Characterization of a New Tissue-Specific Transcription Factor Binding to the Simian Virus 40 Enhancer TC-II (NF- κ B) Element

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We have biochemically and functionally characterized a new transcription factor, NP-TC_{II}, which is present in nuclei from unstimulated T and B lymphocytes but is not found in nonhematopoietic cells. This factor has a DNA-binding specificity similar to that of NF- κ B but is unrelated to this or other Rel proteins by functional and biochemical criteria. It can also be distinguished from other previously described lymphocyte-specific DNA-binding proteins.

The TC-II motif in the simian virus 40 (SV40) enhancer [5'-(246)TGGAAAGTCCCCA(234)-3'] mediates a strong transcriptional response to stimulation by interleukin 1 (IL-1). Previous work indicates that this response is due to activation of NF- κ B (10, 33, 43), which specifically binds to the TC-II sequence. We have shown that in an immature T-cell line, PC60 (7), IL-1 induces the appearance of maximal NF- κ B activity in less than 20 min and induces a rapid increase in the expression of a β -globin reporter gene whose promoter contains four copies of the TC-II enhancer. Although nuclei from nonstimulated PC60 cells contain no detectable NF- κ B, the TC-II motif also acts as a constitutive enhancer in these cells. As previously reported (10), the TC-II sequence not only binds NF- κ B but also forms specific complexes with other proteins that are constitutively active in PC60 cells but were not detected in a pre-B-cell line, 70Z/3.

In this article, we describe a more detailed characterization of this TC-II-specific protein(s) (here called NP-TC_{II}). We show that NP-TC_{II} binding activity is constitutively present in T and B lymphocytes but not in nonhematopoietic cell types, which suggests a role for NP-TC_{II} in lymphocyte differentiation. We present evidence that the constitutive enhancer activity of the TC-II motif is indeed due to NP-TC_{II}. The biochemical and functional properties of NP-TC_{II} rule out a close relationship with NF- κ B and other proteins of the Rel family and show that NP-TC_{II} is different from other lymphocyte-specific DNA-binding proteins.

MATERIALS AND METHODS

Plasmids and probes. TC-II probe. The TC-II-containing, EcoRI-HindIII 73-bp fragment of pSPT-TCII was end labelled as previously described (10).

pG1 plasmids. pG1 contains the rabbit β -globin gene from positions -109 to +1650 inserted into pBR322. pG1.TC-30

contains an inverted head-to-tail tetramer of the SV40 TC-II motif (bp 233 to 247 [49]) inserted upstream of the β -globin gene. pG1.TC-33 to -TC-46 differ from pG1.TC-30 by point mutations in the TC-II motif (18). Plasmids were purified by polyethylene glycol precipitation (10), and the TC-II tetramers were resequenced. For competition experiments, tetrameric fragments were excised and purified on a 4% low-melting-point agarose (Nusieve; FMC, Rockland, Maine) gel.

pGBAc β G1D plasmid contains a version of the β -globin gene carrying a 40-bp deletion in the second exon and is transcribed under the control of the constitutive chicken β -actin promoter (46).

Cell culture and cytokine induction. Cells were grown exponentially to 4×10^5 to 8×10^5 cells per ml. An aliquot was treated for 30 min with human recombinant IL-1 β (1 ng/ml; a gift of Glaxo) or with tumor necrosis factor (TNF) (10^4 U/ml; a gift of W. Fiers, Gent University). Adherent cells were grown to near confluency and induced on the plate. Thymocytes and splenic lymphocytes were prepared by gentle homogenization of the tissues. Nylon wool columns were used to separate Ficoll-purified spleen cells into a nonadherent ($\approx 90\%$ T-cell) and an adherent ($>90\%$ B-cell) fraction (17).

Nuclear extracts and gel retardation assay. Nuclear extracts were prepared at 4°C by the method of Schreiber et al. (40). 4-Amidinophenylmethylsulfonyl fluoride (50 μ g/ml) was added to buffer A. Adherent cells were lysed directly on the plate. Extracts were aliquoted and stored in liquid nitrogen. The protein concentration was determined by the Bio-Rad protein assay (Bio-Rad, Richmond, Calif.). For proteolysis experiments, nuclear extract was incubated with trypsin (50 ng/4 to 15 μ g of total protein; Boehringer Mannheim) at room temperature for 5 min. The reaction was stopped by addition of 500 ng of aprotinin (Boehringer Mannheim).

I κ B and antibodies. I κ B α was purified as previously described (55). The polyclonal antibodies against the human

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p50 subunit of NF- κ B (antiserum 2 against amino acids 19 to 969 of p50) have been described by Kieran et al. (20), and those against a segment of the chicken *v-rel* protein expressed in bacteria (antisera 1 and 2 against amino acids 148 to 316 and 346 to 438 of p59) have been described by Simek et al. (45).

Gel filtration chromatography. Nuclear extract of EL4 cells (3.6 mg of total proteins) was applied to a calibrated Superose 12 column (Pharmacia) equilibrated with nuclear extraction buffer (buffer C) containing 0.4 M NaCl. During elution with the same buffer, 300- μ l fractions were collected, and a 1.5- μ l aliquot of each fraction was used to test TC-II binding activity.

Heparin chromatography. Nuclear extracts prepared from PC60 cells induced by IL-1 (2 mg of total protein) were passed over a heparin-Sepharose column (1 ml of wet resin; Pharmacia). The column was eluted at a flow rate of 0.2 ml/min with a step gradient (2 ml/50 mM change in KCl concentration) in a buffer containing 20 mM HEPES (*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid) (pH 7.9), 1 mM β -mercaptoethanol, 0.1% Nonidet P-40, and 0.2 mM EDTA. Fractions (0.3 ml) were assayed for NP-TC_{II} and NF- κ B.

Band shifts, footprints, and DNA methylation protection. Binding reactions and electrophoresis conditions for gel retardation assays have been described previously (10). About 300 pg (= 6 fmol) of labeled TC-II fragment from pSPT-TCII was added in all reaction mixtures. For competition assays, 250 to 300 fmol of unlabeled DNA was included.

Footprints were obtained as previously described (10), with the following modifications. Two or 4 μ l of heparin-fractionated NP-TC_{II} was incubated with 6 ng of labeled SV40 *Hae*III-*Nco*I fragment (nucleotides [nt] 331 to 40) in 12 μ l containing 4 μ g of poly(dI-dC) · poly(dI-dC), 10 mM HEPES (pH 7.9), 20 mM NaCl, 0.5 mM β -mercaptoethanol, 0.2 mM EDTA, and 0.05% Nonidet P-40. After 30 min, 1 μ l (0.2 U) of DNase I (Pharmacia) in 10 mM Tris (pH 7.5)–10 mM CaCl₂–10 mM MgCl₂–50% glycerol was added. The reaction was stopped after 1 min. Competitions included a 22 M excess of TC-II motif excised from pG1.TC-30.

Transfections. PC 60.21.14.4 cells (7, 44) were transfected with mixtures of a pG1 plasmid and the reference plasmid pG β Ac β G1D, treated with IL-1, and processed as previously described (10). The transcriptional activities of different plasmids were compared by using the polymerase chain reaction-based assay developed by Sperisen et al. (46). Briefly, the RNA from transfected cells was reverse transcribed, and the cDNA was amplified in the presence of [α -³²P]dATP with primers spanning the first intron of the β -globin reporter gene as well as the deletion in the pG β Ac β G1D reference plasmid. Polymerase chain reaction products were fractionated on a sequencing gel, and signals due to pG1 and pG β Ac β G1D mRNA were quantified by densitometric analysis. Their ratio is a linear function of the ratio of transfecting plasmid DNAs (46). Thus, when cells are transfected with the same ratio of different pG1.TC plasmids and pG β Ac β G1D, the signal ratios obtained are proportional to the promoter strength of the pG1.TC plasmids.

Computer-assisted determination of a putative consensus sequence. A novel computer algorithm has been developed in order to find a hypothetical consensus sequence description for the DNA-binding specificity of NP-TC_{II}. The principle of the algorithm is to evaluate a set of candidate motifs by correlating their best matches in a series of competitor

fragments with the binding efficiencies of these fragments and then to rank them by a quality score. The input data consist of a set of (typically short) DNA sequences, each one associated with a number reflecting its relative protein binding efficiency in band shift experiments. Here, these numbers were determined by titrating the capacity of the unlabelled sequences to compete with the labelled TC-II probe in reaction mixtures containing a constant amount of nuclear extract from PC60 and an excess of TC-II probe. Addition of 5 M excess of cold TC-II probe reduced the amount of specifically retarded probe by 50%. For the other sequences, relative activity was determined by measuring the amount of competitor needed to give the same reduction. The binding activity of TC-II was given an arbitrary value of 6 on a scale in which a difference of 1 U corresponds to a twofold change in competitor efficiency. The set of candidate sequence motifs is defined by the length (*l*) of the motif and the number (*k*) of explicitly specified bases (A, C, G, and T) (i.e., the number of positions not occupied by *N*). Here, we considered all motifs of the following types: *l* = 6, 4 ≤ *k* ≤ 6; *l* = 7, 4 ≤ *k* ≤ 7; *l* = 8, 5 ≤ *k* ≤ 7; *l* = 9, 5 ≤ *k* ≤ 7. The algorithm determines for each competitor fragment the quantity *m_{ij}*, defined as the number of matches between the sequence motif *i* and the most similar subsequence in fragment *j*. A classical correlation coefficient is used as quality score for ranking:

$$r_i = \frac{\sum_{j=1}^N (m_{ij} - \bar{m}_i)(x_j - \bar{x})}{\left[\sum_{j=1}^N (m_{ij} - \bar{m}_i)^2 \sum_{j=1}^N (x_j - \bar{x})^2 \right]^{1/2}}$$

where *x_j* is the competitor efficiency of fragment *j* and *N* is the number of fragments tested. The software performing this analysis is available under the name CONSCOR from the ftp servers ncbi.nlm.nih.gov (130.14.20.1) at the National Center for Biotechnology Information, Bethesda, Md., and expasy.hcuge.ch (129.195.254.61) at the University of Geneva or by request from us (contact pbucher@isrec-sun1.unil.ch by electronic mail).

RESULTS

Previous characterization of specific TC-II binding proteins in PC60 nuclei had revealed, besides IL-1-inducible NF- κ B, complexes that were formed by extracts from cells growing in normal as well as in IL-1-supplemented medium (10). We call the protein(s) giving rise to these complexes NP-TC_{II}. The formation of both NF- κ B and NP-TC_{II} complexes is specifically inhibited by the addition of unlabelled TC-II DNA. As described previously, the addition of nucleotides to the binding reaction strongly reduces the NP-TC_{II} band shift but has the opposite effect on NF- κ B. Both NF- κ B and NP-TC_{II} complexes are heat labile, since incubation for 5 min at 65°C completely suppresses their binding to DNA. They have similar electrophoretic mobilities, but the latter shows a much more heterogeneous pattern. This is unlikely to be due to proteolytic degradation during nuclear extraction, as it is not reduced by different protease inhibitors (0.2 μ M aprotinin; 200 μ M phenylmethylsulfonyl fluoride; and 1 μ M each pepstatin, antipain, leupeptin, and chymostatin). Attempts to reduce heterogeneity by changing composition of the binding reaction or electrophoresis buffers, acrylamide percentage, or migration conditions were

FIG. 1. Heparin chromatography. Gel retardation assays with TC-II probe of heparin-agarose chromatography fractions of nuclear extract from IL-1-treated PC60 cells, assayed in the presence (A) or absence (B) of ATP. I, column input without (-) or with (+) ATP; 0.20 to 0.70, KCl concentration (M) in elution buffer.

unsuccessful. NP-TC_{II} complexes were not retained on a wheat germ agglutinin column which, as expected, bound glycosylated Sp1 (data not shown). During heparin chromatography, NF-κB elutes at between 0.35 and 0.50 M KCl as previously described (54), whereas NP-TC_{II} starts to elute already at 0.25 M KCl, with a peak at 0.30 (Fig. 1). Note that heparin-fractionated NP-TC_{II} gives DNA-binding complexes that migrate slightly faster than complexes formed by unfractionated material. We have no explanation for this reproducible change, which is not prevented by the addition of various protease inhibitors.

NP-TC_{II} and NF-κB yield distinct tryptic DNA-binding fragments. Limited trypsin digestion of NF-κB produces two major DNA-binding fragments, κ1 and κ2 (Fig. 2). In other experiments, only fragment κ1 is present after trypsin diges-

FIG. 2. NP-TC_{II} and NF-κB and their DNA-binding trypsin fragments. Nuclear extracts from PC60 or 70Z/3 cells, cultured in normal or in IL-1-supplemented medium, were treated for 5 min with 10 μg of trypsin per ml. Undigested and trypsin (TRYPS)-treated extracts were incubated with TC-II probe in the presence or absence of ATP and/or a 45-fold excess of unlabelled competitor (COMP) DNA containing the wild-type TC-II motif (w) or the mutant TC-42 (m). Retarded complexes were detected on a non-denaturing polyacrylamide gel. κ1 and κ2, complexes formed by trypsin fragments of NF-κB; T1 and T2, complexes formed by trypsin fragments of NP-TC_{II}; F, free probe.

FIG. 3. Gel filtration chromatography of NP-TC_{II}. Nuclear extract from EL4 cells was fractionated on a calibrated Superose column. TC-II binding activity in the indicated fractions was assayed by gel retardation before (A) or after (B) trypsin digestion. The probe used here is the tetramerized wild-type TC-II motif, and the more slowly migrating bands, a and a', are due to complexes containing more than one NP-TC_{II} molecule. Thyroglob., thyroglobulin; b, complexes containing a single NP-TC_{II} molecule; T1 and T2, complexes due to trypsin fragments of NP-TC_{II}.

tion (for examples, see Fig. 4 and 5). Trypsin-treated nuclear extracts from noninduced and IL-1-treated PC60 cells form two major specific complexes with the TC-II probe (T1 and T2) that migrate faster than the ones due to NF-κB fragments. As for NP-TC_{II}, the T1 and T2 complexes are inhibited by ATP, and comparison of the fine specificities (see Fig. 5) clearly shows that T1 and T2 are derived from NP-TC_{II}. We do not know what the relationship between T1 and T2 is. Digestion kinetics do not provide any evidence that T1 might be a precursor of T2. Note that the relative predominance of T1 and T2 consistently varies among different cell lines (see Fig. 4).

NP-TC_{II} consists of heterogeneous proteins with the same DNA-binding moieties or domains. To determine whether the heterogeneity of NP-TC_{II} complexes observed on non-denaturing band shift gels reflects size heterogeneity of the DNA-binding proteins, we fractionated nuclear extract from NP-TC_{II} containing EL4 thymoma cells on a Superose column. Figure 3A shows that NP-TC_{II} activity coelutes with proteins with molecular masses of between 550 and 370 kDa. There is a good correlation between elution volume and electrophoretic mobility of NP-TC_{II} complexes. This indicates that at least part of the heterogeneity in the band shift pattern is due to molecular weight variation of NP-TC_{II}. All fractions, when digested with trypsin, gave rise to the T1 and T2 complexes (Fig. 3B). Attempts to dissociate possible oligomeric complexes, by treatment with increasing concentrations of deoxycholate (DOC) followed by the addition of Nonidet P-40, were unsuccessful. Beyond concentrations of 2% DOC, binding activity of NP-TC_{II} was gradually inhibited, but no shift to a faster-migrating complex was observed (data not shown).

NP-TC_{II} activity is restricted principally to lymphocytes. NP-TC_{II} was first observed in extracts of PC60 cells which have phenotypic characteristics of immature T cells. The pre-B-cell line 70Z/3 and HeLa cells contained no detectable NP-TC_{II} activity (10). This suggested a restricted tissue distribution of NP-TC_{II} DNA-binding activity. To investi-

FIG. 4. NP-TC_{II} binding activity in different cell lines. TC-II complexes formed by nondigested (A) or trypsin-treated (B to E) nuclear extracts from human (h) or mouse (m) cell lines or normal mouse lymphocytes (E), without or in the presence of 25 M excess of unlabelled TC-II sequence (COMP). (A and B) T and B cells; (C) nonlymphoid hemopoietic cells; (D) nonhemopoietic cells; (E) normal mouse lymphocytes.

gate its tissue specificity, we screened nuclear extracts from different cell lines and normal tissues for NP-TC_{II} activity. Cell lines were grown in normal medium or stimulated with IL-1 or TNF. Untreated or trypsin-digested extracts were used for band shift assays with the TC-II probe (Fig. 4). Specificity of presumptive NP-TC_{II} activity was tested by addition of unlabelled TC-II competitor. Results are summarized in Table 1.

As expected, strong constitutive NF- κ B activity was found in all B-cell lines. Extracts from most T-cell lines also contained detectable constitutive NF- κ B. As previously reported (10, 33), IL-1 or TNF α strongly increased NF- κ B activity in almost all T-cell lines and induced its appearance in most NF- κ B-negative cells regardless of tissue origin. Constitutive NP-TC_{II} binding activity is present in extracts of all human and rodent T-cell lines tested. NP-TC_{II} was also detected in all pre-B- and mature B-cell lines tested. 70Z/3 cells contain little NP-TC_{II} activity. Although there was some variation in the electrophoretic mobilities of undigested NP-TC_{II} from the different cell lines, complexes T1 and T2 formed by trypsin-treated extracts migrated with the same mobilities. However, there were notable differences in the relative abundances of T1 and T2. Mouse T1 and T2, as well as κ 1 complexes, migrate slightly faster than their human counterparts. Among normal mouse lymphocytes, thymocytes are quite rich in NP-TC_{II}. Splenic B and T lymphocytes contained both NF- κ B and NP-TC_{II}. As expected, NF- κ B predominated in B cells, whereas T cells contain more NP-TC_{II}.

We could detect strong NP-TC_{II}-like activity also in extracts from K562, a cell line representing precursors of the erythrocytic, granulocytic, and monocytic lineages. Other cell lines belonging to the monocyte-macrophage lineage or

representing myeloid precursor stages contained small amounts of NP-TC_{II}-like activity. In a variety of cell lines of nonhematopoietic origin, we detected no NP-TC_{II} (Table 1). We found some NP-TC_{II}-like activity in the rat hepatoma cell line Fao but not in HepG2, a human hepatoma, or in mouse liver extract.

Treatment with IL-1 had no effect on NP-TC_{II} activity in any of the cell lines tested.

NP-TC_{II} and NF- κ B differ in their fine specificities. Both NP-TC_{II} and NF- κ B bind specifically to the TC-II motif. To compare more precisely the sequence specificities of NF- κ B and NP-TC_{II}, we used TC-II oligonucleotides containing single point mutations in each of the 13 bp forming the TC-II motif of SV40 as competitors for the TC-II probe (Fig. 5). This experiment revealed an overall similarity, but also some clear differences, between the specificities of NF- κ B and NP-TC_{II}. The binding of both factors is reduced by mutations in nt 242 to 245 and somewhat less by mutations in nt 241 and 239. However, NF- κ B binding is also reduced by an alteration of nt 236 and is most strongly diminished by the mutations in nt 237. These two mutations have no or only a marginal effect on NP-TC_{II} binding. The point mutations have the same effect on the binding of NF- κ B and NP-TC_{II} as on that of their proteolytic fragments (T1 and T2 for NP-TC_{II} and κ 1 for NF- κ B). This validates the assignment of the origin of the tryptic DNA-binding fragments (Fig. 1). The results obtained with NF- κ B confirm earlier studies by Macchi et al. (25).

We have previously (10) shown that G237, G238, and G240 were protected against DNase I cleavage and methylation by dimethyl sulfate (DMS) only by extracts from IL-1-treated PC60 cells, whereas G244 and G245 were protected by extracts from both induced and uninduced cells. This agrees

TABLE 1. Tissue distribution of NP-TC_{II} and NF- κ B^a

Cell type	Species	Name	Reference no. or ATCC designation ^b	Activity of:		
				NP-TC _{II}	NF- κ B ^c	
					-IL1	+IL1
Lymphoid cells						
Pre-T cells	Rat	C58	(11)	+++	+	+
	Mouse	MbC12	(29)	+++	(+)	+
		BW5147	TIB 47	+++	+	+
T cells	Human	HSB-2	CCL 120.1	++	+	+
		Jurkat	(19)	+++	-	-
		KT	(22)	++	-	+
	Mouse	B6.1	(51)	++	+	+
		CTLL	TIB 214	+++	+	+
		EL4	TIB 39	+++	-	+
		S49	TIB 28	++	+	(+)
		PbCS.F12	(36)	++	+	+
Pre-B cells	Human	Nalm1	(26)	++	+	+
	Mouse	70Z/3	TIB 158	+	+	+
B cells	Human	Raji	CCL 86	+++	+	+
		Daudi	CCL 213	+++	+	+
		Namalwa	CRL 1432	++	+	+
		SB	CCL 120	+	+	+
	Mouse	LBB	(31)	++	+	+
		A20.2J	(14)	++	+	+
		PC61	TIB 222	++	+	+
		P3X63Ag8	TIB 9	+	+	+
Other hemopoietic cells						
Myeloid	Human	K562	CCL 243	++	+	+
Granulocyte precursor	Mouse	FDC-P	(9)	(+)	-	+
Promyelocytic	Human	HL-60	CCL 240	(+)	-	-
Monocytic	Human	U-937	CRL 1593	(+)	(+)	+
Macrophages	Mouse	P388	CCL 46	(+)	-	-
Mastocytoma	Mouse	P815	TIB 64	(+)	-	+
Nonhemopoietic cells						
Cervical carcinoma	Human	HeLa	CCL 2	-	+	+
Fibrosarcoma	Human	HT-1080	CCL 121	-	(+)	+
Hepatomas	Human	HepG2	HB 8065	-	+	+
	Rat	Fao	(12)	+	-	+
Pancreas tumor	Rat	AR42J	CRL 1492	-	-	-
Fibroblasts	Human	FEK4	(50)	-	(+)	+
	Monkey	COS-1, COS-7	CRL 1650, 1651	-	-	+
	Mouse	WOP32, 3027	(8)	-	+	+
		L-cell	CRL 6362	-	(+)	+

^a Results of gel retardation assays. Abundance of constitutive NP-TC_{II} is estimated for each cell line. The presence of NF- κ B is also indicated. Symbols: +++, strong; ++, medium; +, weak; (+), barely detectable activity; -, not detectable.

^b Reference numbers are in parentheses.

^c Note that IL-1 treatment strongly increased NF- κ B activity in most cell lines, but in the band shifts shown, constitutive and induced NF- κ B activities cannot be compared because of the addition of ATP only to induced extracts.

with the relative importance of nt 237 for the binding of NF- κ B compared with that of NP-TC_{II}. DNase I footprinting and DMS methylation protection performed with heparin-fractionated NP-TC_{II} from EL4 cells confirmed these results (Fig. 6A). In addition to the TC-II site (nt 236 to 249), a region between nt 261 and 288 on the late strand was protected from DNase I. DMS treatment revealed that NP-TC_{II} bound to each of the three repeats of the motif G(T/C)GGAA(T/A)G (TC-II, site I, and site II in Fig. 6B). In TC-II and site I, the first guanine in the repeat is also protected, whereas the last guanine is hypermethylated in TC-II and site II.

Consensus sequence for NP-TC_{II}. We searched for NP-TC_{II} binding sites in a number of sequences from the 5' flanking regions of cellular promoters (Table 2) by testing their capacity to compete with the TC-II probe. We used these data, together with similar data from the TC-II point mutations TC-33 to -46, to derive a consensus NP-TC_{II} binding

sequence by applying to them the novel computer algorithm CONSCOR (see Materials and Methods). The competition efficiency estimates for the point mutations were 6 for TC-40; 5 for TC-34, -35, -36, -38, and -46; 4 for TC-33 and -37; 3 for TC-39 and -41; and 1 for TC-42, -43, -44, and -45. The efficiencies of the other fragments are shown in Table 2. A comprehensive collection of candidate sequence motifs between 6 and 9 bases long (see Materials and Methods) has been compared with these data. The presence of four closely related motifs, ANANTTTCC ($r = 0.886$), CANTTTCC ($r = 0.882$), CNANNTTCC ($r = 0.871$), and AGTTTCC ($r = 0.850$), correlated well with binding strength. The first three motifs are in perfect qualitative agreement with the data, meaning that each competitor fragment containing a better match than another also has a higher or equal competitor efficiency. The "best" motif, ANANTTTCC, has been used to indicate putative binding sites in Table 2, but, with very few exceptions, all four variants map to the same locations in

FIG. 5. Fine specificities of NP-TC_{II} and NF-κB. Nuclear extracts from PC60 cells, cultured in normal medium or treated with IL-1, were used undigested or after treatment with trypsin (TRYPS). They were incubated with TC-II probe in the presence of a 45 M excess of unlabelled competitor (COMPET). Competing DNA was unrelated (NS) or contained TC-II motifs with a point mutation in the indicated position (position 34 corresponds to nt 234 in the SV40 enhancer). Competitor * combines mutations in positions 234 (T → A) and 246 (A → G). κ1, major trypsin fragment of NF-κB; T1 and T2, major trypsin fragments of NP-TC_{II}.

the competitor fragments. Good matches to all four motifs are also found in the three NP-TC_{II} binding sites detected by footprinting (Fig. 6B) and in the GTIIc + GTI segment of the SV40 enhancer. This constitutes independent confirmation of the NP-TC_{II} consensus binding site found by CONSCOR.

NP-TC_{II} is a transcriptional activator. Previous transfection experiments had shown that the tetramerized TC-II motif in pG1.TC-30 conferred a very strong and rapid IL-1 response on the rabbit β-globin promoter (10). Baseline promoter activity of the same construct was also much stronger than those of constructs containing no or other enhancer elements. To determine whether this constitutive activity is due to NP-TC_{II}, we compared the expression of the pG1.TC point mutant in transiently transfected PC60 cells. For each pG1.TC plasmid, we compared constitutive expression with expression after IL-1 treatment (Fig. 7A). As expected, pG1 expression was not affected by IL-1 and expression of all other plasmids was normalized to this level. Figure 7B shows that the pG1.TC plasmids fall into two groups: the first group includes pG1.TC-30 (containing the wild-type TC-II motif) and plasmids with mutations in nt 234, 235, 236, 238, 240, 243, and 246, which are more strongly expressed in IL-1-treated cells. This reflects, presumably, induction of NF-κB. For these plasmids, it is impossible to decide whether expression in the absence of IL-1 is due to NP-TC_{II} or a low level of NF-κB in nuclei of noninduced cells. However, expression of plasmids belong-

ing to the second group and carrying mutations in nt 237, 239, 241, 242, 244, and 245 is not affected by IL-1 treatment, indicating that the plasmids do not respond to NF-κB. Comparison of the expression of these plasmids with the capacity of their TC-II motif to compete for NP-TC_{II} binding (Fig. 5) reveals a striking correlation: the mutation of nt 237 has very little effect on NP-TC_{II} binding, and this TC-II variant has a 10-fold constitutive enhancer activity. The mutation in nt 239 reduces NP-TC_{II} binding and enhancer activities partially. Mutations in nt 241 and 245 reduce both binding and enhancer activities further, while alterations in nt 242 and 244 diminish both almost to background. The same result was obtained in three independent transfection experiments. By plotting the competitor efficiency of the IL-1-unresponsive TC-II variants against the logarithm of their transcriptional activity, we obtained a correlation value of 0.94 (Fig. 7C). While we have no specific explanation for the linear relationship between the logarithm of transcriptional activity and binding activity, these results strongly suggest that NP-TC_{II} is responsible for at least part of the constitutive enhancer activity of the TC-II motif.

NP-TC_{II} differs from Rel-like proteins. Both NF-κB subunits (p50 and p65) belong to the *rel* proto-oncogene family (13, 20, 38). Proteins belonging to this family can dimerize with each other. Our previous observations that NP-TC_{II} had a binding specificity similar to that of NF-κB led to the suggestion that NP-TC_{II} was related to NF-κB. The experi-

TABLE 2. Occurrence of ANANTTTC motif^a

Gene	Sequence (5'-3')	NP-TC _{II} binding activity
Cellular genes ^b		
H-2K ^b (21) (nt -363 to +50)	-273 ... GTTTTG <u>AGAGTTTC</u> <u>GAATCAGAACTCGAGACGACAGAGGTTCA</u> <u>GCAAAGTCT</u> TAGTGCC AGGACGTGAGGTGAGGGGTGGGAAAGCCAGAGGCTGGGGATTGCCCATCTCC <u>ACAGTTTCA</u> CTTCTGACAG CTACCTGGGTCAAGGTCTCTCTGTCTG <u>GGACACTGT</u> TGAAGGG... -95	7
mIgκ (34) (nt 251 to 263)	AGG <u>GGAGTTTC</u> G AGGAGGATC <u>AGAGATTCC</u> <u>AGCAATTTC</u> CAT CGTGAGGTCCGTGAATTCGAGGGGCTGAG AACAGGGGGCTTCCCTCT ATGTACAGGATGTTCTACTAGTTGTACAGGATGTTCTAG	7
hG-CSF (30) (nt -233 to -205)	TGCATGCTTCAGGTGAGCTGATAGAGATCTCTGT <u>GGAAGTTC</u> <u>CGAAGGATCCCGGGTACCGAGCTCG</u> 233 247 248 232	6
mTNFα (41) (nt -666 to -639)	TCCTGAGCTGG <u>GGAGTTTC</u> <u>ACCGAC</u> ...	6
mTNFα (41) (nt -508 to -498)		0
GRE (39) (GRE21GRE)		0
SV40 enhancer ^c		6
TC-II probe		6
TC-30 (TC-II wt)		6

^a ANANTTTC motifs (≥6 matches shown by boldface letters) are shown as boxes. Known κB sites are underlined.
^b Reference numbers are in parentheses immediately after the gene names and before the nucleotide positions.
^c The nucleotide numbers above each sequence indicate the borders of the sequence derived from the SV40 enhancer. For TC-30 and mutated TC-II tetrameric fragments, molar excess is expressed in terms of the monomer.

FIG. 6. DNase I and DMS footprints on the SV40 enhancer. (A) End-labelled DNA samples were incubated with heparin-fractionated NP-TC_{II} from EL4 cells and treated with DNase I or DMS and piperidine. Open boxes indicate protected regions; limits are given. wt, presence of unlabelled TC-II wild-type sequence. (B) Schematic representation of the results. Symbols: ○, G's protected against methylation by DMS; ●, hypermethylated G's. DNase I footprints are underlined. Boxes indicate the occurrence of the NP-TC_{II} binding motif found by the CONSCOR algorithm (see Table 2). TC-II, site I, and site II indicate the repeat of the motif G(T/C)GGAA(T/A)G.

ments described above show that NP-TC_{II} is not identical to NF-κB, but they do not exclude a close relationship with NF-κB or Rel.

To explore this possibility, we examined whether NP-TC_{II} could interact with the NF-κB-specific cytoplasmic inhibitor IκB, which associates with NF-κB and inhibits its binding activity (1, 2, 32). Purified IκBα (40 pg) (55) could completely abolish NF-κB binding activity in extracts from PC60 and 70Z/3 cells (Fig. 8) but failed to inhibit or modify NP-TC_{II} binding activity.

We also checked whether anti-NF-κB or anti-Rel antibodies cross-reacted with NP-TC_{II} (data not shown). While an antiserum against the p50 subunit of human NF-κB causes partial disappearance and supershifting of NF-κB complexes formed by extracts from TNF-treated Jurkat cells, it does not modify the patterns of the complexes due to NP-TC_{II} or

FIG. 7. Relative enhancer activity of TC-II wild-type or mutant tetramers. (A) Results from a single transfection experiment. Each lane shows the mRNA-derived polymerase chain reaction fragments from PC60 cells cotransfected with the indicated amounts of the reference plasmid pGβAcβGD and a pG1 plasmid. pG1.TC plasmids are identified by the position of the mutated TC-II nucleotide (Fig. 4). pG1.TC-30 (w) contains the nonmutated TC-II motif; pG1 contains no enhancer motifs. M, size markers. (B) Comparison of the expression of the different plasmids in untreated or IL-1-treated PC60 cells. For each sample, the ratio between pG1.TC and pGβAcβGD mRNA signals is measured (see Materials and Methods). Expression values standardized in this way are normalized to the expression of enhancerless plasmid pG1 in unstimulated cells. (C) Correlation between enhancer activity and NP-TC_{II} binding. The amount of cold competitor needed to inhibit 50% of NP-TC_{II} binding was determined for IL-1-unresponsive TC-II mutants. The correlation between NP-TC_{II} binding activities and constitutive enhancer activities (normalized to pG1) is 0.942.

its digested fragments. Likewise, polyclonal antibodies raised against a segment of v-Rel and also recognizing mammalian c-Rel (45) partially upshifted NF-κB as well as κ1 complexes but not NP-TC_{II} or T1 or T2 complexes.

A characteristic feature of most so far described Rel-related proteins is their localization as an inactive complex in the cytoplasm of noninduced cells and their activation and translocation into the nucleus upon appropriate stimulation (28). Cytoplasmic Rel complexes can be disrupted and binding activity can be revealed by treatment with dissoci-

ating agents such as DOC. DOC treatment of cytosolic extracts from PC60 cells or unstimulated 70Z/3 or HeLa cells whose nuclei contain little or no detectable NP-TC_{II} revealed, as expected, NF-κB but no NP-TC_{II} binding activity (data not shown).

DISCUSSION

NP-TC_{II}-DNA complexes migrate as a broad band. This heterogeneity is due to size variation, as it can be partially

FIG. 8. Effect of I κ B on NF- κ B and NP-TC_{II}. Nuclear extracts (1 μ g of protein) were incubated with TC-II probe in the presence or absence of ATP and/or 40 μ g of I κ B α . F, free DNA.

resolved by gel filtration chromatography. It is unlikely to be due to proteolytic degradation, as it is not reduced by a range of protease inhibitors. The observation that limited trypsin digestion of different chromatographic fractions of NP-TC_{II} yields the same well-defined DNA-binding fragments with identical fine specificities suggests either that NP-TC_{II} is a family of related proteins with the same DNA-binding domain or that it consists of different oligomeric complexes with the same DNA-binding subunit. So far, our attempts to dissociate such complexes without destroying the DNA-binding activity have been unsuccessful.

NP-TC_{II} is a transcriptional activator. NP-TC_{II} is a constitutive nuclear protein that specifically recognizes the TC-II motif in the SV40 enhancer. TC-II is a protoenhancer that strongly responds to NF- κ B. This has complicated analysis of the *in vivo* function of NP-TC_{II}. But the very close correlation between NP-TC_{II} binding capacity and enhancer activity of those TC-II mutants which do not respond to NF- κ B indicates that NP-TC_{II} is a transcriptional activator. The expression of the strongest IL-1-unresponsive plasmid, pG1.TC-37, is much weaker than the constitutive activity of pG1.TC-30 containing the wild-type TC-II motif, suggesting that NP-TC_{II} is a weaker transcriptional activator than NF- κ B.

NP-TC_{II} may be a marker of early lymphoid differentiation. NP-TC_{II} activity is strongest in nuclei of T- and pre-T-cell lines and normal thymic and peripheral T cells. It is present also in most B- and pre-B-cell lines and splenic B cells, as well as in a primitive hemopoietic precursor line (K562), but was undetectable in a variety of nonhemopoietic cell lines or tissues. This tissue distribution suggests that NP-TC_{II} plays a role in lymphocyte differentiation.

NP-TC_{II} is not closely related to the Rel proteins. Preliminary characterization of NP-TC_{II} had indicated similarity of its DNA-binding specificity to that of NF- κ B. Here, we show that NP-TC_{II} is not related to NF- κ B and is unlikely to be another member of the Rel family.

Five lines of evidence argue specifically against a relationship of NP-TC_{II} with NF- κ B. (i) Limited trypsin digestion of the two proteins yields different DNA-binding fragments. (ii) Both proteins elute at different ionic strengths from a heparin

column. (iii) DNA binding of NP-TC_{II} cannot be inhibited by I κ B α , the protein forming an inactive complex with NF- κ B by binding to its p65 subunit. (iv) Antibodies against the p50 subunit of NF- κ B do not react with NP-TC_{II}. (v) The fine specificities of the two proteins show clear differences despite an overall similarity. Comparison of the capacity of other TC-II-related sequences to compete for binding of NP-TC_{II} and NF- κ B to the TC-II motif has confirmed this picture (data not shown).

Both NF- κ B subunits (p50 and p65) belong to the *rel* proto-oncogene family. One of the characteristics of most Rel-related proteins is their presence as inactive precursors in the cytoplasm and their translocation into the nucleus in response to extracellular stimuli. In contrast, NP-TC_{II} is either constitutively active (in most lymphoid cells and cell lines) or undetectable in nucleus or cytoplasm, even after treatment with dissociating agents which reveal the DNA-binding activity of cytoplasmic Rel proteins (28).

Evidence against a close relationship between NP-TC_{II} and Rel is also provided by the finding that, unlike NP-TC_{II}, Rel protein p75 does not bind to the κ B motif in the H-2K^b gene (28) and that anti-chicken Rel antibodies that cross-react with mammalian Rel and NF- κ B do not affect NP-TC_{II} band shifts.

Derivation of a tentative consensus sequence for NP-TC_{II}. A novel algorithm, CONSCOR (see Materials and Methods), has been used to derive a consensus recognition sequence for NP-TC_{II} which is in perfect qualitative agreement with the experimental evidence. The advantage of CONSCOR over other techniques based on alignment of putative protein binding regions (23, 48) is that it uses "negative results" to penalize motifs that occur on sequences which do not bind the protein. Since the NP-TC_{II} recognition motif was derived from data with relatively few competitor fragments, the candidate consensus sequences had to satisfy correspondingly few constraints. However, the results of the CONSCOR analysis are confirmed by the footprinting experiments, which identify completely independently two binding sites that contain good matches with the highest-scoring NP-TC_{II} recognition motif determined by CONSCOR (Fig. 6B). Further experiments are necessary to discriminate among the four variants with virtually identical scores.

NP-TC_{II} differs from other previously described lymphocyte-specific DNA-binding proteins. Several other nuclear proteins that recognize the TC-II motif or related sequences have been described, but each of them has characteristics that distinguish it from NP-TC_{II}. The TC-II sequence overlaps with the AP2, AP3, and EBP1 motifs, but some of the mutations that affect binding of NP-TC_{II} are located outside the binding sites of these factors (5, 15, 27). Furthermore, these proteins are present in unstimulated HeLa cells.

Some members of the C/EBP family (EBP20 [16], AGIEBP1 [37], AGP/EBP [6], H [4], IL-6DBP [35], and NF-IL6 [42]) bind κ B-like sequences. However, these proteins have been found in nonlymphoid cells, and, in contrast to NP-TC_{II}, most of them are heat stable. Another C/EBP-related factor, NF-M, is present in various hemopoietic cells belonging to the myeloid and monocytic lineages, whereas its activity in B and T lymphocytes is very weak (47).

MBP1, which binds to the H2-K^b κ B-like sequence (3), was cloned from a B-cell line, but its mRNA is highly expressed in HeLa cells and present only at a very low level in Jurkat cells. The *ets-1* proto-oncogene product has a tissue distribution similar to that of NP-TC_{II} but does not bind to the TC-II motif (10a). LyF-1 binds to several lymphocyte-specific gene promoters but is present in HeLa

and not in the BW5147 or K562 cell line (24). TCF-1 α binds to related sequences in promoters of several genes expressed early in T-cell development. But it is not found in mature B cells, including Namalwa cells, which contain large amounts of NP-TC_{II} (52, 53).

We conclude that NP-TC_{II} is most likely a new transcription factor that may play a role in lymphocyte differentiation. To determine definitively its role, we need to further characterize and molecularly clone the constituent protein(s).

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REFERENCES

- Baeuerle, P. A., and D. Baltimore. 1988. I κ B: a specific inhibitor of the NF- κ B transcription factor. *Science* **242**:540–546.
- Baeuerle, P. A., and D. Baltimore. 1989. A 65-kD subunit of active NF- κ B is required for inhibition of NF- κ B by I κ B. *Genes Dev.* **3**:1689–1698.
- Baldwin, A. S., Jr., K. P. LeClair, H. Singh, and P. A. Sharp. 1990. A large protein containing zinc finger domains binds to related sequence elements in the enhancers of the class I major histocompatibility complex and kappa immunoglobulin genes. *Mol. Cell. Biol.* **10**:1406–1414.
- Brasier, A. R., D. Ron, J. E. Tate, and J. F. Habener. 1990. A family of constitutive C/EBP-like DNA binding proteins attenuate the IL-1 α induced, NF- κ B mediated transactivation of the angiotensinogen gene acute-phase response element. *EMBO J.* **9**:3933–3944.
- Bruder, J. T., and P. Hearing. 1989. Nuclear factor EF-1A binds to the adenovirus E1A core enhancer element and to other transcriptional control regions. *Mol. Cell. Biol.* **9**:5143–5153.
- Chang, C.-J., T.-T. Chen, H.-Y. Lei, D.-S. Chen, and S.-C. Lee. 1990. Molecular cloning of a transcription factor, AGP/EBP, that belongs to members of the C/EBP family. *Mol. Cell. Biol.* **10**:6642–6653.
- Conzelmann, A., P. Corthsy, M. Cianfriglia, A. Silva, and M. Nabholz. 1982. Hybrids between rat lymphoma and mouse T cells with inducible cytolytic activity. *Nature (London)* **298**:170–172.
- Dailey, L., and C. Basilio. 1985. Sequences in the polyomavirus DNA regulatory region involved in viral DNA replication and early gene expression. *J. Virol.* **54**:739–749.
- Dexter, T. M., J. Garland, D. Scott, E. Scolnick, and D. Metcalf. 1980. Growth of factor-dependent hemopoietic precursor cell lines. *J. Exp. Med.* **152**:1036–1047.
- Espel, E., C. Fromental, P. Reichenbach, and M. Nabholz. 1990. Activity and interleukin 1 responsiveness of SV40 enhancer motifs in a rodent immature T cell line. *EMBO J.* **9**:929–937.
- Flores, P., and B. Wasylyk. Personal communication.
- Geering, G., L. J. Old, and E. Boyse. 1966. Antigens of leukemias induced by naturally occurring murine leukemia virus: their relation to the antigens of gross virus and other murine leukemia viruses. *J. Exp. Med.* **124**:753–772.
- Geiger, T., T. Andus, J. Klapproth, H. Northoff, and P. C. Heinrich. 1988. Induction of α 1-acid glycoprotein by recombinant human interleukin-1 in rat hepatoma cells. *J. Biol. Chem.* **263**:7141–7146.
- Ghosh, S., A. M. Gifford, L. R. Riviere, P. Tempst, G. P. Nolan, and D. Baltimore. 1990. Cloning of the p50 DNA binding subunit of NF- κ B: homology to *rel* and *dorsal*. *Cell* **62**:1019–1029.
- Glimcher, L. H., K. J. Kim, I. Green, and W. E. Paul. 1982. Ia antigen-bearing B cell tumor lines can present protein antigen and alloantigen in a major histocompatibility complex-restricted fashion to antigen-reactive T cells. *J. Exp. Med.* **155**:445–459.
- Imagawa, M., R. Chiu, and M. Karin. 1987. Transcription factor AP-2 mediates induction by two different signal-transduction pathways: protein kinase C and cAMP. *Cell* **51**:251–260.
- Johnson, P. F., W. H. Landschulz, B. J. Graves, and S. L. McKnight. 1987. Identification of a rat liver nuclear protein that binds to the enhancer core element of three animal viruses. *Genes Dev.* **1**:133–146.
- Julius, M. H., E. Simpson, and L. A. Herzenberg. 1973. A rapid method for the isolation of functional thymus-derived murine lymphocytes. *Eur. J. Immunol.* **3**:645–649.
- Kanno, M., C. Fromental, A. Staub, F. Ruffenach, I. Davidson, and P. Chambon. 1989. The SV40 TC-II(κ B) and the related H-2K^b enhancers exhibit different cell type specific and inducible protoenhancer activities, but the SV40 core sequence and the AP-2 binding site have no enhancer properties. *EMBO J.* **8**:4205–4214.
- Kaplan, J., T. C. Shope, and W. D. Peterson, Jr. 1974. Epstein-barr virus-negative human malignant T-cell lines. *J. Exp. Med.* **139**:1070–1076.
- Kieran, M., V. Blank, F. Logeat, J. Vandekerckhove, F. Lottspeich, O. Le Bail, M. B. Urban, P. Kourilsky, P. A. Baeuerle, and A. Isral. 1990. The DNA binding subunit of NF- κ B is identical to factor KBF1 and homologous to the *rel* oncogene product. *Cell* **62**:1007–1018.
- Kimura, A., A. Isral, O. Le Bail, and P. Kourilsky. 1986. Detailed analysis of the mouse H-2K^b promoter: enhancer-like sequences and their role in the regulation of class I gene expression. *Cell* **44**:261–272.
- Lanzavecchia, A. 1985. Antigen-specific interaction between T and B cells. *Nature (London)* **314**:537–540.
- Lawrence, C. E., and A. A. Reilly. 1990. An expectation maximization (EM) algorithm for the identification and characterization of common sites in unaligned biopolymer sequences. *Proteins Struct. Funct. Genet.* **7**:41–51.
- Lo, K., N. R. Landau, and S. T. Smale. 1991. LyF-1, a transcriptional regulator that interacts with a novel class of promoters for lymphocyte-specific genes. *Mol. Cell. Biol.* **11**:5229–5243.
- Macchi, M., J. M. Bornert, I. Davidson, M. Kanno, R. Rosales, M. Vigneron, J. H. Xiao, C. Fromental, and P. Chambon. 1989. The SV40 TC-II(κ B) enhancer binds ubiquitous and cell type specifically inducible nuclear proteins from lymphoid and non-lymphoid cell lines. *EMBO J.* **8**:4215–4227.
- Minowada, J., T. Tsubota, M. F. Greaves, and T. R. Walters. 1977. A non-T, non-B human leukemia cell line (NALM-1): establishment of the cell line and presence of leukemia-associated antigens. *J. Natl. Cancer Inst.* **59**:83–87.
- Mitchell, P. J., C. Wang, and R. Tjian. 1987. Positive and negative regulation of transcription in vitro: enhancer-binding protein AP-2 is inhibited by SV40 T antigen. *Cell* **50**:847–861.
- Molitor, J. A., W. H. Walker, S. Doerre, D. W. Ballard, and W. C. Greene. 1990. NF- κ B: a family of inducible and differentially expressed enhancer-binding proteins in human T cells. *Proc. Natl. Acad. Sci. USA* **87**:10028–10032.
- Nabholz, M., M. Cianfriglia, O. Acuto, A. Conzelmann, A. Weiss, W. Haas, and H. von Boehmer. 1981. The production of murine cytolytic T-cell hybrids, p. 539–547. *In* G. J. Hmmerling, U. Hmmerling, and J. F. Kearney (ed.), *Monoclonal antibodies and T-cell hybridomas: perspectives and technical advances*. Elsevier/North Holland Biomedical Press, Amsterdam.
- Nagata, S., M. Tsuchiya, S. Asano, O. Yamamoto, Y. Hirata, N. Kubota, M. Oheda, H. Nomura, and T. Yamazaki. 1986. The chromosomal gene structure and two mRNAs for human granulocyte colony-stimulating factor. *EMBO J.* **5**:575–581.
- Nicolas, J. F., D. Wegmann, P. Lebrun, D. Kaiserlian, J. Tovey, and A. L. Glasebrook. 1987. Relationship of B cell Fc receptors to T cell recognition of M1s antigen. *Eur. J. Immunol.* **17**:1561–1565.

32. Nolan, G. P., S. Ghosh, H. C. Liou, and P. Tempst. 1991. DNA binding and I κ B inhibition of the cloned p65 subunit of NF- κ B, a *rel*-related polypeptide. *Cell* **64**:961–969.
33. Osborn, L., S. Kunkel, and G. J. Nabel. 1989. Tumor necrosis factor α and interleukin 1 stimulate the human immunodeficiency virus enhancer by activation of the nuclear factor κ B. *Proc. Natl. Acad. Sci. USA* **86**:2336–2340.
34. Picard, D., and W. Schaffner. 1984. A lymphocyte-specific enhancer in the mouse immunoglobulin κ gene. *Nature (London)* **307**:80–82.
35. Poli, V., F. P. Mancini, and R. Cortese. 1990. IL-6DBP, a nuclear protein involved in interleukin-6 signal transduction, defines a new family of leucine zipper proteins related to C/EBP. *Cell* **63**:643–653.
36. Romero, P., G. Eberl, J. L. Casanova, A. S. Cordey, C. Widmann, I. F. Luescher, G. Corradin, and J. L. Maryanski. 1992. Immunization with synthetic peptides containing a defined malaria epitope induces a highly diverse CTL response. *J. Immunol.* **148**:1871–1878.
37. Ron, D., A. R. Brasier, and J. F. Habener. 1991. Angiotensinogen gene-inducible enhancer-binding protein 1, a member of a new family of large nuclear proteins that recognize nuclear factor κ B-binding sites through a zinc finger motif. *Mol. Cell. Biol.* **11**:2887–2895.
38. Ruben, S. M., P. J. Dillon, R. Schreck, T. Henkel, C. H. Chen, M. Maher, P. A. Baeuerle, and C. A. Rosen. 1991. Isolation of a *rel*-related human cDNA that potentially encodes the 65-kD subunit of NF- κ B. *Science* **251**:1490–1493.
39. Schmid, W., U. Strähle, G. Schütz, J. Schmitt, and H. Stunnenberg. 1989. Glucocorticoid receptor binds cooperatively to adjacent recognition sites. *EMBO J.* **8**:2257–2263.
40. Schreiber, E., P. Matthias, M. M. Müller, and W. Schaffner. 1989. Rapid detection of octamer binding proteins with “mini-extracts,” prepared from a small number of cells. *Nucleic Acids Res.* **17**:6419.
41. Shakhov, A. N., M. A. Collart, P. Vassalli, S. A. Nedospasov, and C. V. Jongeneel. 1990. κ B-type enhancers are involved in lipopolysaccharide-mediated transcriptional activation of the tumor necrosis factor α gene in primary macrophages. *J. Exp. Med.* **171**:35–47.
42. Shimizu, H., K. Mitomo, T. Watanabe, S. Okamoto, and K.-I. Yamamoto. 1990. Involvement of a NF- κ B-like transcription factor in the activation of the interleukin-6 gene by inflammatory lymphokines. *Mol. Cell. Biol.* **10**:561–568.
43. Shirakawa, F., M. Chedid, J. Suttles, B. A. Pollok, and S. B. Mizel. 1989. Interleukin 1 and cyclic AMP induce κ immunoglobulin light-chain expression via activation of an NF- κ B-like DNA-binding protein. *Mol. Cell. Biol.* **9**:959–964.
44. Silva, A., H. R. MacDonald, A. Conzelmann, P. Corthésy, and M. Nabholz. 1983. Rat $\times$ mouse T-cell hybrids with inducible specific cytolytic activity. *Immunol. Rev.* **76**:105–129.
45. Simek, S. L., R. M. Stephens, and N. R. Rice. 1986. Localization of the *v-rel* protein in reticuloendotheliosis virus strain T-transformed lymphoid cells. *J. Virol.* **59**:120–126.
46. Sperisen, P., S. M. Wang, P. Reichenbach, and M. Nabholz. 1992. A PCR based assay for reporter gene expression. *PCR Methods Appl.* **1**:164–170.
47. Sterneck, E., C. Müller, S. Katz, and A. Leutz. 1992. Autocrine growth induced by kinase type oncogenes in myeloid cells requires SP-1 and NF-M, a myeloid specific C/EBP-like factor. *EMBO J.* **11**:115–126.
48. Stormo, G. D., and G. W. Hartzell III. 1989. Identifying protein-binding sites from unaligned DNA fragments. *Proc. Natl. Acad. Sci. USA* **86**:1183–1187.
49. Toozé, J. (ed.). 1982. DNA tumor viruses. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
50. Tyrrell, R. M., and M. Pidoux. 1986. Quantitative differences in host cell reactivation of ultraviolet-damaged virus in human skin fibroblasts and epidermal keratinocytes cultured from the same foreskin biopsy. *Cancer Res.* **46**:2665–2669.
51. von Boehmer, H., H. Hengartner, M. Nabholz, W. Lernhardt, M. H. Schreier, and W. Haas. 1979. Fine specificity of a continuously growing killer cell clone specific for H-Y antigen. *Eur. J. Immunol.* **9**:592–597.
52. Waterman, M. L., W. H. Fischer, and K. A. Jones. 1991. A thymus-specific member of the HMG protein family regulates the human T cell receptor α enhancer. *Genes Dev.* **5**:656–669.
53. Waterman, M. L., and K. A. Jones. 1990. Purification of TCF-1 α , a T-cell-specific transcription factor that activates the T-cell receptor α gene enhancer in a context-dependent manner. *New Biol.* **2**:621–636.
54. Yano, O., J. Kanellopoulos, M. Kieran, O. Le Bail, A. Israël, and P. Kourilsky. 1987. Purification of KBF1, a common factor binding to both H-2 and β 2-microglobulin enhancers. *EMBO J.* **6**:3317–3324.
55. Zabel, U., and P. A. Baeuerle. 1990. Purified human I κ B can rapidly dissociate the complex of the NF- κ B transcription factor with its cognate DNA. *Cell* **61**:255–265.