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Bidirectional transcription from the human immunoglobulin V_H6 gene promoter

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ABSTRACT

The human immunoblobulin (Ig) heavy chain V_H6 gene promoter contains an imperfect octamer (AgGCAAAT) and is not dependent on the Ig heavy chain enhancer for activity; reporter constructs containing this promoter are very active in non-B cells. In experiments designed to characterize regions upstream of the transcriptional start site that are important for promoter function, we produced a series of deletion constructs, including one containing sequences between −74 and −146. Surprisingly, this fragment had promoter activity in both orientations. Inspection of the V_H6 promoter sequence indicated that there was a possible TATA box in the proper orientation upstream of the imperfect octamer. The −74 to −146 fragment functioned as a promoter in the reverse orientation in three B cell lines and in non-B (HeLa) cells, with a much higher level of activity seen in the HeLa cells. To determine if the promoter could work in both directions simultaneously, reporter genes were positioned up- and downstream of a V_H6 promoter fragment. Reporter gene activity was found for both genes in B cells and HeLa cells. Using a reverse transcriptase-polymerase chain reaction procedure (RT-PCR), we found a transcript corresponding to sequences upstream of the V_H6 promoter in RNA from both the lymphoblastoid cell line ML-1, which actively transcribes the V_H6 promoter, and the REH cell line, which does not. No transcripts were found in the KB epithelial cell line. Two or three mRNA 5′ ends were found that mapped between −137 to −143 from the authentic V_H6 transcription site, 31–37 nucleotides upsteam of the putative TATA box. Inspection of the sequence upstream of the V_H6 promoter demonstrated the presence of an open reading frame capable of coding for 96 amino acids. The V_H6 promoter represents the second Ig promoter with bidirectional activity.

INTRODUCTION

The human V_H6 gene is one of 120 to 200 germline variable (V_H) region genes (1, 2 ,3) that are subdivided into 7 families based on homology. The V_H6 gene is the sole member of its family. The process of heavy chain gene rearrangement involves the orderly opening of the chromatin on chromosome 14, with the first known event being transcription of the region around the heavy chain enhancer located between the 3′ end of the joining region (J_H) and the mu switch region (4). Transcription then begins upstream of one of the diversity regions (D_H), followed by rearrangement of the D_H segment to one of the J_H genes. Prior to a rearrangement that juxtaposes a V_H gene with DJ_H, transcription begins upstream of the V_H gene to be rearranged (5–7). Transcription can therefore occur from V_H promoters in immature pre-B cells before rearrangement (4, 8, 9), but in mature B-cells, only the rearranged V_H genes are transcribed (10). The elements within the promoter region of V_H genes responsible for transcriptional activity have been extensively studied (11–13). One element present in virtually all Ig heavy chain gene promoters is the octamer sequence ATGCAAAT; the inverse sequence is found in most human and mouse variable light chain gene promoters. The octamer is considered to be the target of a B-cell specific transactivator protein (14–16), and it is both necessary and sufficient for the activity of immunoglobulin promoters in B cells (12, 13).

The V_H6 gene is the V_H region most proximal to the D_H region (17). The V_H6 promoter contains an imperfect octamer (AgGCAAAT), making it one of 17 Ig heavy and light chain promoters that contain an altered octamer sequence (18). The V_H6 promoter does not contain sequences resembling the heptamer recognition sequence for the octamer binding protein—a common feature of other Ig promoters (19). These findings, combined with the possibility that V_H6 is the first variable region promoter activated in ontogeny, strongly suggest that requirements for optimal activity of this promoter may differ from those of other variable region promoters. During the course of our studies on the V_H6 promoter, we found that when a DNA fragment located between 74 and 146 bp upstream of the transcriptional start site was reversed, the signals for transcription were still present and the activity of the CAT reporter was found in both B- and non-B cells. Examination of the DNA sequence of the V_H6 promoter indicated that there were two divergently oriented TATA-like sequences, one on either side of the imperfect octamer element, with the upstream TATA sequence oriented in the opposite direction. Such a symmetrical organization raises the possibility of bidirectional transcription in this region.

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In the studies described here, we found that the upstream sequence can function as a promoter, and that the V_H6 promoter can apparently direct simultaneous transcription in both directions. Using RT-PCR, we also demonstrated that an RNA from the upstream transcription unit can be found in two B-cell lines. Examination of the upstream region of the V_H6 promoter showed that one reading frame is open for 288 bp, indicating the potential for a protein coding transcript from this region.

MATERIALS AND METHODS

Cell lines

Three B cell lines were used in this study. Nalm-1, a human acute lymphoblastic leukemia (ALL) pre-B cell line that expresses CD10, CD20, CD24 and cytoplasmic Ig (cIg), and contains a functionally rearranged V_H4 gene family member (20). ML-1 is an Epstein–Barr virus (EBV)-transformed human early-B cell line that expresses surface Ig (sIg), with the heavy chain being from the V_H6 gene family (21). BJA-B cells are derived from a human African Burkitt's lymphoma, but is EBV negative and expresses sIgM (22). For studies in non-B cell lines, we used HeLa cells, a human cervical carcinoma cell line. Cell culture conditions were as described in the accompanying paper (Sun and Kitchingman).

Construction of plasmids

Two DNA fragments, V_H6-R74 (from −74 to −146) and V_H6-76 (from −8 to −82) , were generated by PCR using the M2 subclone of the V_H6 promoter as the substrate (Sun and Kitchingman, accompanying paper), then cloned into a T-vector derived from pBluescript KS (+) (Stratagene) as described by Marchuk *et al.* (23). These fragments were transferred into the pCAT-Basic plasmid (Promega) following digestion with *Hin*dIII or *Hin*dIII and *Pst*I, respectively (Fig. 1A). For studies of possible bidirectional V_H6 promoter activity, reporter plasmids containing both the β-galactosidase and CAT reporter genes were prepared. Briefly, the *Bam*HI, *Sal*I and *Pst*I sites within the pSV-β-galactosidase plasmid (Promega) were destroyed by restriction digestion and blunt ending followed by ligation and cloning. Both pSV-β-galactosidase and pCAT plasmids were cleaved completely by *Hin*dIII and partially by *Eco*RI, and the 1.8 kb *Hin*dIII/*Eco*RI fragment from pCAT-Basic and the 6.6 kb fragment from pSV-β-galactosidase were isolated by agarose gel electrophoresis and electroelution. The fragments were then ligated with T4 DNA ligase and cloned. A DNA fragment from positions −16 to −139 of the V_H6 promoter was synthesized by PCR using primers containing *Hin*dIII ends, digested with *Hin*dIII, and cloned between the CAT and β-gal reporter genes in both orientations (Fig.2).

DNA transfections

Transfection of plasmid DNA was performed by electroporation essentially as described by Doffinger *et al.* (24). All transfections used 20 μg of test plasmids and 10 μg of a reference plasmid expressing β-galactosidase (Promega) or human growth hormone (Nichols Institute Diagnostics), as an internal control. Briefly, cells for electroporation were spun down and resuspended in fresh medium 24 h prior to electroporation. Immediately prior to electroporation, cells were collected by centrifugation, and resuspended at a concentration of 1×10^7 cells per 250 μl per cuvette. Electroporation was performed using the cell porator (BRL), with the voltage and capacitance set at 250 V and 800 μFarad. Twenty-four hours after electroporation, cells were collected by centrifugation and washed twice with phosphate-buffered saline, and lysates were prepared by freezing-thawing in lysate buffer (250 mM Tris−HCl pH 7.8, 0.1% Triton X-100). Cell lysates were centrifuged for 10 min at 14 000 rpm at 4°C, and the supernatant was used for either β-galactosidase or CAT assays.

Detection of CAT and β-galactosidase activities

The protein concentrations of cell lysates were measured, and 50−100 μg of protein from each sample were used for either CAT or β-galactosidase assays. CAT activity assays were carried out as described by Gorman (25). To detect β-galactosidase activity, the Promega detection kit was used according to the manufacturer's instructions. Briefly, cell extracts containing 50−100 μg of protein from each sample were first added to flat bottom well plates (Costar Inc.) and adjusted to 100 μl with deionized H_2O. Then, 100 μl of 2× β-galactosidase assay buffer (200 mM sodium phosphate, pH 7.3, 2 mM $MgCl_2$, 100 mM β-mercaptoethanol and 1.33 mg/ml ONPG) were added and the samples incubated at 37°C. The plates were read at 1 h, 6 h and 12 h intervals at an OD of 415 nm on an automatic plate reader. The CAT conversion percentage was adjusted based on the measured β-galactosidase activity when the latter was used as the internal standard. For transfection experiments involving the vector capable of expressing both the CAT and β-gal genes, human growth hormone activity in the culture supernatant was measured using the human growth hormone detection kit from Nichols Institute Diagnostics. The results presented represent the mean activity of two to four independent experiments.

RNA analysis

Total RNA was prepared from ML-1, REH and KB cell lines as described by Chomczynski and Sacchi (26). Poly(A)$^+$ and poly(A)$^-$ fractions were prepared by standard oligo(dT)−Sepharose chromatography (27). For the Northern blot experiment, 50 μg of total RNA or 5 μg of poly(A)$^+$ RNA were electrophoresed on a 1% agarose/6.6% formaldehyde gel, transferred to nylon membranes by capillary blotting in 20×SSC as described (28), and hybridized with the upstream DNA probe.

RT PCR assay

Published methods (29, 30) were used to detect the upstream transcript. Briefly, total RNA was first treated with RNase-free DNase I. After removal of the DNase I by extracting twice with phenol, cDNA was synthesized with 9 U of AMV reverse transcriptase using 0.1 μM of the reverse primer, M2T7.2, in a total volume of 20 μl. Then 10 μl of the cDNA were added to a standard PCR mix containing 1 μM of each primer, M2T7.2 and M2J5.3. PCR was performed on a thermal cycler (MJ Research Inc.); the first cycle was 1.5 min at 94°C, 1 min at 56°C and 1 min at 72°C, and the following 35 cycles were 1 min at 94°C, 1 min at 56°C and 30 s at 72°C. The final polymerization step was extended an additional 10 min at 72°C. One tenth of each reaction was electrophoresed, blotted, and probed with the upstream DNA fragment (27).

RNase protection assay

The V_H6 upstream fragment from −67 to −416 bp was cloned into the pGEM-T-vector (Promega) following PCR amplification.

The complementary sense RNA (cRNA) probe was synthesized with SP6 RNA polymerase using linearized plasmid. Ten μg of poly(A)$^+$ RNA or 50 μg of total RNA from the ML-1 cell line were hybridized overnight at 45°C with a [^{32}P]-UTP labeled cRNA probe in 40 μl of 80% formamide−40 mM PIPES, pH 6.5, 400 mM NaCl, and 1 mM EDTA. After hybridization, unhybridized cRNA probe was removed by digestion for 5 to 10 min at room temperature with 25 μg/ml RNase A in 350 μl of 10 mM Tris−HCl, pH 7.5, 300 mM NaCl, 5 mM EDTA, with 5 μg of carrier RNA added (31). Protected fragments were ethanol precipitated and analyzed on a 6% polyacrylamide/7 M urea sequencing gel. Dideoxy sequencing reactions from the cloned 350 bp upstream fragment of the V_H6 promoter were used for size markers. Correction was made for the difference in mobility of single-stranded RNA and DNA fragments under these conditions (27).

RESULTS

To determine if the imperfect octamer and upstream elements in the V_H6 promoter could function in the absence of downstream elements including the TATA box, we constructed a 3′ deletion that contained sequences from −74 to −146. This construct retained 30−60% of the activity of the 146 bp fragment in the four cell lines in which it was tested (Sun and Kitchingman, accompanying paper). This fragment actually functioned better when the orientation was reversed. We examined the upstream sequence for elements that might promote transcription and found a TATA-like sequence on the opposite strand between −101 and −105 (Fig. 1). To determine if this sequence functions to promote transcription, we compared the promoter activity of the −74 to −146 fragment reversed, V_H6-R74/0-CAT, to a minimal V_H6 promoter containing sequences from −8 to −82 (V_H6-76/

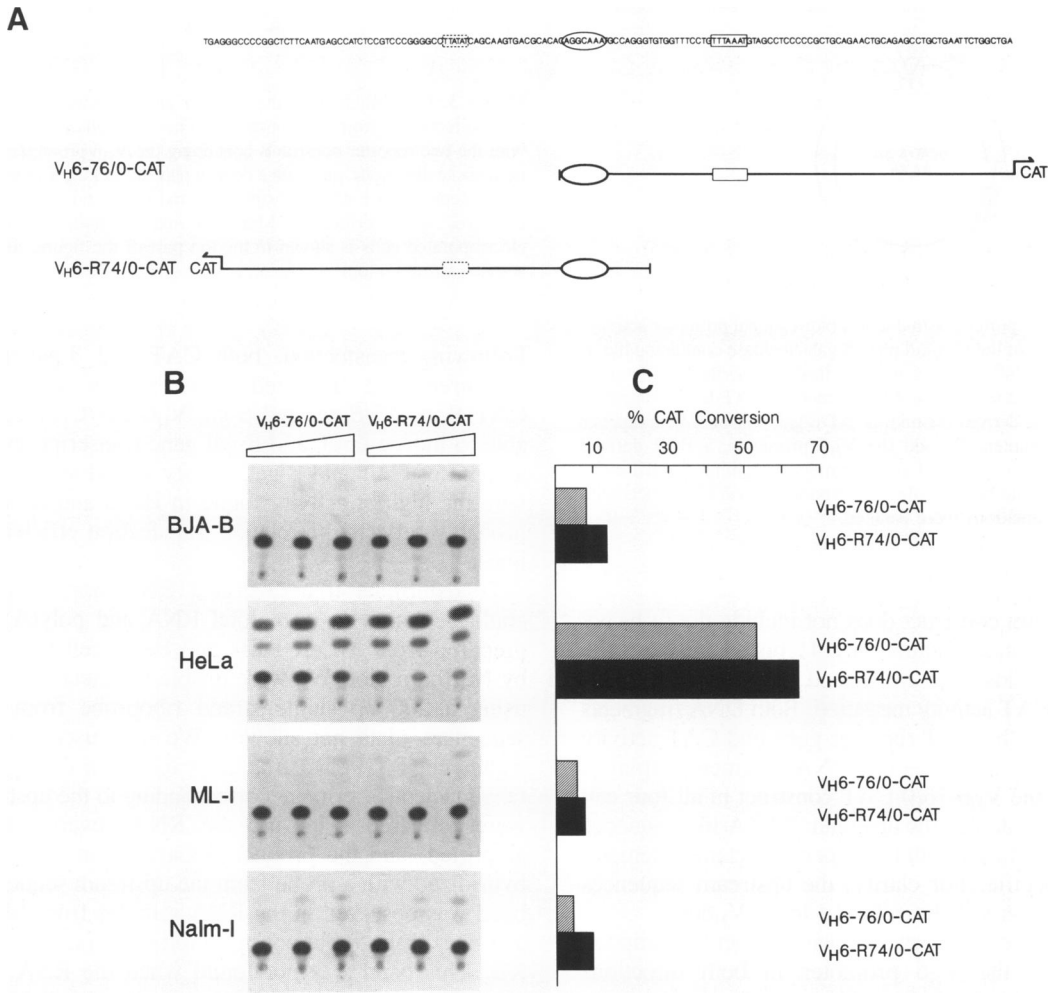

Figure 1. Structure of V_H6 promoter constructs and CAT activity in four cell lines. (**A**) The nucleotide sequence of the V_H6 promoter between −1 and −148 is shown. The locations of the downstream TATA box (−55 to −49), the imperfect octamer (−82 to −75), and the upstream TATA box (−101 to −105) are denoted by the solid box, the oval, and the dashed box, respectively. The structures of the constructs used to determine the promoter activity of the up- and downstream sequences are shown below. The V_H6-76/0-CAT construct contains sequences between −8 to −82 in front of the CAT reporter gene, while the V_H6-R74/0-CAT construct contains sequences from −74 to −146 in the reverse orientation. The direction of transcription relative to the CAT reporter genes is indicated by the arrows. (**B**) Autoradiographs of the CAT assay. CAT reporter plasmids were introduced into the four cell lines as described in Materials and Methods, and cell extracts prepared. CAT activity was assayed using the thin-layer chromatography technique described by Gorman (24). Increasing amounts of CAT plasmid were added to separate cultures. (**C**) Acetylated chloramphenicol was quantified by imaging the autoradiographs shown in part B. The level of activity represents the amount of input chloramphenicol converted to the acetylated forms.

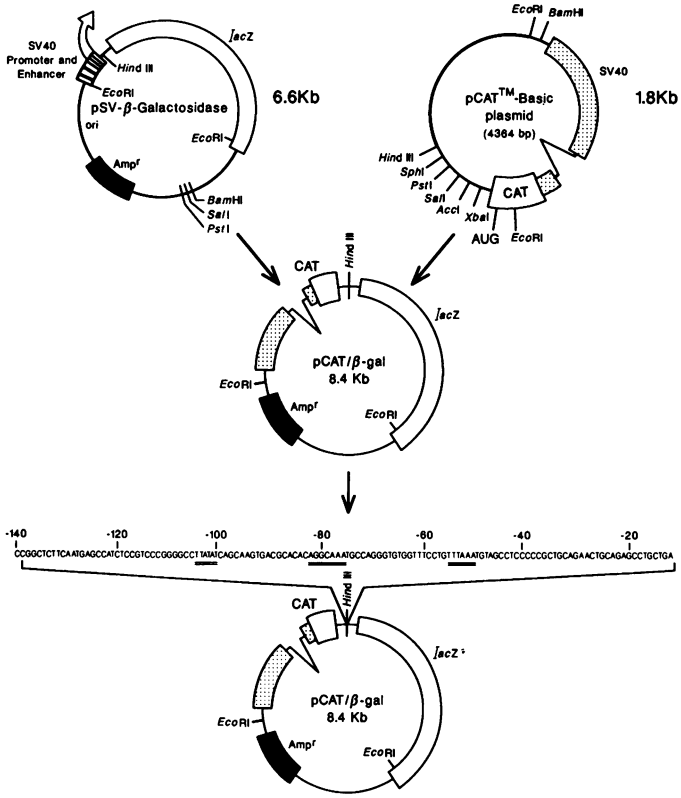

Figure 2. Construction of reporter plasmid to test bidirectional promoter activity. A *Hin*dIII/*Eco*RI fragment of the plasmid pSV-β-galactosidase containing the β-gal sequence without the SV40 promoter was obtained as described in Materials and Methods, and ligated to a *Hin*dIII/*Eco*RI fragment of pCAT-Basic containing the CAT reporter gene. The derived plasmid, pCAT/β-gal, contained both reporter constructs without a promoter. To add the V_H6 promoter, a PCR derived fragment of the promoter was prepared using primers containing *Hin*dIII ends, and ligated to the *Hin*dIII site between the two reporter genes. Constructs with the promoter in both orientations were isolated.

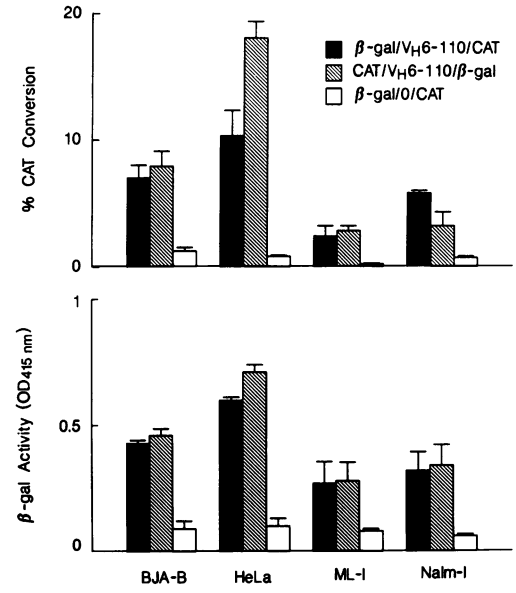

Figure 3. Determination of the reporter gene activity in cells transfected with the bidirectional promoter constructs. The four cells lines were electroporated with the two reporter constructs containing the V_H6 promoter fragment oriented in opposite directions, and with a control plasmid lacking a promoter. Cell extracts were prepared 24 h after transfection , and CAT and β-galactosidase activities measured as described in Materials and Methods. CAT activity of the electroporated cells is shown in the top part of the figure, and β-galactosidase activity at the bottom.

0-CAT). The upstream construct does not include the Ig heavy chain gene enhancer and contains only 41 bp upstream of the TATA box. The plasmids were transfected into three B-cell lines and HeLa cells and CAT activity measured. Both DNA fragments led to transcription of the CAT reporter gene and CAT activity (Fig. 1B and C). The −74 to −146 DNA fragment displayed higher activity than the V_H6-76/0-CAT construct in all four cell lines. The results indicate that the upstream TATA-like sequence is functional when combined with the imperfect octamer element, and is not tissue specific. For clarity, the upstream sequences promoting transcription will be referred to as V_H6up.

A question that remained unanswered was whether transcription could be driven by the V_H6 promoter in both directions simultaneously. To address this question, we designed a two-reporter vector (Fig. 2). One reporter was positioned such that its transcriptional orientation was in the natural position downstream of the imperfect octamer, while the second reporter was put in the opposite orientation upstream. A 122 bp fragment of the V_H6 promoter, containing sequences from −16 to −139 (to include all the elements necessary for full promoter activity), was cloned between these two reporter genes in both orientations to avoid bias toward a particular promoter/reporter combination.

Following transfection, both CAT and β-gal activities were measured and analyzed. The results showed that both CAT/V_H6-127/β-gal and β-gal/V_H6-127/CAT constructs were able to induce CAT and β-gal gene transcription (Fig. 3). The amount of CAT and β-gal activity varied among the cell lines, with the highest activity found in HeLa and BJA-B cells. This probably reflects the relative transfection efficiency of the cell lines.

To determine if a divergent RNA transcript from V_H6up could be found *in vivo*, total RNA and poly(A)$^+$ RNA were prepared from ML-1, REH and HeLa cell lines and analyzed by Northern blot. We were unable to detect any specific signal using a 200 bp single strand riboprobe from the upstream sequences (data not shown). We then used a more sensitive technique, RT-PCR, to attempt to find a divergent transcript. Oligonucleotide primers corresponding to the upstream sequence were prepared (Fig. 4), the RNA reverse-transcribed and amplified, and the products separated on an agarose gel and hybridized with a probe from the upstream sequence. A 200 bp band was observed in the RNA samples from ML-1 and REH cell lines (Fig. 5) but not in samples from the epithelial cell line KB. This band was not found when the RNA samples were amplified without prior reverse transcription (RT$^-$; Fig. 5). We also examined whether this transcript could be found in HeLa cells, since promoter constructs are quite active for bidirectional transcription in these cells. RT-PCR analysis failed to show the presence of transcripts, even when up to 5 μg of poly(A)$^+$ RNA were used (data not shown).

To map the transcriptional start site of this divergent transcript, an RNase protection assay was used. RNA from ML-1 cells protected a series of fragments that clustered around 130 bp in

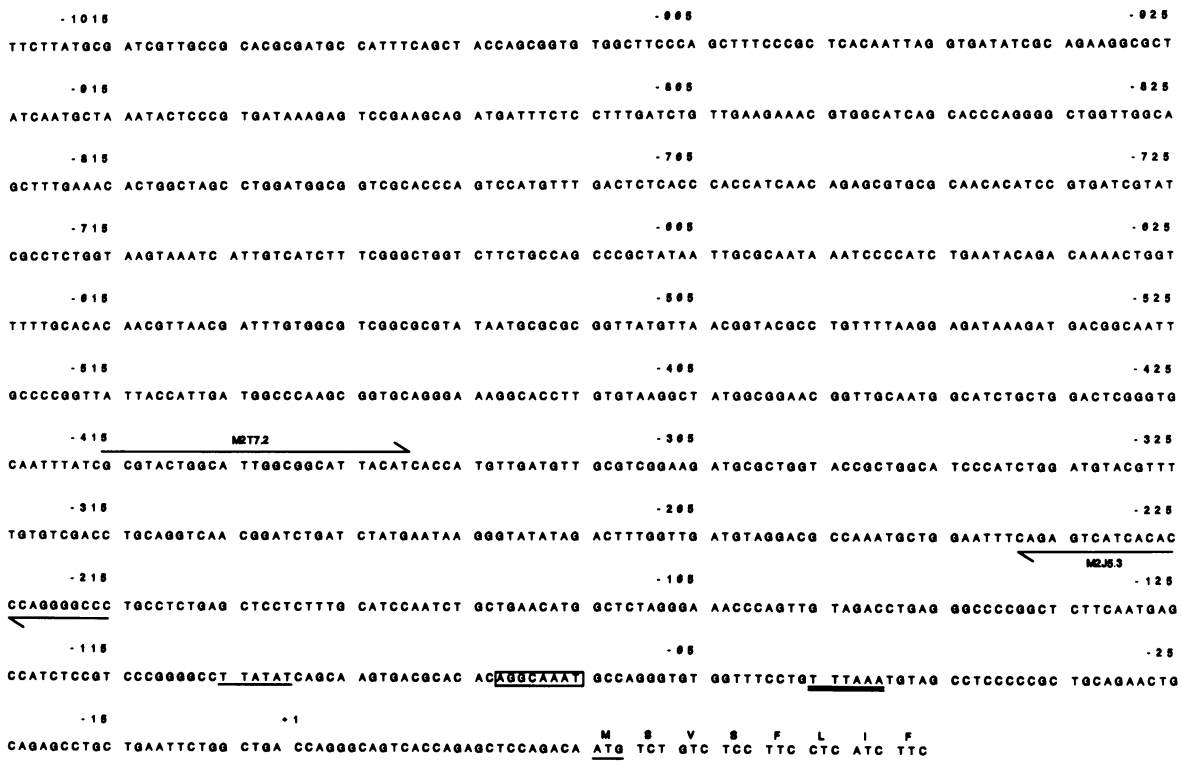

Figure 4. Nucleotide sequence of the V_H6 promoter. The nucleotide sequence of 1024 bp upstream of the transcription start site is shown. The imperfect octamer sequence is boxed, while the upstream and downstream TATA boxes are underlined, and double underlined, respectively. The locations of the two primers (M2T7.2 and M2J5.3) used to generate the DNA fragment used to prepare a riboprobe are indicated by arrows.

length relative to the DNA standard (Fig. 6). This positioned the 5′ ends of the divergent transcript between 31 and 37 bp upstream of the divergent TATA-like sequence at around bp −137 to −143. No protected fragments were found when either yeast RNA or RNA from KB cells was used (data not shown).

The divergent transcript was detected in both total and poly(A)$^+$ RNA preparations from ML-1 cells. Hence, this divergent transcript is probably polyadenylated and may encode a protein. When the sequence upstream of the divergent octamer was translated in all three reading frames, we found that an ATG codon at position −176 was followed by an open reading frame of 288 bp, potentially coding for 96 amino acids (Fig. 7).

DISCUSSION

The experiments described in this paper demonstrate that the Ig heavy chain V_H6 promoter can function bidirectionally *in vivo*, and that the divergent transcript produced from $V_H6$up has the characteristics expected of a protein-coding mRNA. This is the first human Ig promoter shown to have such characteristics, and the second Ig promoter shown capable of initiating transcription bidirectionally. The bidirectional promoter activity, combined with the absence of tissue specificity and the lack of a requirement for the Ig heavy chain J-Cμ intron enhancer (Sun and Kitchingman, accompanying paper), make the V_H6 promoter unique among all Ig promoters studied thus far. Most Ig promoters analyzed to date have not been examined for their

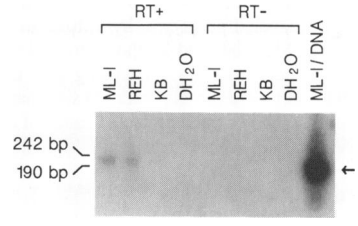

Figure 5. RT-PCR demonstration of a transcript from the upstream sequences. First strand cDNA was prepared from polyA$^+$ RNA isolated from the ML-1 and REH B cell lines, and from the KB epithelial cell line. First strand cDNA was prepared using the M2T7.2 primer as described in Materials and Methods, followed by PCR amplification using the M2T7.2 and M2J5.3 primers. The PCR product was run on an agarose gel, blotted to a nylon membrane, and probed with sequences lying between the two primers. As a control, ML-1 DNA was amplified by PCR using the same primers. RT$^+$ reactions were reverse transcribed first; no reverse transcription step was performed in the RT$^-$ samples. The arrow on the right side of the figure indicates the size of the expected product. The numbers on the left side of the figure indicate the position of two DNA fragments of known size.

ability to promote bidirectional transcription, so we do not know how unique the V_H6 promoter is in this respect.

The mouse Ig heavy chain gene V_H441 is the other immunoglobulin promoter that has been demonstrated to have bidirectional activity (32). This promoter also has two TATA-like sequences, one upstream and one downstream of a perfect

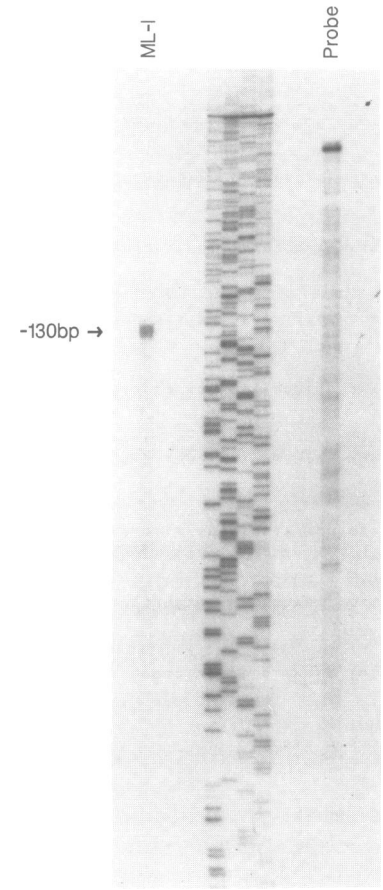

Figure 6. RNase protection assay to locate the 5′ ends of the upstream transcript. A plasmid clone containing sequences between the M2T7.2 and M2J5.3 primers was isolated, linearized, and transcribed with SP6 RNA polymerase in the presence of [α-^{32}P]-UTP. The radiolabeled probe was hybridized to polyA$^+$ RNA from ML-1 cells as described in Materials and Methods, treated with RNase, and the protected fragments separated on a 6% sequencing gel. Dideoxy sequencing reactions of the plasmid insert were run in parallel as size markers. The protected fragments run as a cluster of two or three protected bands of about 130 bp. Probe not subjected to hybridization or RNase treatment is shown on the far right side of the figure.

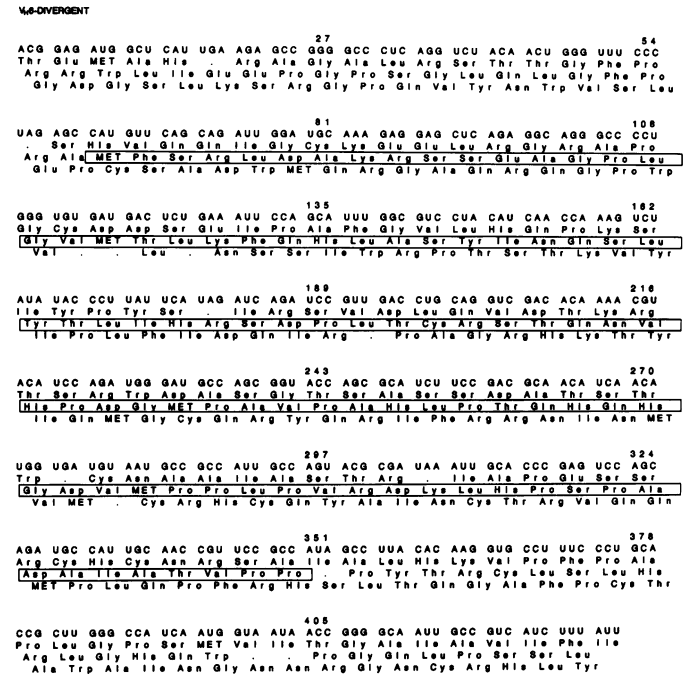

Figure 7. Translation of the DNA sequence upstream of the V_H6 promoter in all three reading frames. The nucleotide sequence of the upstream sequence from −115 to −544 is shown reversed and complemented, and converted into an RNA sequence. The protein coding potential for each of the three reading frames is shown below the nucleotide sequence. The 96 amino acid peptide potentially encoded in open reading frame 2 is boxed, starting with an initiator methionine. The methionine is located at position −176 in the DNA sequence shown in Figure 4, which is 34 to 40 nucleotides away from the transcriptional start sites.

octamer element (32). Like the V_H6 promoter, the V_H441 promoter can direct transcription in both directions in reporter constructs and transcripts from upstream of the promoter can be found in cells expressing the V_H441 Ig heavy chain transcript (32). The upstream transcript from this promoter is not polyadenylated and does not contain an open reading frame of significant size, indicating that it does not encode a protein. Its function is unknown.

For both the V_H6 and the V_H441 promoters, the question remains as to whether both the upstream and downstream transcripts are produced in the same cells. The results presented here (Fig. 3) suggest that the V_H6 promoter can direct transcription in both directions in the same construct, but this finding should be interpreted with caution. It is possible that each plasmid can be transcribed either upstream or downstream, but not both. The same could be true *in vivo*, with some cells producing the Ig heavy chain gene transcript while others produce the V_H6up transcript. Further work at the single cell level is necessary to confirm that the promoter functions simultaneously in both directions. Currently, we can state with certainty only that the presence of an upstream sequence capable of being expressed does not inhibit expression of the downstream transcript.

A number of promoters from both prokaryotes and eukaryotes have been shown to function bidirectionally. These can be placed into three general categories with respect to the divergent transcript. The first category is where the bidirectional promoter produces an upstream transcript that is apparently non-coding. In prokaryotic cells, the ompC/micRNA (33) is one example, while in eukaryotes the upstream sequence of the rat insulin gene (34) and the Ig V_H441 transcript are examples of this type of transcriptional unit. The only one of these for which a function is known is the ompC/micRNA transcription unit, where the micRNA regulates expression of the ompC gene by binding to the mRNA, thereby inhibiting translation, which feeds back to inhibit transcription (33). The second general category of bidirectional promoter is where both the upstream and regular transcripts are polyadenylated and potentially code for proteins, but there is no known interaction between the two gene products. Examples of this type of transcription unit include the dhfr/Rep-1 genes from mice (35) and perhaps the upstream partner of the chicken skeletal α-actin gene (36). The third type of bidirectional transcription unit is one where protein products are produced from both genes, and one protein either interacts with or regulates expression of the other. For example, the araC/araBAD (37, 38) locus is transcribed bidirectionally, and the araC protein regulates

transcription of its own mRNA and that for the araBAD gene products (37). The same is true for several phage genes (39), for the yeast mating type genes (40), and for Gal1−Gal10 (41, 42) locus. In higher eukaryotes, bidirectional transcription units coding for interacting proteins include the Surf-1 and Surf-2 locus of mice (43) and the mouse α1(IV)/α2(IV) collagen genes (44, 45). The divergent transcription units code for proteins of similar sequence to the downstream transcripts, and in the case of the collagen genes, interacting proteins. Where the upstream transcript from the V_H6 gene lies in this spectrum of potential roles for the alternative product from bidirectional promoters awaits cloning and characterization of the cDNA for V_H6up.

The steady-state level of the V_H6up transcript is very low, as indicated by our inability to detect it by Northern blots and the very weak signal following RT-PCR. It accumulates at levels several hundred times lower than that of the V_H6-D-J-Cμ transcript in ML-1 cells (Z.Sun and G.R.Kitchingman, unpublished data), but we do not know whether this disparity is due to transcriptional or post-transcriptional events. This disparity is not recapitulated in the expression constructs, where the levels of activity of the two reporter genes are quite similar. This observation could reflect equal transcription of the V_H6 and V_H6up genes in ML-1 cells, with rapid turnover of the V_H6up transcript leading to the large difference in steady-state levels, or it may be that the bidirectional promoter functions differently in the context of the genome and the plasmid, producing artificially high levels of reporter gene activity. The latter case has been shown for the 3-hydroxy-3-methylglutaryl coenzyme A reductase gene promoter from mice (46), which produces bidirectional transcripts in plasmid constructs but only downstream sequences are transcribed from the endogenous gene. However, it is possible that low levels of transcripts would be missed under the conditions used, and nuclear run-ons were not performed to demonstrate the lack of divergent promoter activity (46). Further work is clearly needed to discriminate between these two, and perhaps other, possible reasons for the difference in the steady-state levels of the two RNAs transcribed from the V_H6 promoter.

Several features about the V_H6 promoter make it worth pursuing the identity and function of the upstream transcript. It is probably the first heavy chain variable region promoter utilized during ontogeny; thus, opening of the chromatin in this area upstream of the diversity region may have special requirements which could include the product of the V_H6up transcript. The requirement could be for a sterile transcript, as is found when chromatin first opens around the enhancer region (4), and around the constant regions before class switch occurs (6), or as a protein that may facillitate the process. Another possibility is that bidirectional transcription may bring about negative supercoiling (unwinding) of double-stranded DNA between the two divergent transcription complexes (47), as speculated on by Nguyen *et al.* for the V_H441 promoter (32). Negative supercoiling has also been implicated in recombination (48, 49), and could thus facilitate recombination of the V_H6 gene to a DJ_H. Also, there is approximately 50 kb between the V_H6 gene and the next closest upstream V_H gene, the largest spacing found for any of the 64 V_H genes mapped thus far (3). Perhaps this large distance reflects the presence of a large gene, potentially the V_H6up transcript, that is required for recombination. Further investigation of bidirectional transcription from the V_H6 promoter should provide insight into a novel mechanism for controlling gene expression.

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