled DNA fragments that represented the DNA within the 1.3-kb Sau 3AI fragment described above. For two restriction fragments (fragments I and II in Fig. 1C) in addition to the X-Y-containing fragment (IV in Fig. 1C), a retarded band was present when nuclear extracts from spleen cells were used (lanes 3 and 10 in Fig. 1D). Formation of these proteinase K–sensitive (*7*) complexes was blocked by competition with excess unlabeled probe DNA (lanes 4 and 11 in Fig. 1D), but not by several unrelated competitor DNAs (lanes 5, 6, 12, and 13). To determine whether B lymphocytes contain the binding activity, we prepared splenic nuclear extracts from athymic nude mice, whose spleens lack T cells and consist primarily of B cells. The factor was found in nude mice (lanes 7 and 14 in Fig. 1D). B lymphoma lines that express Ia constitutively were also assayed. Nuclear extract from M12.4.1, a class II–positive mature B cell lymphoma line, generated a DNA-protein complex that comigrated with the band from normal spleen cell extract (lanes 8 and 15).

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These results suggested that the presence of this DNA-binding activity may be correlated with the ability of the cells to express class II MHC genes. Since preparation of sufficient quantities of normal pre-B and plasma cells for these experiments was not feasible, we studied cultured cell lines that represented these developmental stages of the B lineage. Nuclear extracts from 70Z/3 and R8205 (pre-B), from M12.4.1 (mature B), and from three myeloma cell lines were assayed against fragments I (7) and II (Fig. 2). Two of three extracts from the 70Z/3 pre-B cell line failed to bind fragment II. The third extract contained the factor (lane 2 in Fig. 2), and cytoplasmic RNA from this cell preparation contained Aα and Aβ RNA. The nuclear extract from Ia^+ R8205 cells generated two complexes (lane 3), of which one comigrated with the complex found using spleen cell (lane 4) and M12.4.1 (lane 6) nuclear extracts (*10*). In contrast, in the three myeloma cell extracts, no band was observed with fragment II (lanes 7 to 9 in Fig. 2), and no band comigrating with the complexes formed by spleen extracts was observed with fragment I. Therefore, this DNA-binding activity correlated with the developmental state and class II MHC gene expression of B-lineage cells.

IL-4 influences B cell development (*11*) and stimulates increased MHC class II mRNA levels in B cells (*12*). We prepared nuclear extracts from normal (Fig. 3A) or athymic (Fig. 3B) mouse spleen cells, cultured with or without recombinant IL-4. When these extracts were assayed for proteins that bind fragments I and II, more label was present in complexes generated by IL-4–treated spleen cells (Fig. 3A, lanes 2 and 6; Fig. 3B, lanes 2 and 4) than by matched control cells (Fig. 3A, lanes 1 and 5; Fig. 3B, lanes 1 and 3). Unlabeled fragment I or fragment II competed for binding to fragments I and II by IL-4–induced splenic nuclear extracts (Fig. 3A, lanes 3 and 7), but other DNA fragments did not compete for binding (Fig. 3A, lanes 4 and 8). In four of five paired experiments, a detectable level of Aα DNA-binding protein was present in unstimulated spleen cell extract; the small amount of DNA-binding activity is consistent with the low level of Aα transcripts present (*7, 13*). IL-4 treatment increased the level of this binding activity significantly. We conclude that a binding protein is present in B cells when constitutive (IL-4–independent) class II MHC gene transcription is active and that the level of binding activity in normal B cells can be increased by IL-4 treatment.

To determine whether the IL-4–mediated increase in class II gene expression in splenic B cells was a transcriptional effect, we as-

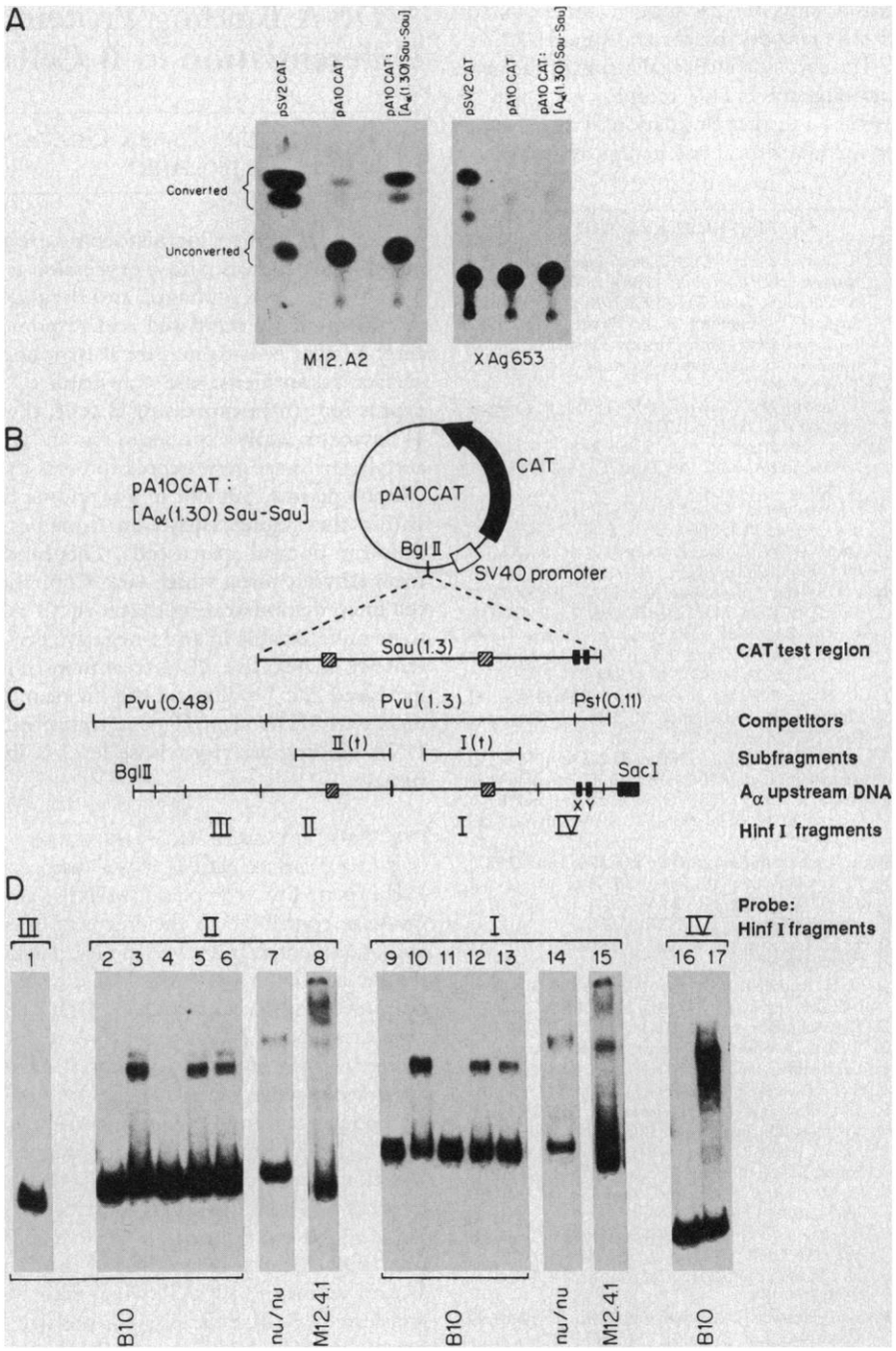

Fig. 1. (**A**) The B lymphoma cell line M12.A2 and the myeloma cell line XAg653 were transfected (*22*) with pSV2CAT (*23*); with pA10CAT (*4*), which lacks an enhancer; and with pA10CAT containing a 1.3-kb Sau 3AI fragment from the Aα upstream DNA and assayed for chloramphenicol acetyltransferase activity (*23*). (**B**) A 1.3-kb Sau 3AI restriction fragment from the Aα upstream DNA was ligated into pA10CAT to generate pA10CAT[Aα(1.3)Sau-Sau]. The position of the X and Y sequences (solid boxes) and of the target sequences described below (cross-hatched boxes) are diagrammed. (**C**) The upstream region of $A\alpha^k$. A solid box marks the beginning of exon I leader sequence. Hinf I sites are marked by vertical lines; the fragments used as probe DNAs are indicated (*24, 25*). Competitor DNAs are shown above the gene. Pvu = Pvu II, Pst = Pst I; approximate fragment sizes in kilobases are in parentheses. Cloned subfragments of fragments I and II are shown as I(t) and II(t). (**D**) Equal portions of nuclear extracts (*8, 17*) were added to radiolabeled Hinf I fragments from $A\alpha^k$ upstream DNA, followed by nondenaturing polyacrylamide gel electrophoresis and autoradiography (*9, 26*). The fragments used were approximately 580 bp (fragment I, lanes 9 to 15), 500 bp (fragment II, lanes 2 to 8), 300 bp (fragment III, lane 1), and 220 bp (fragment IV, lanes 16 and 17). Lanes 2, 9, and 16 contained no nuclear extract; the other lanes contained 1.2 μg of protein. Comparable masses (50 ng) of unlabeled competitor DNAs were included in the binding mixtures in lanes 4 to 6 and lanes 11 to 13. The competitor DNAs were Pvu(1.3) (lanes 4 and 11), Pvu(0.48) (lanes 5 and 12), and a 270-bp dimer of the 110-bp X-Y consensus region Pst I fragment [Pst(0.11), lanes 6 and 13] in pUC18 polylinker (*17*).

sayed spleen cells by nuclear run-on transcription (*14*). In a B lymphocyte–enriched spleen cell population from athymic mice, addition of recombinant murine IL-4 to the culture medium resulted in increased Aα gene transcription relative to actin transcription. The ratio of Aα to actin transcription was 4.4-fold and 9.0-fold greater in IL-4–treated cells than in control cells in two independent experiments (*15*). The absolute prevalence of Aα run-on transcripts also increased in all experiments. Each cell transcribed approximately four times as much Aα when treated with IL-4 as when cultured without IL-4. This value is comparable to the fourfold increase in steady-state RNA levels reported previously (*12*). Thus, IL-4 concurrently increases transcription of Ia genes and induces an MHC DNA-binding activity.

To identify the target sequence of this developmentally regulated and IL-4–inducible nuclear protein, we performed deoxyribonuclease I (DNase I) footprint analyses (*16*). Subfragments of the Hinf I fragments I and II [I(t) and II(t) in Fig. 1C] that compete for all binding to full-length fragments I and II (Fig. 3A) were used. The differences in DNase I reactivity between free DNA and DNA bound to protein are summarized in Fig. 4A. A single region protected from DNase I attack was identified on each probe. The DNase I footprint of the band generated with the M12.4.1 nuclear extract was indistinguishable from that of the IL-4–inducible factor in spleen cells (*7*). Competition experiments had shown that fragment I sequences could

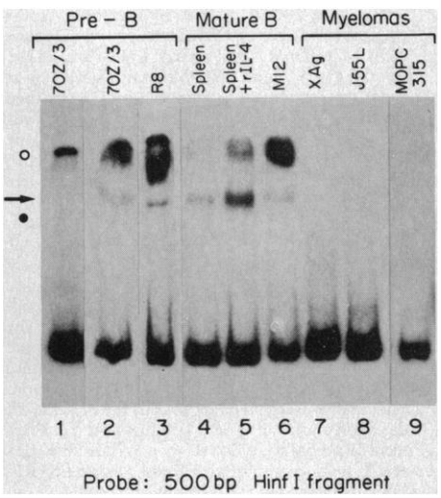

Fig. 2. DNA binding assays of cell lines representing stages of the B cell lineage. Nuclear extracts were prepared from pre-B cell lines 70Z/3 (lane 1 and lane 2, an independent extract) and R8205 (lane 3), *nu/nu* spleen cells cultured with (lane 5) and without (lane 4) IL-4; M12.4.1, a mature B lymphoma line (lane 6); and three murine myelomas (XAg653, lane 7; J55L, lane 8; and MOPC315, lane 9). Gel retardation assays were performed with these extracts and fragment II probe DNA. Results with fragment I probe were identical to those with fragment II, except that with J55L a band was occasionally present (indicated by a solid dot to the left of the lanes) that migrated faster than the spleen band (indicated by an arrow). Each DNA binding reaction with myeloma cells represents about 6×10^5 nuclei. The IL-4–inducible band in spleen nuclear extract is readily detectable at an input of 1.5×10^5 nuclei (*7*).

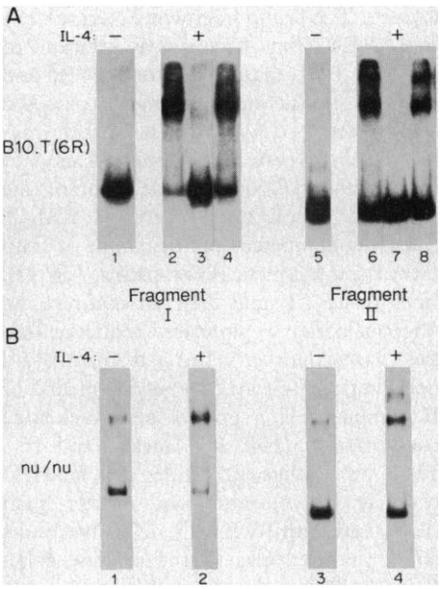

Fig. 3. IL-4 induces the splenic protein that binds Aα DNA. Splenic nuclear extracts were prepared from normal [B10.T(6R) in (A)] and outbred athymic (*nu/nu*) mice [in (B)] after overnight culture of the spleen cells either with (+) or without (–) 230 U/ml (*27*) recombinant murine IL-4. Matched pairs of extracts were assayed for binding activity with fragments I and II as above. As a control, extracts were shown to contain comparable activities of binding to the Aα X box and to a probe containing the Aα X and Y box consensus region (*7, 17*). (**A**) Extracts of uninduced (lanes 1 and 5) or induced (lanes 2 to 4 and 6 to 8) spleen cells. Probe fragments were as indicated. Competition experiments were performed with unlabeled I(t) (lane 3) and II(t) (lane 7) subfragments, as well as with Pvu(0.48) (lanes 4 and 8) and Pst(0.11) dimer (not shown), as in Fig. 1C. (**B**) Extracts of uninduced (lanes 1 and 3) or induced (lanes 2 and 4) spleen cells with fragment I (lanes 1 and 2) or fragment II (lanes 3 and 4).

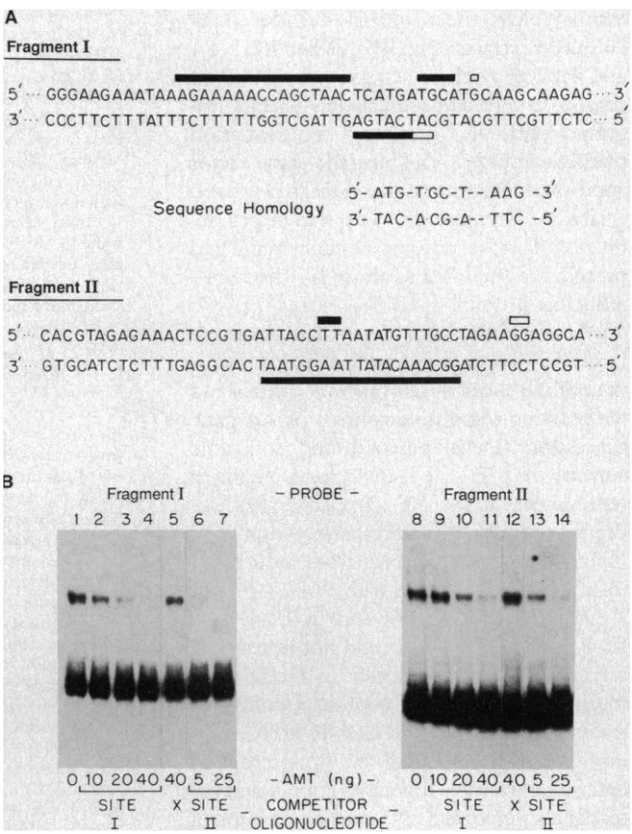

Fig. 4. (**A**) Recognition targets of the binding protein. DNase I footprint analyses (*16*) were performed with I(t) and II(t) subfragments of fragments I and II (*28*). Solid bars mark regions of protection from DNase I attack and open bars signify increased reactivity of the coding (lower) and anticoding (upper) strands. The DNA sequences in these target regions of fragment I and II are shown (*25*). The sequences are aligned to demonstrate the core of identity (ATG-T·GC-T-AAG.) shown between the fragment I and fragment II double-strand sequences and labeled "sequence homology." (**B**) Oligonucleotide competition for binding to probe fragments I and II. Oligonucleotides representing the targets defined by DNase I footprint experiments with fragments I and II were prepared (site I, or BRE-1,5′-gatcTAAAGAAAAACCAGCTAACTCATGATGCATGCAAG-3′ and its complementary strand gatcCTTGCA....TCTTTA; and site II, or BRE-2, 5′-gatcCCGTGATTACCTTAATATGTTTGCCTAGAAGGAGGCAAA-3′ and its complementary strand gatcTTTGCCT....TCACGG). Oligonucleotide BRE-1 (cloned as a dimer in pUC18 polylinker), oligonucleotide BRE-2, and a 29-bp oligonucleotide with Eα X consensus sequences (*17*) were used as competitors in DNA binding assays with fragment I and fragment II probes and nuclear extracts from IL-4–treated *nu/nu* spleens. Competitors added were none (lanes 1 and 8); dimer BRE-1 (10, 20, and 40 ng, lanes 2 to 4 and 9 to 11); X(Eα) oligonucleotide (40 ng, lanes 5 and 12); BRE-2 (5 ng, lanes 6 and 13; 25 ng, lane 7).

compete with fragment II probe for formation of complexes, and vice versa (7). To analyze the basis for this cross-competition, we prepared oligonucleotides spanning the protected and hypersensitive residues of fragments I and II and tested them in competition experiments using several nuclear extracts. The 35-bp fragment I oligonucleotide BRE-1 (Fig. 4 legend) is sufficient to bind an IL-4–inducible protein (7), and these oligonucleotide sequences competed for formation of complexes with both fragments I and II (Fig. 4B, lanes 2 to 4 and 9 to 11). A 39-bp oligonucleotide spanning the protected region of fragment II, BRE-2, efficiently competed for formation of fragment I and fragment II complexes (Fig. 4B, lanes 6, 7, 13, and 14). In contrast, an oligonucleotide of unrelated sequence [the Eα X box sequence, $X(E\alpha)_{33}$, from (17)] did not compete for formation of fragment I or II complexes at a greater oligonucleotide concentration (Fig. 4B, lanes 5 and 12). Three other oligonucleotides also failed to compete at concentrations greater than those used with BRE-2 (7, 18). The results with uninduced spleen extracts and with IL-4–induced normal spleen extracts (7) were comparable to those with IL-4–induced *nu/nu* spleen extracts (Fig. 4B). When M12.4.1 and R8205 nuclear extracts were used in parallel experiments, unlabeled BRE-2 oligonucleotide also competed for formation of this complex (7). Thus, the same factor appears to recognize a site in fragment I and a site in fragment II, and the target sequences for the developmentally regulated protein are the same as those for the IL-4–inducible protein.

We conclude from these data that the target sequences we identified may contribute both to B cell developmental control and lymphokine-responsive control of Aα gene expression. Developmental and inducible control of a B cell transcription factor is reminiscent of the NF-κB factor (19). Although T cells do not transcribe immunoglobulin light chains, NF-κB is induced in these cells by activation with PMA (19, 20). T lymphocytes also respond to IL-4. The IL-4–inducible factor would not necessarily activate class II transcription in T cells since murine T lymphocytes contain a dominant trans-acting repressor of class II MHC gene expression (21). It will be of interest to determine whether this IL-4–inducible protein is also involved in transcription control in T lymphocytes.

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5. For cotransfection assays, 3×10^6 (M12.A2) or 6×10^6 (XAg653) cells were transfected (22) with 4 μg pCH110 (29) and 4 μg of CAT construct {pSV2CAT (23), pA10CAT (4), or pA10CAT[Aα-(1.30)Sau-Sau], referred to as pA10CAT[Aα]}. Cellular extracts were assayed for β-galactosidase activity, and volumes of extract with equal β-galactosidase activity were analyzed for CAT activity and quantitated by liquid scintillation counting (23). In comparable transfections of M12.A2 without pCH110 cotransfection, the pA10CAT[Aα] construct directed the synthesis of 70 times more CAT activity than did the pA10CAT control (mean value from three experiments) and 0.5 times the CAT activity directed by pSV2CAT.
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10. An additional DNA-protein complex (open dot in Fig. 2) that migrated more slowly than the reference band (arrow in Fig. 2) was also present in a number of lanes. This complex was not reproducible with spleen cell extracts and did not uniformly correlate with development, as it was generated by one nuclear extract from the myeloma cell line XAg653. In addition, the migration of the slower mobility complex differed in assays of various nuclear extracts (Fig. 2, lane 3 versus lanes 2, 5, and 6). We did not characterize these complexes further.
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15. Spleen cells were isolated from *nu/nu* athymic mice or B10.A mice and divided equally. The media were either unsupplemented (no IL-4; control), or supplemented with recombinant murine IL-4 to a final concentration of 150 U/ml. After overnight culture at 37°C, nuclei were prepared from the cells (17). Equal concentrations of nuclei were suspended at 3×10^8 to 8×10^8 per milliliter in 50 m*M* tris (*p*H 7.6), 5 m*M* $MgCl_2$, 0.1 m*M* EDTA, and 40% glycerol, and stored at −70°C. Run-off transcription and purification of RNA were performed as described (14). Filter-bound DNAs were prepared from single-strand DNA preparations of bacteriophage M13 without insert or with a 1-kb insert of $A\alpha^k$ cDNA in each orientation. The filters were hybridized in 10 m*M* TES, 10 m*M* EDTA, 0.2% SDS, 0.4*M* NaCl, 10 μg/ml tRNA for 4 to 8 hours at 65°C. Hybridizations were then performed for 60 hours at 65°C in 3 ml of 10 m*M* TES, 10 m*M* EDTA, 0.2% SDS, and 0.4*M* NaCl. Autoradiographic exposures of the filters were quantitated by densitometry. Within each experiment, the control Aα/actin signal ratio was assigned a value of 1 and the Aα/actin signal ratio in the treated sample was compared to this value. Stimulation of transcription was observed with three independent paired cell preparations.
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25. Full sequence data are available on request. DNA sequence analysis was performed with algorithms of the BioNet system of Intelligenetics.
26. DNA binding assays were performed as described (16), with 0.01-ml mixtures of nuclear extract and 4 μg of poly(dI-dC)·(dI-dC). For competition with unlabeled DNAs, each DNA was mixed with nuclear extract and placed at room temperature for 4 min; the rest of the reaction ingredients were then added. Complexes were analyzed by electrophoresis on 4% polyacrylamide gels containing 45 m*M* tris-borate and 1 m*M* EDTA, for 2 hours at 11 V/cm with cooling.
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28. DNA binding reactions were performed in 30 to 40 μl of total volume under the conditions described above, with 2×10^5 to 5×10^5 Cerenkov counts per minute of labeled DNA. The reactions were then adjusted to 5 m*M* $MgCl_2$, 5 m*M* $CaCl_2$, and DNase I at 0.5 to 1.0 μg/ml (Worthington). Incubation at room temperature was continued for 2 min; then EDTA and EGTA were added to attain a 5 m*M* total concentration, and the samples were analyzed by electrophoresis as described above. Radioactive bands representing free and complexed DNA were processed as described (17) and analyzed by electrophoresis on 6% linear sequencing gels. Fragments I, II, and IV were sequenced in both directions by the dideoxy method (24), and these sequences were correlated with G+A and A>C channels of chemical degradation sequences determined from the end-labeled DNAs used for DNase I footprints. The subfragments used are diagrammed in Fig. 1A and were generated in subcloning fragments I and II into the M13mp19 (30) Sma I site. Probes for footprint experiments were prepared by Eco RI or Hind III digestion and 5′ or 3′ end labeling with ^{32}P. Fragment II probes were Eco RI–Sau 3AI or Hind III–Ava II fragments; fragment I probes were Eco RI–Hae III fragments. DNA manipulations and chemical degradation reactions were performed according to standard procedures (31).
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