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The Immunoglobulin Octanucleotide: Independent Activity and Selective Interaction with Enhancers

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The thymidine kinase (*tk*) promoter of herpes simplex virus includes an octanucleotide sequence motif (ATTTGCAT) that is also an essential component of immunoglobulin kappa gene promoters. In the absence of an enhancer, *tk* promoter derivatives that contain this element support a higher rate of transcription than those that lack it. The action of the kappa enhancer augments that of the octanucleotide in B lymphoid cells; when both elements are present, *tk* promoter activity is increased by more than an order of magnitude. In contrast, the presence of the octanucleotide in this promoter markedly reduces its response to a nonimmunoglobulin enhancer. These results suggest that the octanucleotide may mediate a selective interaction among promoters and enhancers.

AT LEAST TWO DISTINCT DNA sequence elements influence the transcription of immunoglobulin kappa light chain genes. One of these, occupying a 250–base pair (bp) region of an intron adjacent to the constant region exon (*1, 2*), is a transcriptional enhancer; it can act over distances of several kilobases to increase the activity of a promoter in cis regardless of its orientation relative to the promoter (*3*). The kappa enhancer is preferentially active in B lymphoid cells, undergoes a localized change in chromatin structure that correlates with the onset of kappa transcription (*2*), and is required for maximal expression of kappa genes transfected into B lymphocytes (*4, 5*). A second essential element is the octanucleotide motif ATTTGCAT, which is found 70 ± 10 bp upstream from the transcriptional initiation sites of all light chain genes, and in inverted orientation (ATGCAAAT) at the same location in heavy chain gene promoters (*6, 7*). The octanucleotide has been stringently conserved in vertebrate evolution, and deletions of DNA segments that encompass this element abolish immunoglobulin promoter activity (*5, 7, 8*).

Recent studies suggest that the octanucleotide locus is included in the binding site for a specific nuclear protein (*9*) and that similar or identical octameric motifs function in the promoters of the histone H2B (*10*) and *Xenopus* small nuclear RNA (snRNA) genes (*11*). Little is known, however, about the precise role of the octanucleotide in promoter function or the extent to which it might interact with the enhancer to influence the level of kappa gene expression. In this report we demonstrate that the same octanucleotide is also a functional component of the thymidine kinase promoter of herpes virus and is required for maximal promoter function in both lymphoid and nonlymphoid cells. The presence of the octanucleotide markedly reduces the responsiveness of this promoter to a nonimmunoglobulin enhancer, whereas the kappa enhancer and the octanucleotide element act in concert to produce a stimulatory effect far greater than that of either element alone.

The described sequence elements of the thymidine kinase (*tk*) promoter (Fig. 1A) include a TATA box at residues −21 through −26 relative to the transcriptional initiation site, as well as a cluster of distal sequences between residues −46 and −105 that encompasses at least three protein-binding domains. These elements alone are sufficient to establish promoter function either in vivo or in vitro (*12, 13*). Farther upstream, between residues −131 and −138, we noticed a perfect copy of the light chain octanucleotide. To determine whether this sequence might also contribute to the function of the *tk* promoter, we compared the activity of a DNA fragment termed promoter A, which comprised only sequences +55 through −109, to that of promoter B, which extended an additional 39 bp upstream to position −148 and so included the octanucleotide motif (Fig. 1A).

We constructed a series of recombinant plasmids (Fig. 1B), fusing each promoter fragment to the coding sequences of the bacterial chloramphenicol acetyltransferase (CAT) gene (*14*). In most instances, the plasmids also contained the polyoma virus early region and origin of replication, which enable plasmids to replicate in mouse cells and thereby greatly increase the sensitivity of the transient expression assay (*4*). The plasmids were transfected into the mouse myeloma cell lines P3X63Ag8 and SP2/0, and relative *tk* promoter activity was inferred by comparing the levels of CAT enzyme activity in lysates of the transfected cells. Plasmids containing promoter A yielded a relatively low basal level of enzyme expression, whereas those containing promoter B generated CAT activity at three to four times the basal level (Table 1). To determine whether this difference resulted from the light chain octanucleotide present in promoter B, we inserted a synthetic 12-bp DNA oligomer containing that sequence immediately upstream from promoter A (Fig. 1C); this modification (promoter AL) increased CAT expression to a level equivalent to that obtained with promoter B.

We next examined the effect of the kappa enhancer element on *tk* promoter derivatives that either contained or lacked the octanucleotide. When inserted 0.6 kb upstream from promoter A, the kappa enhancer increased CAT expression to three to five times the basal level (Table 1). In contrast, fusion of this enhancer with promoter B yielded CAT activity that averaged 15 times the basal value; similar levels (11 times basal) were achieved with a combination of the kappa enhancer and promoter AL. These values correspond to a three- to fivefold increase over the activity of promoters B or AL alone, implying that the kappa enhancer augments *tk* promoter activity by a fixed proportion regardless of the presence of the octanucleotide and that the combined action of both these sequence elements is approximately equal to the algebraic product of their individual effects. The results obtained were essentially identical for both of the myeloma lines tested and were not influenced by the presence of the polyoma sequences (*15*). Moreover, the octanucleotide produced similar quantitative effects on CAT expression in transfections of the mouse 3T3 or rat XC fibroblast lines (Table 1). Thus, the stimulatory activity of this

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sequence is not confined to lymphoid cells.

Strikingly different results were obtained when the kappa enhancer was replaced by the Moloney murine sarcoma virus (MoMSV) enhancer. In transfected myeloma cells, this element increased CAT expression from promoter A more than sevenfold, but had little or no effect on the activity of promoter B (Table 1). Although the MoMSV enhancer was clearly functional on both promoters in 3T3 cells, the response of promoter B to this enhancer was only threefold, in contrast to the 13- to 23-fold response of promoter A. The basis for this unfavorable interaction is unknown; nevertheless, it is clear that not all enhancer elements produce multiplicative effects with the light chain octanucleotide.

We confirmed and extended the CAT assay results by analyzing the CAT transcripts with a primer extension assay. Unfractionated RNA from the transfected myeloma cells was annealed to an end-labeled DNA primer complementary to sequences near the 5′ end of the CAT coding region, and the primer was elongated with reverse transcriptase. Figure 2A shows an autoradiogram of the elongation products after fractionation by gel electrophoresis. For each of the plasmids tested, nearly all detectable transcripts initiated within a 4-bp region spanning residues −1 through +3, a pattern of initiation that is characteristic of the *tk* promoter in various cell types and in cell-free transcription (*12, 13*). Thus, all of our promoter constructs directed accurate transcriptional initiation. In addition, the relative abundance of correctly initiated transcripts, as determined by scintillation counting, paralleled closely the observed differences in CAT enzyme activity (Fig. 2B). Because identical sequences are transcribed from each plasmid, the relative levels of accumulated transcripts or of enzyme activity presumably reflect differences in the rate of initiation. We therefore conclude that promoters containing the light chain octanucleotide (promoters B and AL) were approximately three times as active as promoter A. The activities of all three promoters increased by a factor of approximately four when linked to the kappa enhancer; in constructs containing both the kappa enhancer and the light chain octanucleotide, promoter activity exceeded the basal value by more than an order of magnitude. We found, however, that the octanucleotide had no effect when placed in the inverted orientation immediately upstream from promoter A (promoter AH). The octanucleotide was also inactive when inserted 55 bp downstream from the initiation site (promoter AD).

Our findings indicate that the light chain octanucleotide motif is a functional component of the *tk* promoter. Deletion of the

Table 1. Effects of the octanucleotide and enhancer elements on CAT enzyme expression in transiently transfected cells. Enzyme activity was assayed 48 hours (myeloma cells) or 12 hours (fibroblasts) after transfection with plasmids that contained the promoters and enhancers indicated. Each value given is the mean ± SEM; the number of determinations is listed in parentheses. The data are normalized to one construct for each cell line, corresponding to specific activities of 560 U, 10 U, and 0.1 U per gram of protein for myeloma, 3T3, and XC cell extracts, respectively. In most cases, more than one preparation of each plasmid was tested. Data shown for myeloma cells are a composite of results from two different cell lines that adhere to tissue culture plates; these were transfected by the method of Banerji *et al.* (*22*). Fibroblasts were transfected by the calcium phosphate method (*21*). The CAT enzyme assay was performed essentially as described (*14*), and quantified by scintillation counting.

Promoter	Enhancer	Relative CAT enzyme specific activity		
		Myeloma cells	3T3 cells	XC cells
A	None	1 (7)*	1 (4)	
B	None	3.4 ± 0.1 (2)	3.9 ± 0.7 (4)	
AL	None	3.4 ± 0.5 (4)	3.6 (1)	
A	Kappa	4.0 ± 0.1 (9)*		1 (4)*
B	Kappa	15.4 ± 1.4 (4)*	3.5 ± 0.4 (2)	2.8 ± 0.6 (4)*
AL	Kappa	10.9 ± 2.4 (5)		
A	MoMSV	7.3 ± 1.3 (3)*	18.3 ± 4.8 (3)	
B	MoMSV	3.8 ± 0.1 (2)	12.1 ± 0.9 (3)	

*Tested both with and without polyoma viral sequences (*15*).

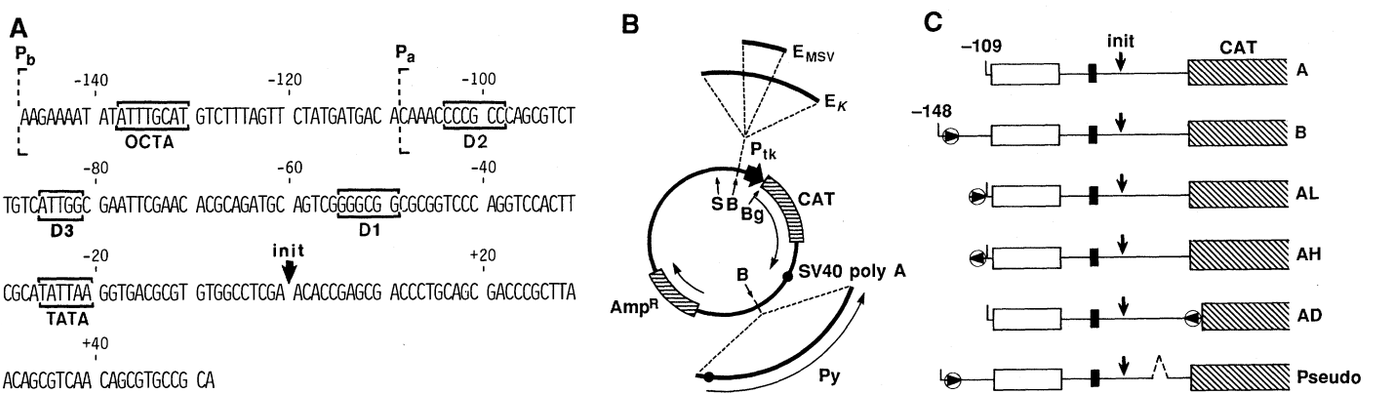

Fig. 1. Plasmid and promoter constructs. (**A**) Organization of the *tk* promoter. The transcription initiation site (init), TATA box, and octanucleotide (OCTA) are indicated; distal elements include two GC-rich regions (D1 and D2) and a CCAAT element (D3) (*12, 13*). The promoter fragments extended from a Bgl II site at +55 to Bam HI linkers at −109 (P_a) or −148 (P_b). (**B**) Plasmid structures. The plasmid pCAT3M (4.4 kb) contains a promoterless CAT gene and the SV40 polyadenylation region (*20*). We replaced the Xba I site of pCAT3M with a Sal I linker to produce pCAT3MS, and inserted a 3.6-kb fragment (*5*) encompassing the origin and early region of polyoma virus into the unique Bam HI site of pCAT3MS to produce pCAT3MSP. Promoter fragments were inserted between the unique Sal I and Bgl II sites of pCAT3MS or pCAT3MSP after ligating the 275-bp Sal I–Bam HI fragment of pBR322 onto the upstream (Bam HI) end of each promoter. Enhancers were also ligated into the Bam HI site at the 5′ end of the promoter. The mouse kappa enhancer (E_k) was contained in a 1.3-kb Hind III–Hpa I genomic fragment (*3*) with Bgl II linkers. The MoMSV enhancer (E_{MSV}) was a 0.35-kb Bam HI fragment (*21*). Enhancers and polyoma sequences were used in inverted orientation only. Restriction sites: B, Bam HI; Bg, Bgl II; and S, Sal I. (**C**) Promoter constructs. Locations of the TATA box (closed rectangle) and *tk* distal elements (open rectangle) are shown; the light chain octanucleotide (circle) lies at −138 in promoter B and in the pseudo-wild-type construct (Pseudo). A double-stranded oligonucleotide whose sequence (TATTTGCATGCA) includes the octanucleotide was ligated onto the Bam HI end of promoter A in either the light chain or heavy chain orientation to form promoters AL and AH, respectively, or inserted in heavy chain orientation into the Bgl II site at +55 to produce promoter AD. The pseudo-wild-type gene contains a derivative of promoter B with residues +16 to +36 deleted and replaced by a 10-bp Bam HI linker.

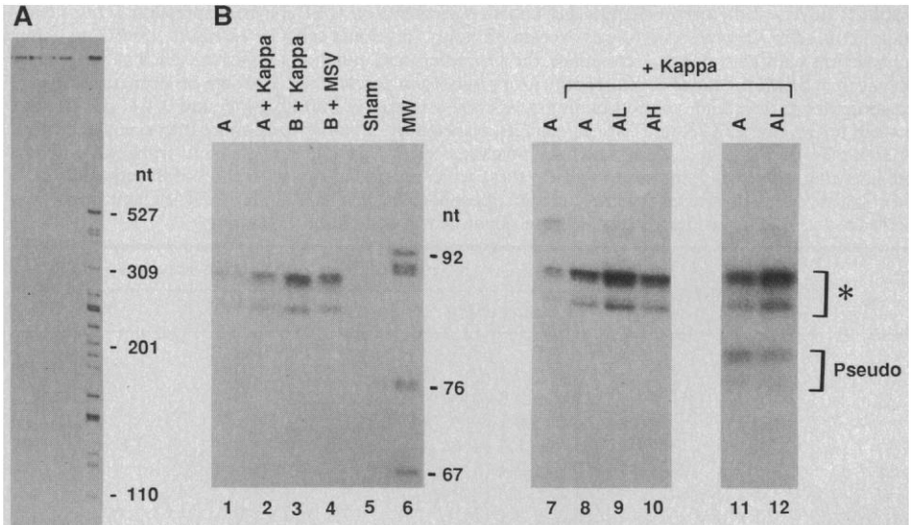

Fig. 2. Analysis of *tk* promoter activity in transfected myeloma cells. RNA was isolated as described (*23*) from P3X63Ag8 myeloma cells 48 hours after transfection; a ^{32}P end-labeled synthetic DNA primer complementary to sequences 5 to 31 bp upstream from the CAT initiation codon was annealed to 25 μg of RNA and subjected to primer extension. (**A**) Accuracy of initiation. In cells transfected with each of four plasmids [corresponding to those in lanes 1 to 4 of (B)], initiation is confined to a discrete 4-bp region (asterisk). (**B**) Transcript abundance. Cells were transfected with plasmids containing the promoters and enhancers indicated (*22*). Briefly, cultures at 10 to 20% confluency were washed in tris-buffered saline (TBS), exposed to 1 μg of plasmid DNA in 0.6 ml of TBS containing DEAE-dextran (0.5 mg/ml) for 30 minutes at room temperature, and then treated with 0.1 m*M* chloroquine in growth medium at 37°C for 3.5 hours. Sham-transfected cells (lane 5) were treated identically, but received no DNA. Lanes 11 and 12 show cotransfections of 1 μg of the test plasmid and 0.25 μg of a second plasmid that contained the pseudo-wild-type gene (see Fig. 1C) and the kappa enhancer; the latter plasmid produced slightly truncated transcripts which provide an internal standard for quantifications. Lengths (in nucleotides) of molecular weight standards are indicated. Accurately initiated transcripts from test plasmids correspond to labeled fragments 86 to 89 nucleotides long (asterisks); those from the pseudo-wild-type gene (Pseudo) are 10 nucleotides shorter.

octanucleotide reduced *tk* promoter activity by approximately 70% in our assay, an effect comparable to that produced by inactivation of any other distal element in this promoter (*12*). Perhaps because different experimental systems were used, the effect of the octanucleotide was not detected in earlier studies of *tk* promoter function; consequently, detailed analyses of this promoter have emphasized the region downstream from residue −110. The possible influence of the octanucleotide on the previous findings should now be assessed. Data from one study (*13*) suggested that HeLa cell extracts contain factors that bind near the octanucleotide in the *tk* promoter.

The octanucleotide is normally situated 256 bp, 138 bp, and 60 to 80 bp upstream from the initiation sites in the snRNA, *tk*, and immunoglobulin promoters, respectively, suggesting that its activity, like that of an enhancer, may be relatively independent of position. The octanucleotide is also a functional component of the immunoglobulin heavy chain enhancer (*7, 16*). Unlike an enhancer, however, the octanucleotide elements of both the *tk* and snRNA promoters (*17*) fail to function when inverted or placed downstream from the initiation site. The functional significance of the differing orientations of this element in immunoglobulin heavy chain and light chain promoters therefore remains a matter of conjecture. The octanucleotide does not appear to play a primary role in specifying the site of initiation at the *tk* promoter, as shifts in the location of this element (to position −138 in promoter B, −120 in promoter AL, or +55 in promoter AD) do not affect the fidelity of initiation.

The role of the octanucleotide in *tk* promoter activity is evident in both lymphoid and nonlymphoid cells, a finding compatible with the observation that proteins capable of binding this sequence are expressed in a variety of cell lineages (*9, 10*). Evidence that the heavy chain octanucleotide plays a similar quantitative role in certain snRNA promoters of *Xenopus* (*11*) further supports the notion that it is a phylogenetically ancient and highly conserved genetic signal that may influence the activity of a variety of genes in diverse species and cell types. Any direct contribution of the octanucleotide to the tissue-specific transcription of immunoglobulin genes would therefore require a B lymphocyte–specific interaction of this sequence with other nuclear components, presumably DNA-binding factors. Indirect evidence of such interactions has been reported (*18*), but the factors involved have not yet been characterized.

In both myeloma cells and fibroblasts, the presence of the octanucleotide substantially diminishes the responsiveness of the *tk* promoter to the MoMSV enhancer. In contrast, the actions of the octanucleotide and the kappa enhancer augment one another in myeloma cells, producing a multiplicative effect on promoter activity. Thus, promoters containing the octanucleotide may be less responsive to certain types of enhancers than are promoters lacking the octanucleotide. This, in turn, suggests that the octanucleotide might have a role in the selective functional interaction observed between the kappa promoter and kappa enhancer (*19*). Such a favorable interaction could have special importance for the immunoglobulin genes, which are encoded as widely separated gene segments in germline DNA. During the course of B-cell differentiation, sequences encompassing the promoter and 5′ exons of an immunoglobulin kappa gene become fused to genomic loci containing the enhancer and downstream coding regions of the gene. Conceivably, these DNA rearrangements may serve not only to juxtapose the necessary coding sequences, but also to bring together two dissimilar and independent regulatory elements whose actions then magnify one another, and so contribute to the prodigious transcriptional activity that is characteristic of this family of genes.

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Characterization and Clinical Association of Antibody Inhibitory to HIV Reverse Transcriptase Activity

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Reverse transcriptase activity of the human immunodeficiency virus (HIV) was blocked in vitro by immunoglobulin G (IgG) derived from certain individuals infected with this retrovirus. A heterogeneous immune response for inhibition of enzyme function was noted. Catalytic activity was depressed by 50% or more with the use of 10 micrograms of IgG from 11 of 16 HIV-seropositive asymptomatic carriers, but from 0 of 8 seronegative controls and 2 of 12 patients with acquired immune deficiency syndrome (AIDS) or the AIDS-related complex (ARC). The inhibitor was confined to the F(ab')$_2$ fragment. It was not directed against the poly(rA) · oligo(dT) template, nor against major envelope or structural viral antigens, and did not cross-react with bacterial, avian, or other mammalian DNA polymerases. It did not correlate with recognition of polymerase antigens by radioimmunoprecipitation. Loss of this inhibitor may be associated with development of clinical disease. Ten asymptomatic HIV-seropositive carriers with high titers of IgG antibodies to reverse transcriptase were followed for a mean of 3 years. All of four lost inhibitory capability prior to development of AIDS or ARC, while titers persist in the six who remain clinically healthy.

THE SERUM OF MANY ANIMALS NATURALLY infected with retroviruses contain antibodies capable of blocking the enzymatic activity of particulate reverse transcriptases (*1–4*). In some instances, reactivity occurs in the absence of detectable antibody to viral envelope or structural proteins (*2*). Correlations of antibody to reverse transcriptase (RT) with clinical status have been studied in cattle and cats. Bovine leukemia viral polymerase could be inhibited specifically by antibody from the serum of leukemic cattle, but not from the serum of infected animals experiencing solely a leukocytosis (*2*). In contrast, those cats that were exposed to feline leukemia virus (FeLV) and remained nonviremic or in a low state of viremia had antibody to FeLV polymerase (*1*). No such reactivity occurred in viremic or diseased animals.

Antibodies to the RT of human immunodeficiency virus (HIV), the etiologic agent of the acquired immune deficiency syndrome (AIDS), are readily detectable in the majority of HIV-seropositive individuals (*5–7*). These immunoglobulins, directed against proteins associated with endonuclease and RNA-dependent DNA polymerase functions, can be recognized by immunoblotting and radioimmunoprecipitation techniques and occur independently of the individual's clinical status (*6, 7*). In addition, sera from asymptomatic West Africans infected with the human T-lymphotropic virus type IV (HTLV-IV), a closely related retrovirus, cross-react with HIV polymerase antigens (*7*). Such marked immunogenicity of a polymerase product has not been demonstrated for other mammalian retroviruses, including HTLV-I, in their respective hosts. The significance of these responses is unknown. Some animals infected with retroviruses generate antibody that binds to RT without neutralizing it (*8*).

In one approach to this problem we obtained serum samples from individuals with evidence of exposure to HIV as determined by immunoblotting for antigens in lysates of purified HIV. Immunoglobulin G (IgG) was isolated from each serum by ammonium sulfate fractionation and DEAE-cellulose column chromatography (*9*) or Zeta-Chrom 60 filter separation (AMF Lab Products). F(ab')$_2$ fragments of selected IgGs were prepared by digestion of the IgG with pepsin followed by chromatographic purification (*10*). HIV virions were obtained from culture supernatants of chronically infected H9 CD4$^+$ lymphoblasts (*11*). Virus recovered from clarified supernatants precipitated with polyethylene glycol 4000 were solubilized in 0.8*M* NaCl, 0.5% Triton X-100, 0.5 m*M* phenylmethylsulfonyl fluoride (PMSF), 50 m*M* tris (*p*H 7.9), 1 m*M* dithiothreitol, and 20% glycerol. Neutralization of RT activity was performed by incubating IgG or IgG fragment with disrupted HIV for 4 hours at 4°C. Enzyme assays were based on the method of Goodman and Spiegelman (*12*), as modified for the cation and template preferences of HIV (*13*). None of the IgG samples tested had measurable RT activity in the absence of added HIV polymerase.

As shown in Fig. 1, incorporation of [^{3}H]deoxythymidine monophosphate (dTMP) into DNA by the viral polymerase was blocked by IgG obtained from certain HIV-seropositive individuals. Using a screening dose of 10 μg of whole IgG per reaction mixture, we found that 11 of 16 asymptomatic viral carriers inhibited RT activity by ≥50%. In contrast, 0 of the 8 samples from seronegative controls and 2 of 12 samples from patients with AIDS or AIDS-related complex (ARC) were suppressive at this concentration. The seronegative controls included six male laboratory workers, one female with HTLV-I–associated CD4$^+$ lymphoma, and one female who gave a "false positive" reaction for antibody to disrupted HIV by enzyme-linked immunosorbent assay (ELISA). The erroneous reaction in this pregnant woman was confirmed by negative ELISA competition for viral antigen. The seropositive groups were all homosexual males, age-matched to the control group. The symptomatic group included six AIDS patients (four with *Pneu-*

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