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# Identification of a Novel Factor That Interacts with an Immunoglobulin Heavy-Chain Promoter and Stimulates Transcription in Conjunction with the Lymphoid Cell-Specific Factor OTF2

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**The tissue-specific expression of the MOPC 141 immunoglobulin heavy-chain gene was studied by using in vitro transcription. B-cell-specific transcription of this gene was dependent on the octamer element 5′-ATGCAAAG-3′, located in the upstream region of this promoter and in the promotors of all other immunoglobulin heavy- and light-chain genes. The interaction of purified octamer transcription factors 1 and 2 (OTF1 and OTF2) with the MOPC 141 promoter was studied by using electrophoretic mobility shift assays and DNase I footprinting. Purified OTF1 from HeLa cells and OTF1 and OTF2 from B cells bound to identical sequences within the heavy-chain promoter. The OTF interactions we observed extended over the heptamer element 5′-CTCAGGA-3′, and it seems likely that the binding of the purified factors involves cooperation between octamer and heptamer sites in this promoter. In addition to these elements, we identified a second regulatory element, the N element with the sequence 5′-GGAACCTCCCCC-3′. The N element could independently mediate low levels of transcription in both B-cell and HeLa-cell extracts, and, in conjunction with the octamer element, it can promote high levels of transcription in B-cell extracts. The N element bound a transcription factor, NTF, that is ubiquitous in cell-type distribution, and NTF was distinct from any of the previously described proteins that bind to similar sequences. Based on these results, we propose that NTF and OTF2 interactions (both with their cognate DNA elements and possibly at the protein-protein level) may be critical to B-cell-specific expression and that these interactions provide additional pathways for regulating gene expression.**

The tissue-specific expression of rearranged immunoglobulin genes is subject to control by transcription factors through their cognate DNA sequence elements. In vivo studies have shown that heavy-chain genes require both enhancer (3, 16, 19, 20, 28, 32, 46) and promoter (2, 9, 13, 27, 33, 48) elements for high levels of cell-type-specific expression. Some of the proteins that interact with these sequences have already been detected (5, 6, 10, 23, 25, 26, 41, 42, 46), and most seem to be ubquitous in cell-type distribution. The only well-documented B-cell-specific protein is the octamer transcription factor 2 (OTF2) (23, 39, 45), which is found in varied abundance in cells of B-lymphocyte lineage (41). This transcription factor interacts with the conserved octanucleotide 5′-ATGCAAAT-3′, which is present in all known $V_H$ promoters and in the inverted orientation in the heavy-chain enhancer and all light-chain variable region gene promoters. In vivo and in vitro studies have shown that this element is the minimal requirement for efficient cell-type-specific transcription (4, 8, 11, 13, 15, 18, 25, 29, 34, 48).

Interestingly, a ubiquitous transcription factor, OTF1, mediates the transcription of the ubiquitously expressed histone H2B gene (43) and interacts with octanucleotide elements in small nuclear RNA gene promoters and the simian virus 40 enhancer (5). Both OTF1 (12) and OTF2 (39) have been purified, and the sequence of cloned OTF2 has recently been elucidated (30, 38, 44). The restriction of OTF2 to B cells suggests that it is the primary effector of tissue-specific expression of the immunoglobulin genes. However, the presence of octanucleotide elements in a variety of gene contexts leads to the question of functional specificity of OTFs and suggests that the gene-specific transcription activity of the OTFs may be regulated through differential interactions with other cellular factors.

In the case of the heavy-chain $V_H$ promoters, in vivo studies have identified at least two other sequence elements that play a modulatory role in transcription (2, 9). A pyrimidine-rich region and a heptamer element, 5′-CTCATGA-3′, are generally found in close proximity to the octamer sequence and function in conjunction with the octamer element to stimulate transcription. In studying the in vitro transcription of the MOPC 141 $V_H$ promoter, we identified an additional element, the N element, near the octamer sequence that contributes significantly to the function of the MOPC 141 $V_H$ promoter; this function involves interaction with a novel protein.

## MATERIALS AND METHODS

**Template construction.** Plasmid templates were derived from pSV-$\gamma_{2b}$VC, a gift of S. Morrison. A *Hin*dIII-*Pvu*II fragment containing sequences from positions −100 to +177 of the $\gamma_{2b}$ heavy-chain gene was cloned in pUC13 and served as the wild-type template ($N^+O^+$) in transcription assays. Mutant templates were made by inserting oligonucleotides that contained point mutations in the sequences of interest between positions −71 and −42. Template $N^+O^-$ contained

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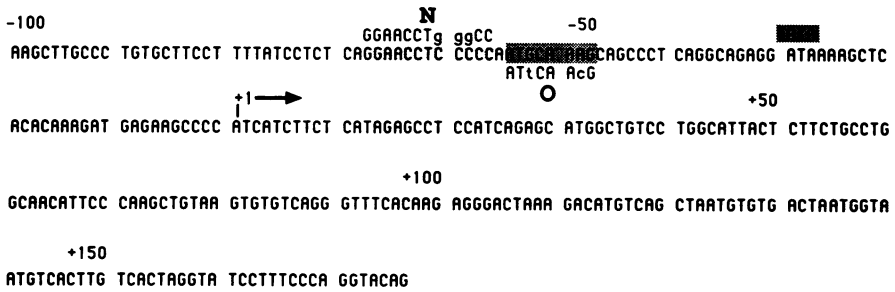


FIG. 1. Promoter region of the MOPC 141 gene. The sequence of the transcribed strand from position −100 to position +177 of the MOPC 141 heavy-chain gene is shown. The N element (N) and octamer element (O) are shown in the shaded boxes. The sequences of the point mutations in these elements are shown above and below the wild-type sequence. The TATA box region, the transcription start site, and a degenerate octamer at position +135 are also shown.

two point mutations in the octamer sequence, template $N^-O^+$ contained three point mutations in the N sequence, and template $N^-O^-$ incorporated an oligonucleotide with point mutations in both the octamer and N-binding sites (Fig. 1). All of these plasmids were confirmed by DNA sequencing.

**Extract preparation.** Namalwa cells (B cells) and HeLa cells were grown as previously described (39), and nuclear extracts were prepared by the method of Dignam et al. (7).

**Transcription assays.** A standard transcription reaction contained 50 mM Tris (pH 8.0), 12% glycerol, 46 mM KCl, 0.12 mM EDTA, 0.3 mM dithiothreitol, 0.16 mM phenylmethylsulfonyl fluoride, 3 mM $MgCl_2$, 600 μM each ribonucleoside triphosphate, and 10 μg of template per ml. B-cell and HeLa-cell nuclear extracts containing 7.5 mg of protein per ml were added in various amounts as indicated in the figure legends. After incubation at 30°C for 40 min, the reactions were stopped by the addition of an equal volume of 20 mM Tris (pH 8.0), 0.6 M sodium acetate, 1% sodium dodecyl sulfate, 20 mM EDTA, and 200 μg of yeast tRNA per ml. Reactions were extracted twice with phenol-$CHCl_3$ and once with ether and then precipitated with ethanol.

In transcription competition reactions, extracts were first preincubated with oligonucleotides for 20 min at 30°C and then added to standard transcription mixtures. After an additional 40 min, reactions were terminated and processed as described below.

**S1 nuclease protection assays.** Transcription reactions were analyzed by the S1 nuclease protection assay as previously described (49). Briefly, RNA from the in vitro transcription reaction was hybridized to a single-stranded, kinase-labeled probe for at least 18 h at 46°C in 80% formamide. The probe used in this analysis was a 90-base oligonucleotide containing sequences from positions −20 to +70 of the $\gamma_{2b}$ heavy-chain gene. Specific transcription resulted in a nuclease-protected product visualized by autoradiography of 6% acrylamide gels containing 8 M urea.

Transcripts were quantitated by scintillation counting of the gel slice containing the protected probe and normalized by comparison to the probe protected by hybridization to readthrough transcripts. In the case of oligonucleotide competition in transcription reactions, the results were normalized by comparison to the readthrough transcripts and to the basal-level transcription as assessed by quantitation of the double mutant templates.

**Electrophoretic mobility shift assays.** Electrophoretic mobility shift assays were performed as previously described (39). Probes used in these assays were the wild-type promoter and oligonucleotides containing the octamer and N sequences. Protein-DNA complexes were formed by using nuclear extracts from HeLa cells, Namalwa B cells, and Jurkat T cells that were either uninduced or induced with phytohemagglutinin and phorbol ester (47). Competitors (at 200-fold molar excess) included the wild-type and mutant heavy-chain promoter oligonucleotides as well as oligonucleotides containing the octamer-binding site of the κ light-chain promoter (39), the H2TF1-binding site of the $H\text{-}2K^b$ major histocompatibility gene promoter (1) or the NFκB-binding site of the light-chain enhancer (21).

**DNase I footprinting and methylation interference analysis.** DNase I footprinting was performed as described by Scheidereit et al. (39) with purified octamer transcription factors and the wild-type $V_H$ promoter as a probe. Methylation interference with protein binding of the N transcription factor (NTF) was assessed by using the bound and free complexes from a mobility shift assay as previously described (21).

## RESULTS

**Sequence requirements for in vitro transcription.** The MOPC 141 $V_H$ promoter was mutated in either or both of the octamer and N elements (Fig. 1). At low concentrations of HeLa nuclear extracts, the double mutant and wild-type templates were each transcribed at comparable low levels (lanes 1 and 2, Fig. 2A). At higher extract concentrations, transcription of the wild-type template was selectively increased (compare lanes 4, 6, and 8 with lanes 3, 5, and 7; Fig. 2A). In contrast, the transcription activity of the wild-type template in B-cell extracts was consistently higher (10-fold) than that of the double mutant over a range of extract concentrations (compare lanes 10, 12, 14, and 16 with lanes 9, 11, 13, and 15; Fig. 2A). Control reactions demonstrated that an adenovirus major late promoter without the upstream stimulatory sequence (37) was transcribed equally well in either extract (Fig. 2B).

To determine the sequence requirements for transcription, templates containing reciprocal mutations in the octamer and N elements were tested by in vitro transcription (Fig. 3). The transcription observed at high HeLa extract concentrations was relatively unaffected by loss of the octamer element (compare lanes 2 and 3, Fig. 3) but was dependent on the N sequence either in the presence or absence of the octamer element (compare lanes 2 and 3 with lanes 1 and 4). In contrast, the elevated transcription observed in B-cell extracts was dependent on both the N and octamer elements; transcription was reduced fivefold by mutation in the octamer sequence (lane 7) and threefold by mutation of the N element (lane 8). The relative contributions of the N and octamer sequences in transcription remained the same over

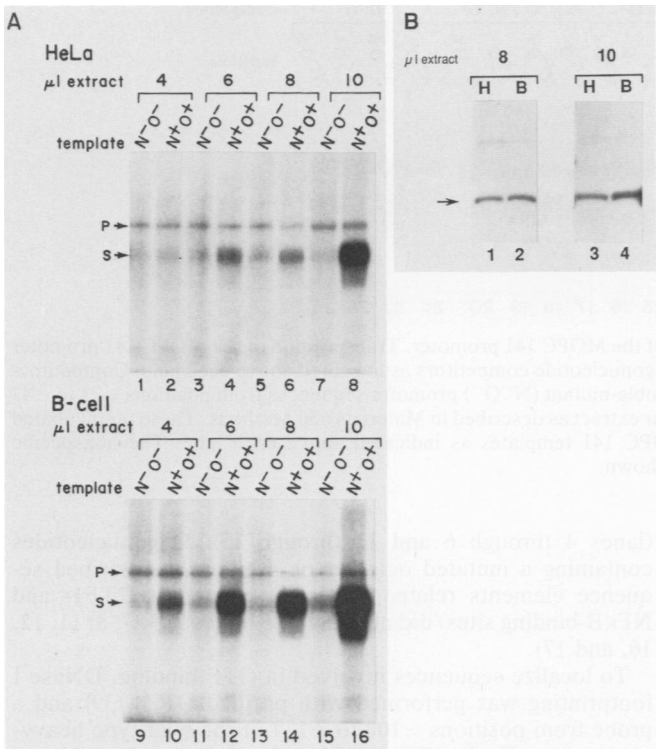


FIG. 2. Titration of various HeLa- and B-cell extracts in transcription reactions. HeLa- and B-cell extracts were added at the indicated amounts to transcription reactions as described in Materials and Methods. (A) Transcription reactions with the double-mutant ($N^-O^-$) or wild-type ($N^+O^+$) templates at increasing extract concentrations. Hybridization of kinase-labeled probe to read-through transcripts resulted in a nonspecific S1-protected product (P). Hybridization of probe to accurately initiated MOPC 141 transcripts resulted in a specific S1-protected product (S). (B) Transcription reactions with the adenovirus major late promoter control template. The indicated amounts of HeLa-cell (H) and B-cell (B) extracts were added to transcription reactions containing the control template. The specific S1-protected product is marked by an arrow.

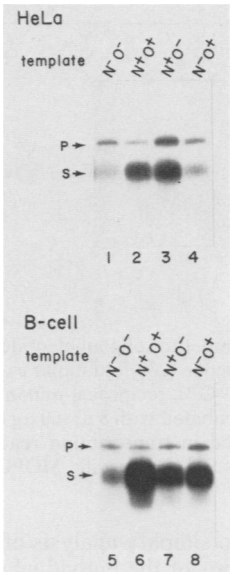


FIG. 3. Effect of N and octamer promoter elements on HeLa- and B-cell transcription. The wild-type MOPC 141 promoter ($N^+O^+$) mutated in either ($N^+O^-$ or $N^-O^+$) or both ($N^-O^-$) the N and octamer elements (Fig. 1) and transcribed with 8 μl (60 μg of protein) of HeLa- or B-cell nuclear extracts as described in Materials and Methods and indicated above each lane. The nonspecific readthrough transcripts (P) and MOPC 141-specific transcripts (S) are shown.

the range of B-cell extract concentrations shown in Fig. 2 (data not shown). These results show that both the N and octamer elements can stimulate transcription independently, and they suggest some cooperativity between these regulatory elements in B-cell-specific expression.

To test for factor interactions with these regulatory sequences, transcription reactions were carried out in the presence of competitor oligonucleotides containing either or both of the N and octamer elements (Fig. 4). The oligonucleotide containing the N element only slightly inhibited transcription of templates containing the wild-type promoter (compare lane 10 with lane 2, Fig. 4) and did not significantly inhibit the already low transcription of templates containing only the N element (compare lane 11 with lane 3). Transcription dependent on the octamer element remained unchanged in the presence of N element containing competitors (compare lane 10 with lane 2 and lane 12 with lane 4). The octamer element oligonucleotide inhibited transcription of templates containing the wild-type promoter or octamer element alone (lanes 14 and 16 versus lanes 2 and 4, respectively). Interestingly, we have consistently observed a higher level of transcription of the template containing the N element only in the presence of octamer competitor when compared with transcription in the absence of any competitor (compare lanes 15 and 3). The implication of this result will be discussed below. The competitor oligonucleotide containing both N and octamer elements (lanes 5 through 8) or a mixture of reciprocal mutant oligonucleotides (lanes 17 through 20) inhibited transcription of all templates containing elements beyond the basic promoter. Transcription in the presence of the double mutant oligonucleotide (lanes 21 through 24) was unchanged from that observed in the absence of any competitor (lanes 1 through 4). These studies suggested that at least two distinct factors mediated transcription of this heavy-chain gene in B cells.

**Electrophoretic mobility shift assays for transcription factor binding.** The suggestion that two factors mediated transcription of this heavy-chain promoter prompted us to investigate the interaction of nuclear factors with the promoter elements. When a fragment containing the wild-type promoter was used in binding assays with extracts from HeLa and B cells, at least five specific complexes were observed (Fig. 5). Binding competition studies with the reciprocal mutant oligonucleotides allowed us to identify octamer-dependent complexes C1 (OTF2 dependent) and C4 (OTF1 dependent) and N-element-dependent complexes C2 and C3. Note that C1 is specific in B cells and comigrates with a nonspecific complex formed in HeLa nuclear extracts. Interestingly, complex C5 appeared to be dependent on the binding to both the octamer and N elements, since either element alone could compete for complex formation. Furthermore, the presence of C5 in both HeLa extracts and B-cell extracts suggests that the complex consists of OTF1 and the N factor bound simultaneously to their cognate sites. The C5 complex was absent in gel shift assays with probes containing only a single element (see below), providing further evidence that this complex is dependent on the binding to multiple elements. Because of the significant overlap of complexes C3 and C4, further studies with probes containing a single

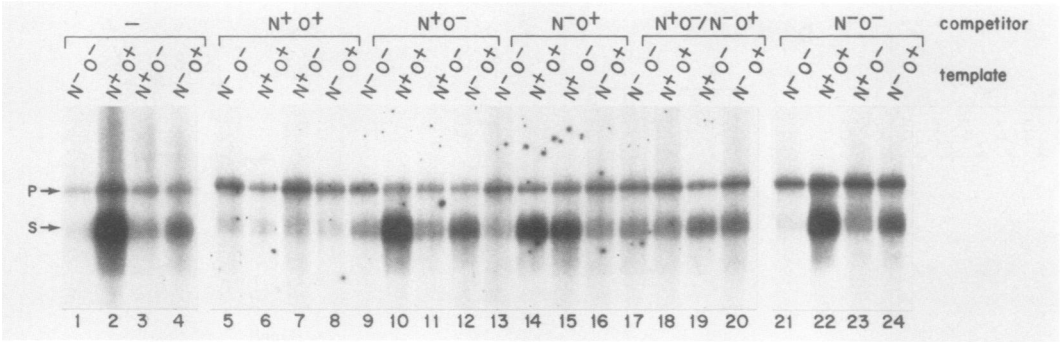


FIG. 4. Effect of competitor oligonucleotides on B-cell transcription of the MOPC 141 promoter. Transcription of the MOPC 141 promoter was assayed in the presence (200-fold molar excess) or absence (−) of oligonucleotide competitors as indicated above each lane. Competitors contained wild-type ($N^+O^+$), reciprocal-mutant ($N^+O^-$ or $N^-O^+$), or double-mutant ($N^-O^-$) promoter sequences from positions −71 to −42 (Fig. 1) and were preincubated with 8 μl (60 μg of protein) of B-cell nuclear extract as described in Materials and Methods. These preincubated extracts were then used in transcription reactions containing the MOPC 141 templates as indicated above each lane. The nonspecific readthrough transcripts (P) and specific MOPC 141 transcripts (S) are shown.

element were used to simplify analysis of this promoter and distinguish interactions of the individual factors.

**Binding of octamer transcription factors.** Using the $N^-O^+$ oligonucleotide probe in gel shift assays (Fig. 6), we observed a prominent OTF1 complex, C4, in HeLa- and B-cell extracts and a minor but reproducible OTF2 complex, C1, in B-cell extracts. The octamer complexes specifically competed with oligonucleotides containing an octamer sequence (lanes 4 through 6 and 13 through 15). Oligonucleotides containing a mutated octamer or previously described sequence elements related to the N element (H2TF1- and NFκB-binding sites) did not compete (lanes 2, 3, 7, 8, 11, 12, 16, and 17).

To localize sequences involved in OTF binding, DNase I footprinting was performed with purified OTFs (39) and a probe from positions −100 to +177 of the wild-type heavy-chain gene. Each OTF (OTF1 from HeLa or B cells and

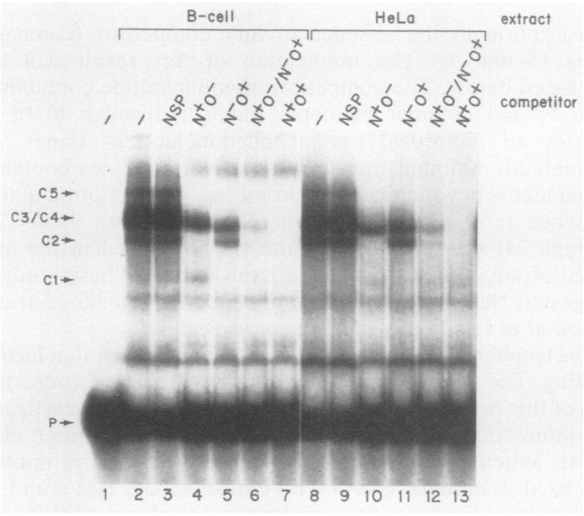


FIG. 5. Electrophoretic mobility shift assay to identify protein-DNA complexes on the MOPC 141 promoter. Mobility shift assays were used to identify ubiquitous and B-cell-specific proteins that bound to the regulatory elements of the MOPC 141 promoter. A promoter fragment (P) containing sequences from positions −100 to +177 was incubated with 1 μl (7.5 μg of protein) of B-cell or HeLa-cell nuclear extract as described in Materials and Methods and indicated at the top of the figure. Under these conditions, five specific protein-DNA complexes (C1 through C5) were observed. Oligonucleotide competitors (200-fold molar excess) containing MOPC 141 promoter sequence from positions −71 to −42 were either wild type ($N^+O^+$), reciprocal mutant ($N^+O^-$ or $N^-O^+$), or double mutant ($N^-O^-$) in the N and octamer elements (Fig. 1). Inhibition of complex formation in the presence of these competitors allowed the identification of OTF-dependent complexes (C1 and C4) and NTF-dependent complexes (C2 and C3). Complex C5 appeared to be dependent on the presence of both OTF and NTF factors. A 17-base-pair oligonucleotide (NSP) containing coding sequence from the histone H1 gene was used as a control.

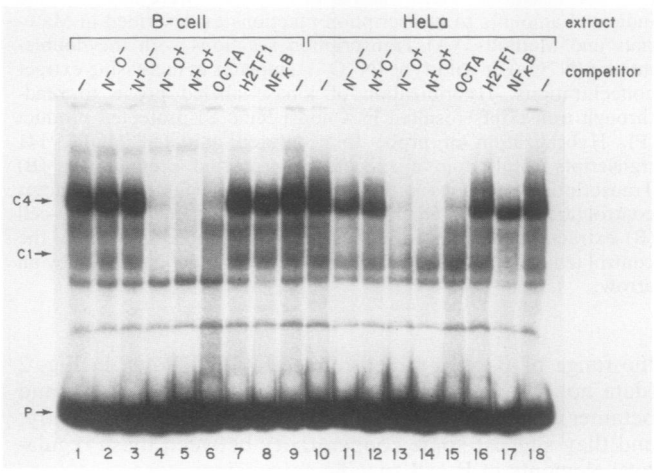


FIG. 6. Electrophoretic mobility shift assays to detect OTF interaction with the MOPC 141 promoter. A DNA fragment of the MOPC 141 promoter with sequences from positions −71 to −42 contained a mutation in the N element (Fig. 1) and flanking polylinker DNA from pUC13. When used as a probe (P) in electrophoretic mobility shift assays, two specific complexes (C1 and C4) were observed in B-cell extracts (lanes 1 through 9) and one specific complex (C4) was observed in HeLa extracts (lanes 10 through 18). Oligonucleotide competitors containing the MOPC 141 promoter sequence from positions −71 to −42 were as described previously (Fig. 1; see legend to Fig. 5). The OCTA oligonucleotide competitor contained sequences from the kappa light-chain promoter from positions −75 to −52, including the octamer element. The H2TF1 oligonucleotide competitor contained sequences from the $H2K_b$ major histocompatibility gene from positions −177 to −154, including the binding site for H2TF1. The NFκB oligonucleotide competitor contained sequences of the kappa light-chain enhancer from position +3923 to position +3954, including the binding site for NFκB. Extract (1 μl; 7.5 μg of protein) and competitors were added to each reaction as indicated at the top of the figure.

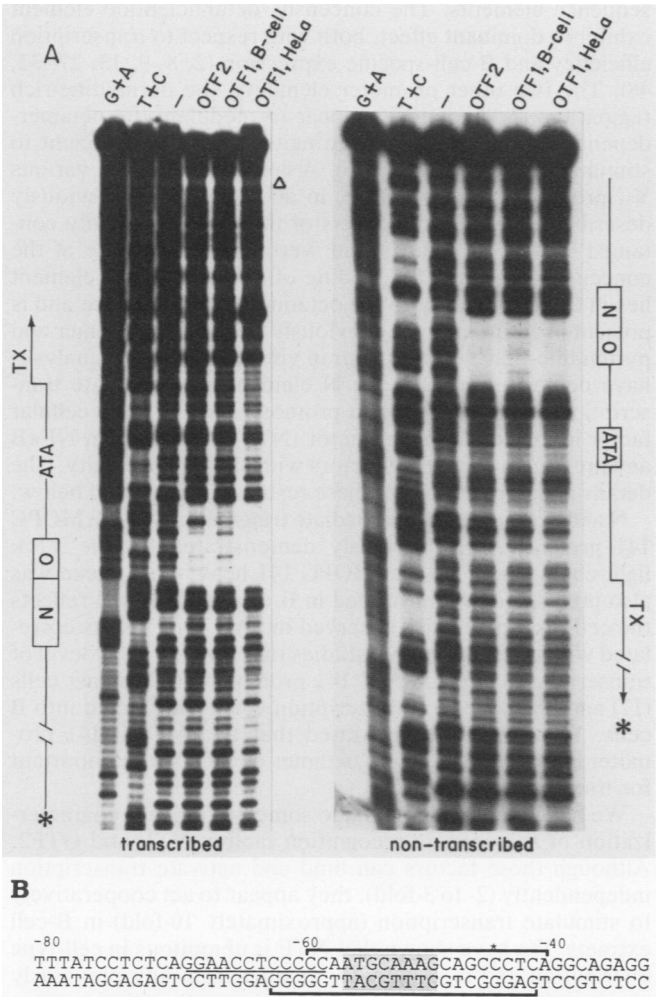


FIG. 7. Interaction of OTFs with the MOPC 141 promoter by DNase I footprinting. The interaction of purified OTF1 from HeLa cells and OTF1 and OTF2 from B cells was studied by DNase I footprinting with a DNA fragment containing wild-type MOPC 141 promoter sequences from positions −100 to +177 (A). The promoter probe was kinase labeled on either strand at restriction sites within the flanking polylinker sequence (*). The relative positions of the N and octamer (O) elements on the transcribed and nontranscribed strands are shown. The TATA (ATA) element and transcription start site are also marked. A second OTF footprint on the transcribed strand is marked (△); with longer electrophoresis times (data not shown), these interactions can be mapped to a degenerate octamer element at position +135 (Fig. 1). (B) Summary on the interactions of OTFs with the upstream octamer.

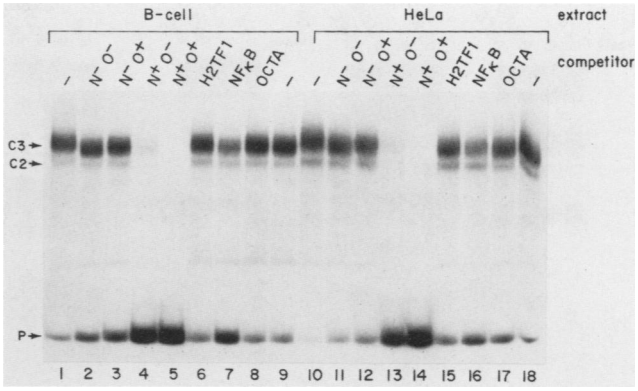


FIG. 8. Electrophoretic mobility shift assays to detect NTF interaction with the MOPC 141 promoter. A DNA fragment containing MOPC 141 promoter sequences from positions −71 to −42 carried a mutation in the octamer element (Fig. 1) as well as some flanking pUC13 polylinker DNA. When used in electrophoretic mobility shift assays, two specific protein-DNA complexes, C2 and C3, were observed in both B-cell and HeLa-cell nuclear extracts as shown. Oligonucleotide competitors (described in detail in the legends to Fig. 5 and 6) were used to demonstrate that these two complexes were NTF dependent. Specificity for the N element is shown by the inhibition of complex formation by N-element-containing competitors and by little or no inhibition of complex formation by oligonucleotides containing similar sequences found in the H2TF1- or NFκB-binding sites.

OTF2 from B cells) showed interactions over positions −58 to −39 on the transcribed strand and over positions −61 to −40 on the nontranscribed strand (Fig. 7). A unique hypersensitive site at position −43 with respect to the transcription start site on the transcribed strand was observed with OTF2 binding. The DNase-protected region covered a sequence that included part of the heptamer element (positions −42 to −36), which has been shown to function cooperatively with the octamer element in mediating in vitro transcription (36) as well as the dimerization of OTFs on the BCL1 heavy-chain promoter (35); thus, it seems likely that the extended footprint we observe in the MOPC 141 promoter represents interaction of an OTF dimer with the octamer and adjacent heptamer elements. We have not compared the DNase protection in the presence and absence of the heptamer(s) in these promoters. Further studies to confirm these results and functional studies on the role of the heptamer elements in mediating MOPC 141 transcription are in progress.

**Binding and characterization of NTF.** Using the $N^+O^-$ oligonucleotide as a probe in gel shift assays (Fig. 8), we observed one minor complex, C2, and one major complex, C3, in both B-cell and HeLa-cell extracts. The amount of C2 complex varied from extract to extract and appeared to increase with repeated freeze-thawing of the extract, indicating that C2 may result from degradation of the protein involved in C3 complex formation. We estimate the molecular mass of NTF to be on the order of 90 kilodaltons, based on correlations with the $R_f$ value of OTF1 (90 kilodaltons)-octamer complexes with probes of similar sizes. Formation of both complexes could be specifically inhibited by oligonucleotides containing the N sequence (lanes 4, 5, 13, and 14). Oligonucleotides containing either a mutated N element (lanes 2, 3, 11, and 12) or an octamer element (lanes 3, 8, 12, and 17) did not compete for binding of the probe. Binding specificity of NTF showed that an oligonucleotide containing the NFκB-binding site (9 of 11 nucleotides were the same as those in the N element) partially inhibited complex formation (lanes 7 and 16), and an oligonucleotide containing the H2TF1-binding site (7 of 11 nucleotides were the same as those in the N element) did not compete for binding of the probe (lanes 6 and 15). The lack of inhibition of complex formation with an oligonucleotide containing the H2TF1-binding site leads us to believe that NTF is not H2TF1, although, like H2TF1, it is present both in HeLa and B cells. In complementary experiments, gel shift analysis failed to reveal any detectable interaction between purified NFκB (21) and the N promoter element and showed that the N element did not effectively inhibit NFκB binding to its cognate site (data not shown). Based on these results we therefore believe that NTF is also distinct from NFκB.

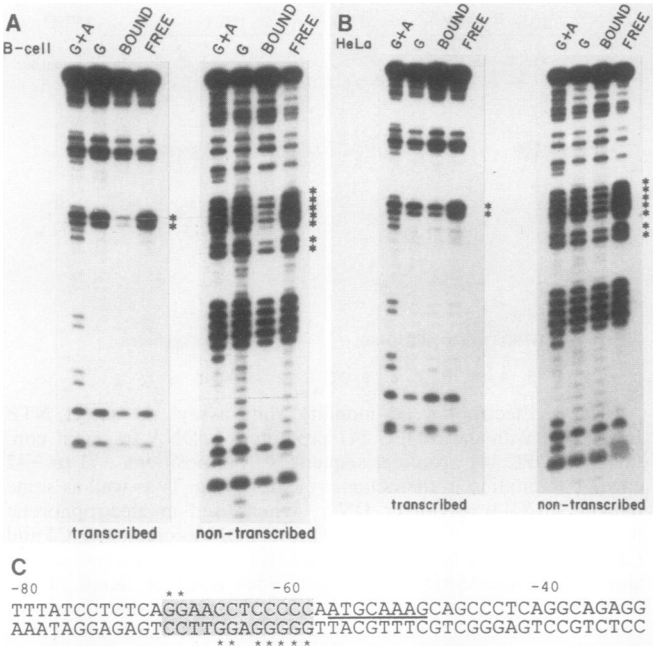


FIG. 9. Methylation interference to detect NTF interactions with the MOPC 141 promoter. Methylation interference with NTF protein binding was assessed using the bound and free complexes from a mobility shift assay with B-cell and HeLa-cell extracts (Fig. 8). DNA probes containing MOPC 141 sequences from positions −71 to −42 were labeled on either strand by using restriction sites found in the flanking pUC13 polylinker DNA. Briefly, the bound and free complexes were separated and isolated from mobility shift gels, eluted, cleaved with piperidine, and electrophoresed on denaturing gels. Methylated G residues, which interfere with NTF protein-DNA interactions in both B cells (A) and HeLa cells (B), are indicated (*). These interactions are identical for NTF complexes in B-cell and HeLa-cell extracts and are summarized in panel C.

Other studies have shown that T-cell induction by phorbol esters and phytohemagglutinin can increase the binding (or amount) or similar or related factors that bind to the NFκB target site (14, 31). We therefore analyzed extracts from uninduced and induced T cells in binding assays with the $N^+O^-$ probe (data not shown). The results showed the formation of the major C3 complex in B-cell, HeLa-cell, and T-cell extracts, with no difference in the binding of NTF in uninduced or induced T-cell extracts. Positive T-cell induction was assessed by other methods (47; data not shown). Based on these results, we believe that NTF is distinct from the previously described induced T-cell protein.

Finally, we used methylation interference to study the interaction of NTF with the heavy-chain promoter. Piperidine cleavage of DNA in the bound complex indicated clear binding interference as a result of methylation of the indicated G residues (Fig. 9). The interference patterns were identical when B-cell (Fig. 9A) and HeLa-cell (Fig. 9B) nuclear extracts were compared (Fig. 9C). It appears, therefore, based on the transcription results showing activation of transcription via NTF in both HeLa and B cells and the ubiquitous distribution of NTF among various cell types, that NTF in distinct from NFκB but may be related to a family of proteins that recognize similar sequences.

## DISCUSSION

Optimal transcription of heavy-chain $V_H$ promoters in vivo has been shown to require at least three upstream sequence elements. The concensus octanucleotide element exhibits a dominant effect, both with respect to transcription efficiency and B-cell-specific expression (2, 8, 9, 13, 27, 32, 48). The two other promoter elements, the pyrimidine-rich region and the heptamer, appear to modulate the octamer-dependent transcription, since neither alone is sufficient to stimulate transcription (9, 36). A comparison of the various $V_H$ promoters revealed that, in addition to the previously described elements, promoters of the Q52 gene family contained 9 of 11 sequences that were similar to those of the concensus sequence for binding of NFκB. This N element lies in close proximity to the octanucleotide sequence and is present in addition to the previously described heptamer and pyrimidine-rich elements. Our in vitro transcription analyses have demonstrated that the N element can modulate transcription of the heavy-chain promoter, but that the cellular factor involved is a novel factor (NTF) distinct from NFκB and previously described factors with similar specificity. The details and implications of these results are discussed below.

**N and octamer elements mediate transcription of the MOPC 141 promoter.** As previously demonstrated for the T1 κ light-chain gene (29), the MOPC 141 heavy-chain gene was also preferentially transcribed in B-cell extracts and reflects the cell-type specificity observed in vivo. Our results correlated with previous in vivo studies that showed a low level of transcription of the MOPC 141 promoter in fibroblast cells (17) and high levels of transcription when transfected into B cells. We further demonstrated that two MOPC 141 promoter elements, the N and octamer elements, are important for transcription in vitro.

We have identified and made some preliminary characterization of the cognate recognition factors NTF and OTF2. Although these factors can bind and activate transcription independently (2- to 3-fold), they appear to act cooperatively to stimulate transcription (approximately 10-fold) in B-cell extracts. We have shown that NTF is ubiquitous in cell-type distribution and is apparently distinct from any previously characterized factors that bind to elements with sequence similarity to the N element. In contrast to the observation with B-cell extracts, the low $V_H$ promoter activity in HeLa extracts is mediated by the N element and the NTF that is present in these extracts. Thus, although the octamer element in the $V_H$ promoter is recognized by both OTF1 and OTF2, it appears that only OTF2 (in B cells) is capable of activating transcription of this promoter under our assay conditions, either alone or in conjunction with NTF. Analysis of the protein-DNA interactions in this promoter showed that OTF1 and OTF2 from B cells and OTF1 from HeLa cells interacted with identical sequences in the promoter. Some qualitative difference in OTF2 binding was apparent from the hypersensitive site at position −43. This difference may reflect differences in the binding of the smaller OTF2 protein or may be due to subtle changes in binding and dimerization of OTF2 that are not seen with OTF1.

**NTF interacts specifically with OTF2 to stimulate transcription.** Transcription of the MOPC 141 promoter in HeLa extracts suggested that the factor(s) enhancing transcription via the N element was ubiquitous and distinct from NFκB, since the latter has not been detected in unstimulated HeLa-cell nuclear extracts and since NFκB did not bind to the N element. The implication is that whereas both OTF1 and OTF2 can bind to this promoter, only OTF2 can functionally interact with the N factor, as observed in B-cell transcription. We did observe a mutimeric complex (C5) that appeared to be composed of NTF and OTF1 bound to this

promoter, but this complex is apparently inactive in stimulating transcription. The absence of an identifiable OTF2-NTF complex does not preclude this possibility, since low levels of complex formation may not be detectable under these electrophoretic mobility shift assay conditions. A low level of productive interaction may be more obvious in the transcription assays.

Methylation interference analysis of NTF interactions with the heavy-chain promoter showed that methylation of any of the G residues in the N element interfered with N-factor binding. These G residues on the nontranscribed strand near the octamer element were also involved in the binding of the octamer factors as determined by DNase I footprinting. Due to limitations resulting from its relative size in comparison with chemical probes, DNase I may overestimate the extent of the protein-DNA interactions, thus suggesting the apparent overlap in binding domains of the octamer and N elements. On the other hand, close contacts between the two proteins may be necessary for their productive interaction, or the simultaneous binding of the two proteins may induce conformational changes favoring transcription activation. The relative size difference between OTF1 (90 kilodaltons) and OTF2 (60 kilodaltons) may limit these interactions, and, as our results suggest, only OTF2 would be capable of positive effects. Based on these results, we propose that the N protein is a bona fide transcription factor, that it is present in many cells (B cells and cells of nonlymphocyte origin), and that, in conjunction with the OTF2, it can mediate the transcription of a family of heavy-chain genes. An intriguing possibility is that all of these NFκB-like proteins are related and that the DNA-binding domains are similar in each of these factors, allowing some flexibility in DNA recognition. It is also interesting to note that many of the NFκB-like sequence elements are found in close proximity to other *cis*-acting elements (e.g., the Sp1-binding site and the two NFκB elements of the human immunodeficiency virus type 1 promoter), perhaps indicating the requirements for functional interaction with other proteins to achieve the desired effects. A further understanding of the relationship between this family of NFκB-like proteins and their specific (or overlapping) functions will be elucidated with the purification and cloning of these factors.

**NTF and OTF2 interactions provide another level of regulation in tissue-specific expression.** The cooperative interaction we propose has important implications in the tissue-specific regulation of this heavy-chain gene. Our current model suggests that the N-transcription factor is present in all cell types and weakly interacts with promoter elements to stimulate a low level of transcription. Consistent with this proposal are previous results showing low levels of expression of this gene when introduced into mouse L cells (17) and the requirement for increased concentrations of HeLa nuclear extract to positively affect in vitro transcription of N-element-containing templates in our experiments. The transcription competition experiments also provide another clue as to N-factor interactions. We have consistently observed a higher level of transcription of the $N^+O^-$ template in the presence of the octamer competitor when compared with transcription in the absence of any competitor. One explanation for this result would be that the N and octamer factors can interact independently of DNA binding and that binding of OTF to DNA competitors releases some of the sequestered N protein. This DNA-independent interaction could provide an additional level of transcriptional control. In any case, it seems apparent that heavy-chain genes can be regulated by combinations of *cis* elements (pyrimidine-rich track, heptamer, octamer, and N elements) and that the interactions between the cognate transcription factors serve to confer an individual program of gene expression. In the case of the MOPC 141 gene, the interplay between the proposed OTF2 dimer (bound to adjacent heptamer-octamer sites by analogy to the BCL-1 promoter) and NTF presents a unique regulatory system for inducing high levels of transcription.

It is clear that the octamer element and its cognate protein play a dominant role in mediating transcription of the immunoglobulin genes. Our results confirm and extend the previous results by demonstrating the importance of the functional interactions, both at the protein-DNA level and possibly between octamer and N factors. The specificity of OTF2 in mediating the activation of these genes has been a point of controversy. Our studies have shown that OTF1 from HeLa cells is not capable of stimulating transcription in our in vitro system, although, within the limits of our characterization, the protein-DNA and potential protein-protein interactions appear to be identical to OTF2. Simple arguments of tissue specificity based on the relative abundance of these proteins, as suggested by LeBowitz et al. (24), do not seem to be applicable in the studies we present. Quantitative differences, as assessed by gel mobility shift binding, in the levels of OTF1 in HeLa- and B-cell extracts appear to be negligible; in contrast to extracts from BJA-B cells, OTF1 is more abundant than OTF2 in the Namalwa B-cell extracts used in these studies. The logical conclusion from our results is that OTF1 from HeLa cells cannot function in stimulating transcription in the context of this heavy-chain promoter; however, we cannot rule out the possibility that the OTF1 in B cells is different and functions in conjunction with the N factor to increase transcription.

Finally, the identification of NTF-mediated transcription of a mouse promoter in a human transcription system may be extended to human heavy-chain immunoglobulin genes as well. The identification of an N element (9 of 12) in the identical position relative to the octamer-binding site in a human heavy-chain immunoglobulin gene promoter (22) may be of similar importance in mediating transcription of this gene. We have not yet tested this promoter in our in vitro system. Further analysis of the potential interaction between the N and octamer factor(s) and probing the mechanisms involved in tissue-specific transcription regulation of these genes requires reconstituting cell-type-specific transcription with purified factors. Current efforts are directed toward developing such in vitro systems.

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