Transcription of the blk Gene in Human B Lymphocytes Is Controlled by Two Promoters*

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Genomic DNA containing the first exon and 5′-flanking region of the human protein tyrosine kinase, blk, was isolated. Sequence analysis identified a TG repeat element in this region with enhancer activity, but no TATA or CCAAT sequences were found. Two blk transcripts of 2.2 and 2.5 kilobases were identified in various B-cell lines by Northern blot analyses, and primer extension experiments demonstrated two clusters of multiple transcription start sites. Subsequent promoter analyses by transient transfection assays with a reporter gene identified two promoter elements in the human blk gene. Promoter P1 contains sequences that have been shown to regulate the expression of immunoglobulin genes and promoter P2 contains elements that are highly conserved in the promoter of major histocompatibility complex class II genes, as well as a B-cell-specific activator protein- (BSAP) binding site. Electrophoretic mobility shift assays demonstrated that the binding of a protein to the BSAP-binding site was correlated with the presence of the 2.5-kilobase blk transcript. These data suggest that the two human blk RNAs arise from the transcription of the blk gene by two distinct promoters and that these promoters may be subject to regulation by different trans-acting factors.

The blk gene is a member of the src family of protein tyrosine kinases (1, 2). The product of the blk gene, as well as other src family members including fyn, lyn, and lck, has been shown to associate with the immunoglobulin receptor complex (3, 4). Since signal transduction via the B-cell antigen receptor is mediated by protein tyrosine phosphorylation (5, 6), blk may play a role in B-cell activation and the initial steps of the intracellular signal pathway.

Little is known about the regulatory mechanisms controlling the restricted expression of blk. In the mouse, blk expression is restricted to B-lymphoid cells and is developmentally regulated (7). blk transcripts are first detected in pro-B-cells and persist through differentiation to mature-B-cells, but are absent in plasma cells. This expression pattern is similar to that of two other B-cell-specific genes, mb-1 and CD19 (8, 9). The control of CD19 gene expression has been shown to involve a B-cell-specific activating protein (BSAP) (10). BSAP is a member of the paired domain family of transcription factors and is encoded by the paired box gene Pax-5 (11, 12). Recently, a BSAP binding site was identified in the murine blk promoter region (13). The correlation of the expression of BSAP and blk suggested that BSAP may, at least partially, account for the B-cell-specific expression of murine blk.

In contrast to the murine blk gene, the human blk gene, although predominantly expressed in B-cells, is also found in some T-cells (14, and this study). In order to understand the mechanisms regulating human blk expression, we have isolated and characterized the first exon and the 5′-flanking region of the human blk gene. Northern blot analyses identified the presence of two blk RNAs in various B-cell lines, and the transcription start sites of these RNAs were mapped to two clusters. Electrophoretic mobility shift assays demonstrated that expression of one of the blk RNAs was correlated with the presence of BSAP. Luciferase reporter gene assays and deletion analyses identified two promoter elements in the 5′-flanking region of the blk gene. An enhancer-like element, containing a BSAP binding site, was identified in the human blk promoter region.

Materials and Methods

Cell Lines and Cell Culture—The following cell lines were obtained from the American Type Culture Collection: Reh (acute B-cell lymphocytic leukemia), ARH-77 (plasma cell leukemia), Raji (Burkitt's lymphoma), RPMI 6666 (B lymphoblastoid cell line), RPMI 7666 (B lymphoblastoid cell line), UC 729 (B lymphoblastoid cell line), CEM (T-cell acute lymphoblastic leukemia), U-937 (histiocytic lymphoma, macrophage/monocyte like), LNCaP (prostate adenocarcinoma), DU 145 (prostate carcinoma), T-47D (ductal carcinoma of the breast), and 293 (transformed primary embryonal kidney cells). SUP-B8 (Burkitt's lymphoma), SUP-B12 (Burkitt's lymphoma), SUP-B17 (Burkitt's lymphoma), SU-DHL4 (diffuse histiocytic lymphoma), Daudi (Burkitt's lymphoma), Nalm6 (precursor-B acute lymphoblastic leukemia), OCI-Ly8 (B cell immunoblastic lymphoma), and Jurkat (acute T cell leukemia) cell lines were kindly provided by Dr. Ron Levy, Stanford University.

All B- and T-lymphoid cell lines were grown in RPMI 1640 medium (Hydine Laboratories, Inc., Logan, UT) supplemented with 10% fetal bovine serum (Hydine Laboratories, 2 mg/ml L-glutamine, 100 units/ml penicillin, and 100 μg/ml streptomycin. DU 145 and T-47D cells were maintained in minimal essential medium (Life Technologies, Inc.) supplemented with 10% fetal bovine serum, 25 mM sodium bicarbonate, 0.006 μg/ml bovine insulin (Sigma), 100 units/ml penicillin G (Life Technologies, Inc.), and 100 μg/ml streptomycin (Life Technologies, Inc.).

Isolation of the blk Promoter—Genomic DNA clones were isolated from a human lymphocyte genomic library in the bacteriophage Lambda Dash (Stratagene, La Jolla, CA), using a 32P-labeled blk cDNA as the probe. Six positive hybridizing clones were isolated after screening approximately 1 × 106 plaques. Two clones containing blk 5′-flanking sequences were identified by restriction mapping and Southern blot analyses with a 32P-labeled first exon region probe of the blk DNA.

Received for publication, July 6, 1995

*This work was supported by Grant 3065 R1 from the Council for Tobacco Research-USA Inc. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

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‡The abbreviations used are: BSAP, B-cell-specific activator protein; PCR, polymerase chain reaction; CAT, chloramphenicol acetyltransferase; MHC, major histocompatibility complex; kb, kilobase(s); bp, base pair(s); PIPES, 1,4-piperazineethanesulfonic acid.

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THE JOURNAL OF BIOLOGICAL CHEMISTRY Vol. 270, No. 43, Issue of October 27, pp. 25968–25975, 1995

Printed in U.S.A.
Transcriptional Regulation of Human blk

CAT plasmid and electrophoresed using 0.4-cm cuvettes in a Bio-Rad gene pulser (Bio-Rad) at 300 V, 960 microfarads for B-cells and 250 V, 960 microfarads for T-cells. After incubation in RPMI complete medium for 24–36 h, the cells were harvested and lysed in 150 µl of 250 mM Tris (pH 7.7) by three freeze-thaw cycles. The cell extracts were then analyzed for luciferase and CAT activities. DU 145, T-47D, and 293 cell lines were transfected using the calcium phosphate precipitation method as described previously (20).

Luciferase activity was measured from 20 µl of the cell extract reacting with the luciferase reagents as described by the supplier (Analytical Luminescence Laboratory, San Diego, CA). The light emission was measured with a Monolight 2010 instrument (Analytical Luminescence Laboratory), reading relative light for 10 s. Luciferase activities were further analyzed for CAT-specific activity. For CAT assays, cell extracts were heated to 65°C for 15 min to inactivate endogenous acetylases. Subsequently, CAT activity was measured by incubating 50 µl of the cell extract with (32)Pchloramphenicol and butyryl-coenzyme A as described previously (21, 22).

Electrophoretic Mobility Shift Assays—Electrophoretic mobility shift assays were carried out as described previously (23). Two pairs of complementary oligonucleotides were designed with 3’ complementarity as follows: 5’-BSAP, 5’-ATTAAAAAGACAAAGACAGTGGT-GCTG-3’; 3’-BSAP, 5’-ACCAGGGCACGCTTCTTCCAGCCTACTG-3’. 5’-mBSAP, 5’-ATTAAAAAGACAAAGAAAGACAGTGGT-GCTG-3’; 3’-mBSAP, 5’-ACCAGGGCACGCTTCTTCCAGCCTACTG-3’. The BSAP and mBSAP oligonucleotides correspond to nucleotides 404 to 391 of the blk 5’-flanking region and span a putative BSAP-binding site. The 5’-mBSAP and 3’-mBSAP oligonucleotides correspond to the same region but contain two mutations, C to A and G to A, as indicated by lower case letters. Each pair of oligonucleotides was annealed and fill-in labeled with Klenow DNA Polymerase (Pharmacia Biotech Inc.) and [α-32P]dCTP. Ten pmol of each labeled DNA probe was incubated with 10 µg of whole cell extract in 1 x binding buffer (40 mM KCl, 20 mM HEPES (pH 7.7), 0.1 mM MgCl2, 0.4 mM dithiothreitol) containing 4% Ficoll and 1 µg of salmon sperm DNA in a volume of 25 µl at room temperature for 30 min. Protein-DNA complexes were analyzed on a native 4% polyacrylamide gel in 0.25 x Tris borate-EDTA. Gels were dried and autoradiographed.

RESULTS

Cloning and Sequence Analysis of Human blk 5’-Flanking Region—To isolate the genetic elements involved in the regulation of blk gene expression, we initially screened a human lymphocyte genomic library with the full-length blk cDNA (24). Five Phase I clones identified by Southern blot analysis are diagrammed in Fig. 1. Restriction enzyme mapping showed that the human blk gene spans greater than 60 kb of genomic DNA, including other members of the src gene family, the first exon of human blk contains only 5’-untranslated sequence. A 2.3 kb Smal-EcoRI fragment containing the 5’-flanking region and exon 1 of the human blk gene was subcloned and sequenced (Fig. 2). Nucleotide position −386 corresponds to the 5’ end of the cDNA (24). Computer analysis demonstrated an absence of TATA or CAAT motifs located at the optimal distance (25–27) from the −386 site or the +1 major transcription initiation site (later identified in this study). However, several potential motifs known to be involved in the expression of other B-cell-specific genes were found in two clusters separated by approximately 400 bp. One cluster contains E (28), PU.1 (29, 30), X- and Y-like boxes (31), and C-MYB (32) motifs. Another cluster located upstream of the +1 position contains E, PU.1, PEA3 (33, 34), and SP1 (35) motifs. In between, a potential BSAP-binding site (10, 12) that has been shown to be important for the expression of the B-cell-specific gene, CD19, was identified at position −404 to −391. The other notable feature of the nucleotide sequence in the 5’-flanking region of the blk gene was the presence of a 36-bp TG repeat (TG element) at position −2071 to −2106.

Two Sizes of blk Transcripts Differentially Expressed in B-cell Lines—The presence of a cluster of B-cell-specific motifs downstream of position −386 suggests that the expression of blk may have two positive regulatory regions. To test this
hypothesis, we first investigated the expression pattern of the blk gene by Northern blot analysis. An RNA blot containing total RNA isolated from various lymphoid cell lines was hybridized with a labeled blk cDNA probe (Fig. 3, upper panel). The same blot was reprobed with a labeled human glyceraldehyde-3-phosphate dehydrogenase DNA probe as a control (Fig. 3, lower panel). Two major blk transcripts with approximate sizes of 2.2 and 2.5 kb were detected (Fig. 3). The relative abundance of these two RNAs was different in the tested cell lines. As shown in Table I, four patterns of blk expression were observed. The 2.2-kb message was expressed at a higher level than the 2.5-kb RNA in ARH-77, SU-DHL4, Daudi, Raji, SUP-B8, and Reh. Both transcripts were detected with equal intensity in SUP-B17, OCI-Ly8, Nalm 6, and normal spleen. SUP-B12, RPMI 6666, RPMI 7666, and J urkat expressed only the 2.2-kb RNA; the 2.5-kb transcript was not detected. Finally, in two B-lymphoblastoid cell lines (PW and UC 729) and several non-B-cell lines (CEM, U-937, LNCaP, T-47D, and 293), no blk transcripts were detected.

Mapping of the blk Transcription Initiation Sites—To determine whether the 2.2- and 2.5-kb transcripts initiate from the same or different start sites, we carried out primer extension experiments using two different DNA primers. The primers blk pro29 (from 143 to 170) and blk EXT (from 2256 to 2287) were designed for the extension of the 2.2- and 2.5-kb RNA species, respectively. Total RNA isolated from Daudi, RPMI 7666, and normal spleen was used for these experiments. The results showed that the 2.2-kb transcript initiates from a different start site than the 2.5-kb transcript.

Fig. 1. Restriction enzyme map of the human blk gene. All clones were isolated from a human genomic DNA library in the bacteriophage vector lambda-DASHTM. Clones blk26A and blk29A contain the first exon (indicated by the filled box) and the 5′-flanking region. The second exon is located in the blk21A clone and contains the translation start site (indicated by the arrow). Restriction enzymes are indicated as follows: B, BamH1; E, EcoR1; H, HindII; S, SalI; Xb, XbaI; Xh, XhoI.

Fig. 2. Nucleotide sequence of the 5′-flanking region of the human blk gene. The major transcription initiation site of the Daudi, ARH-77, and RPMI 7666 cell lines, determined by primer extension experiments, is shown by the solid triangle and is designated nucleotide +1 of the gene. TG repeats are labeled and underlined. Sequences similar to those of the binding sites for controlling the B-cell specificity of immunoglobulin genes (E box, PU.1, and PEA3) and MHC class II genes (X-box and Y-box) are also labeled and underlined. The boxed sequences are potential BSAP-binding sites. The putative motifs for γ-interferon response elements are doubly underlined. c-myb and cyclic-AMP response elements are also labeled and underlined.
Table 1

| Cell lines            | Northern Analysis | Reverse PCR | BSAP |
|-----------------------|-------------------|-------------|------|
|                       | 2.5 kb  | 2.2 kb | 2.5 kb  | 2.2 kb | |
| **Malignant B cell lines** |         |         |         |         | |
| ARH-77                | +       | +       | +       | +       | + |
| SU-DHL4               | +       | +       | ND<sup>d</sup> | ND | + |
| Daudi                 | +       | +       | +       | +       | + |
| Raji                  | +       | +       | +       | +       | + |
| SUP-B8                | +       | +       | +       | +       | + |
| SUP-B17               | +       | +       | ND | ND | ND |
| OCI-Ly8               | +       | +       | +       | +       | + |
| Nalm 6                | +       | +       | ND | ND | ND |
| SUP-B12               | +       | +       | +       | +       | + |
| RPMI 6666             | +       | +       | +       | +       | + |
| RPMI 7666             | +       | +       | +       | +       | + |
| PW                    | –       | –       | ND | ND | ND |
| UC 729                | –       | –       | ND | ND | ND |
| **Non-B cell lines**  |         |         |         |         | |
| CEM (T cell)          | –       | –       | ND | ND | – |
| Jurkat (T cell)       | –       | –       | ND | ND | – |
| U-937 (myeloid)       | –       | –       | ND | ND | ND |
| LNCaP (prostate)      | –       | –       | ND | ND | ND |
| T-47D (Breast)        | –       | –       | ND | ND | ND |
| 293 (kidney)          | –       | –       | ND | ND | ND |
| **Normal Tissue**     |         |         |         |         | |
| Spleen                | +       | +       | +       | +       | + |
|**a** Relative levels of each transcript are indicated by ++ (strong hybridization signal), + (moderate to weak signal), and – (no signal).

**b** + indicates the presence of an amplification product, +/− indicates a weak amplification product, and − indicates no amplification product.

**c** The presence (+) or absence (−) of BSAP was determined by electrophoretic mobility shift assays.

**d** ND, not determined.

The presence of two *blk* transcripts, with approximate lengths of 2.2 and 2.5 kb, are observed in some cell lines and both transcripts in other cell lines. A major transcript start site was found at position +1 in Daudi, ARH-77, and RPMI 7666 cell lines but was absent in the control cell line CEM. This suggests that the 2.2-kb RNA that is highly expressed in Daudi, ARH-77, and RPMI 7666 cells initiates mainly from this start site.

A pattern of heterogeneous start sites was also found using the *blk*EXT primer; however, we failed to detect extension products in the RPMI 7666 cell line using this primer (Fig. 4B). To confirm the lack of expression of the 2.5-kb *blk* message in RPMI 7666 cells and other cell lines, reverse transcribed-PCR was performed. Total RNAs from these cell lines were reverse transcribed and amplified with an upstream primer, either *blk*pro28 (−173 to −192), for the detection of the 2.5-kb RNA, or *blk*pro26 (−51 to +70), for the detection of the 2.2-kb RNA, and a downstream primer, race 1 (from exon 3). The results demonstrated that SUP-B12 and Jurkat cell-line cDNA can be amplified by *blk*pro26, but not by *blk*pro28, whereas RPMI 6666 and RPMI 7666 cell line cDNA can be amplified by both primers (Table I). This suggests that the 2.5-kb transcript, although undetectable by Northern or primer extension analyses in RPMI 6666 and RPMI 7666 cells, can be detected by the more sensitive reverse transcribed-PCR method. In contrast, the SUP-B12 and Jurkat cell lines were found to lack the 2.5-kb transcript by these assays. The presence of only the 2.2-kb *blk* RNA in some cell lines and both transcripts in other cell lines suggested that the expression of these two RNAs may be controlled by different regulatory elements.

Functional Analysis of the Human *blk* Promoters—To determine whether the 5′-flanking region contains regulatory elements that control the tissue-specific expression of the *blk* gene, transient transfection assays were performed in a variety of cell lines. A Smal-EcoRI (−2258 to +75) and KpnI-EcoRI
(about −3000 to +75) fragment containing 5′-flanking sequences and part of the first exon region of the human blk gene were inserted upstream of the luciferase gene in the plasmid pGL2-basic. The resulting plasmids, pl76 and pl78, were transfected into B-cell lines (Daudi and SUP-B8) and non-B-cell lines (DU 145, T-47D, and J urkat), and luciferase activity was determined. Two plasmids, an SV40 promoter-driven luciferase construct (pGL2-control) and a promoter-less luciferase plasmid (pGL2-basic), were used for controls. In addition, each construct was cotransfected with the RSV-CAT plasmid to normalize for transfection efficiency. As shown in Table I, both pl76 and pl78 constructs gave much higher levels of luciferase activity in B cell lines (Daudi or SUP-B8) than in non-B-cell lines (DU 145 or T-47D). These constructs were also active in the T-cell line, J urkat which we have shown expresses blk (Table I).

To identify the regulatory elements responsible for the tissue-specific expression and the promoter activity of the human blk gene, we have constructed 5′ deletion mutations of the pl76 luciferase reporter gene construct (Fig. 5). The different deletion constructs were transfected into Daudi cells, and luciferase activities were measured. The results show that removal of sequences from −2258 to −1628 (pl82, −1628 to +75) significantly reduced luciferase expression (Fig. 5), indicating the presence of an enhancer element in this region. Further deletion to −338 (pl90, −338 to +75) resulted in an increase in luciferase activity, suggesting the presence of a negative regulatory element upstream of −338 and the presence of an element with promoter activity between −338 and +75 (designated P1). Interestingly, combining the P1 promoter fragment with the enhancer containing fragment (−2258 to −1628), which yields plasmid pl84, dramatically increased activity to nearly the same level as the pl76 construct.

Deletion of the pl76 construct from the 3′ end (pl86, −2258 to −338) resulted in a construct with significant promoter activity, albeit weaker than the P1-containing pl90 construct. To map this element further, it was subdivided into two fragments, one containing the enhancer element (pl88, −2258 to −1628) and the other containing a cluster of potential regulatory motifs (pl97, −801 to −338). Neither of these constructs had significant activity, suggesting the presence of a weak promoter that requires an enhancer element for activity.

A TG element within the blk 5′-flanking Region Shows Non-tissue-specific Enhancer Activity—To test whether the fragment from −2258 to −1628 could enhance transcription in a B-cell-specific manner, the fragment was inserted downstream of an SV40 promoter-driven luciferase gene. The resulting construct pl104 was transfected into Daudi, DU 145, T-47D, ARH-77, J urkat, and 293 cells. As shown in Table III, in all of the cell lines tested, the −2258 to −1628 fragment in pl104-enhanced SV40 promoter activity by about 3-fold compared to the pGL2-promoter plasmid. Thus this region contains an enhancer, but it is not B-cell-specific. Deletion analyses localized this enhancer to a TG repeat element (see Fig. 1).2 These results are in agreement with other observations that TG elements can act as enhancers (41, 42).

The Binding of a Protein at the BSAP Site Is Correlated with the Expression of the 2.5-kb blk RNA—Recent studies (13) have shown that the expression of murine blk is correlated with the presence of BSAP and have suggested that BSAP is a positive regulator of murine blk transcription. To determine whether BSAP may regulate the expression of one of the human blk RNAs, we examined the putative BSAP site in the human blk gene (position −404 to −391) by electrophoretic mobility shift assay. A 50-bp probe (from −426 to −375) spanning the BSAP-binding site was prepared from the 5′-flanking region of blk. This probe was mixed with cell extracts prepared from various lymphocytic cell lines. As shown in Fig. 6A, a DNA-protein complex was found in cell extracts of Daudi, ARH-77, SU-DHL4, OCI-Ly8, SUP-B17, Raji, SUP-B8, RPMI 6666, and RPMI 7666 cell lines, whereas it was not present in extract of SUP-B12, J urkat, CEM, or UC 729 cells (Fig. 6, A and B). As summarized in Table I, the presence of this DNA-bound protein correlated with the presence of the 2.5-kb transcript, indicating that this protein may be a positive regulator of the 2.5-kb blk transcript. Interestingly, the expression of the 2.2-kb blk transcript in SUP-B12 and J urkat apparently does not require this protein.

To confirm that the bound protein was BSAP, we mutated the 50-bp BSAP probe at positions −406 (C to A) and −402 (G to A). Mutations at the corresponding positions in the murine blk promoter have been shown to impair the binding of BSAP (13). Data presented in Fig. 6B show that the mutated probe, m-BSAP, was unable to interact with the protein identified by the wild-type (wt-BSAP) probe.

**DISCUSSION**

In previous studies, it has been shown that the expression of the murine blk gene is B-lineage restricted and developmentally regulated (2, 7). The murine blk gene is expressed in pre-B cells through mature B-cell stages of differentiation but not in plasma cells. Our studies and others (14) have demonstrated the expression of the human blk gene in B-cell lines representing all stages of differentiation (pre-B through plasma cells), and in at least one T-cell line (J urkat), but not in any of the non-lymphoid cell lines examined. The expression of human blk in the early stages of T-cell development has been reported previously (14). The function of blk in these T-cells is not understood. It is possible that human blk may play a role in signal transduction early in T-cell development.

In most of the human B-cell lines examined in this study, we found two blk transcripts (2.2 and 2.5 kb). Interestingly, these two transcripts appeared to be differentially expressed in the various cell lines. Some cell lines expressed higher levels of the 2.2-kb message as compared to the 2.5-kb transcript, others expressed relatively equal levels of both transcripts, while still others expressed only the 2.2-kb message. To begin to understand the mechanism(s) by which these two blk transcripts are differentially regulated, we examined their structural differences using Northern and PCR analyses.

We have previously described the isolation of a human blk cDNA clone (24). This cDNA corresponds in length to the 2.5-kb
sequence of the human blk gene lacks TATA box elements near the transcription start sites, contains X-box, CRE, and Y-box motifs. Interestingly, the spacing between these elements is similar to that in the MHC class II promoter (31, 43). The molecular mechanisms controlling transcription of MHC class II genes have been studied extensively, and the nuclear factors binding to these sequences have been identified (44-47). These boxes all contribute to the B-cell-specific expression of MHC class II genes. Another DNA-binding sequence that may contribute to the B-cell-specific expression of blk is the BSAP site at position −404 to −391 (see Fig. 2). Previous studies on the CD19 (10) and murine blk promoters (7, 13) have shown the presence of a site recognized by the B-lymphoid transcription factor BSAP. This factor, like Sp1 and GCN4 (48), may play a role in activating transcription from TATA-less promoters.

Another cluster of sequences, located just upstream of the major transcription start site at +1, contains E, PU.1, and PEA3 boxes. These sequences are present in the promoter and enhancer regions of immunoglobulin genes and are important for the B-cell-specific expression of these genes (49-52). A binding site for the general transcription factor Sp1 (35) is also located in this region. Sp1 sites are often found in TATA-less promoters. Whether cooperation of Sp1 with the other binding factors (E, PU.1, and PEA3) is necessary for the activity of the blk promoter requires further investigation.

To test the functional activity of the 5'-flanking region of the human blk gene, a series of luciferase reporter gene plasmids were constructed and transfected into various cell lines. Results from these assays demonstrated that a 2.3-kb DNA fragment from the 5'-flanking region of the human blk gene contains tissue-specific promoter function (Table II). This fragment drives transcription in Daudi and SUP-B8 B-cell lines and Jurkat T-cells but not in T-47D breast cancer cells or DU 145 prostate cancer cells. Deletion analysis of this fragment in Daudi cells demonstrated three functionally important regions.

One region (−2258 to −1628) does not stimulate transcription independently but does act as an enhancer element. The combination of this region with promoter P1 or P2 (described below) increased transcription activity. However, this region also enhanced expression from the SV40 promoter approximately 3-fold in lymphoid and non-lymphoid cell lines, indicating that this is not a tissue-specific enhancer. We have identified a TG repeat element within this region as being

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**Table III**

Enhancer activity of the blk 5'-flanking region from Smal (−2258) to StuI (−1628)

| Construct | Daudi | DU 145 | T-47D | ARH-77 | Jurkat | 293 |
|-----------|-------|--------|-------|--------|--------|-----|
| pGLO-basic | 1     | 1      | 1     | 1      | 1      | 1   |
| pGLO-SV40b | 6.6 ± 0.5 | 17.4 ± 8.1 | 2.5 ± 0.6 | 5.8 ± 2.4 | 9.0 ± 1.1 | 224 ± 82.9 |
| pl.104d  | 21.4 ± 4.7 | 40.1 ± 20.5 | 6.4 ± 0.9 | 24.2 ± 9.8 | 21.7± | 592.0 ± 156.0 |

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*a* Values for fold increases in luciferase activity over the promoter-less vector pGLO-basic are normalized to CAT activity and are presented as the mean ± S.E. from duplicate or triplicate experiments.

*b* Containing the SV40 promoter (no enhancer) driving the luciferase gene.

*d* Constructed with the putative luciferase enhancer inserted downstream of the luciferase gene.

*Results from a single transfection experiment.*
sites as well as a cluster of potential transcription factor-binding sites as described above. These results are consistent with the hypothesis that the blk gene contains two promoters. We have designated the putative promoter between −338 and +75 as “P1” and the promoter between −1628 to −338 as “P2”. Two promoters controlling single gene expression are also present in another member of the src family, Ick (39). Similarly, the mouse Thy-1.2 glycoprotein gene (57), α-amy1ase (58) rat β2 (59) gene, and B-50/GAP-43 gene (60) are also transcribed from two promoters.

The presence of two promoters in the blk gene was further supported by the data from electrophoretic mobility shift assays. The P2 promoter, which drives the expression of the 2.5-kb blk transcript, contains a possible BSAP-binding site. We have demonstrated that the expression of the 2.5-kb transcript is correlated with the presence of a protein that binds to a probe containing this BSAP site. In contrast, cell lines that express the 2.2-kb blk transcript but lack the 2.5-kb transcript, lack this binding protein. These results suggest that the expression of the 2.5-kb transcript is regulated by BSAP or a related protein whereas the expression of the 2.2-kb transcript is not under regulation by this factor.

In summary, we have reported studies to support the conclusion that the human blk gene is expressed from two distinct promoters. The presence of different clusters of known B-cell-specific regulatory motifs within these two promoter regions and the different expression patterns of the two blk transcripts in various B-cell lines suggest that these promoters may be subject to regulation by different trans-acting factors. Consistent with this hypothesis is our identification of a protein that binds to the BSAP site in promoter P2 and the demonstration that the presence of this protein is correlated with the expression of the 2.5- but not the 2.2-kb blk transcript. The human blk gene is expressed throughout B-cell development, from very immature B-cells (as well as immature T-cells) to plasma cells. Two distinct blk promoters may be required to allow efficient transcription of the blk gene throughout B-cell development, since different trans-acting factors may be present at different stages of differentiation. Investigations are underway to identify these factors and to further elucidate the regulatory mechanisms controlling blk expression in human B lymphocytes.

Acknowledgments—We thank Dr. Susan Dymecki and Dr. Ronald J. Weigel for helpful advice and critical review of this manuscript. We thank Dr. Allison C. Chin for technical assistance.

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