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The Human *Pim-1* Gene Is Selectively Transcribed in Different Hemato-Lymphoid Cell Lines in Spite of a G+C-Rich Housekeeping Promoter

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The expression of the *Pim-1* proto-oncogene was studied by using the K562, Daudi, and Jurkat cell lines. In K562, *Pim-1* mRNA levels were more than 20-fold higher than in Daudi and 50-fold higher than in Jurkat. Nuclear run-on assay data correlated directly with the steady-state mRNA levels, suggesting that the rate of transcription was responsible for the selective expression of this gene. Furthermore, the half-life of *Pim-1* mRNA was shown to be 47 min in K562, 71 min in Daudi, and 35 min in Jurkat. This indicated that selective *Pim-1* mRNA expression did not depend on posttranscriptional regulation. Therefore, 1.7 kilobases of the *Pim-1* promoter was sequenced and studied in detail. The sequence showed that the region from nucleotide −1 to −873 was G+C rich (71%). Study of promoter deletions defined two major functional regions, a proximal element (nucleotide −104 to −1) and a distal element (nucleotide −427 to −336). DNase I protection assays identified binding sites for the Sp1 and AP2 proteins in these elements. A possible new transcription factor binds at position −348 in the distal element. In our study of the 1.7-kilobase *Pim-1* promoter, we found no differences between K562 and Jurkat that could explain large differences in transcription. Therefore, the *Pim-1* promoter appears to function constitutively, and we conclude that distant elements must regulate the tissue-selective expression of this gene. Although the *Pim-1* gene has a G+C-rich housekeeping promoter, expression is carefully regulated at the level of transcription.

Activation of the *Pim-1* proto-oncogene is associated with malignant lymphoma. The *Pim-1* locus was originally described as a site of recurrent retroviral integration in murine T-cell lymphoma (10). Such integration resulted in increased expression of *Pim-1* mRNA. In recent experiments, transgenic mice with *Pim-1* constructs developed T-cell lymphoma at a low frequency (47). This frequency was increased by retroviral infection and chemical mutagenesis (5, 47). In these model systems, *Pim-1* and c-*myc* (or sometimes N-*myc*) are often activated together, suggesting that these genes might cooperate in causing malignancy. It is also known that the *Pim-1* gene codes for a protein kinase of approximately 36 kilodaltons (13, 29, 30, 38, 44).

The *Pim-1* gene is expressed at high levels in hemato-lymphoid tissue and testes, while other tissues express low or undetectable amounts. In our survey of 37 human cell lines, we found the highest expression in myeloid cell lines (K562 and KG-1), intermediate levels in many B-cell lines (including Daudi) and in a choriocarcinoma line, and undetectable levels in 7 T-cell lines (including Jurkat) (30). In the murine system the expression pattern is similar, except that thymocytes are positive, suggesting *Pim-1* expression at some stage of T-cell differentiation (10, 47).

The 5′ end of the *Pim-1* gene appears to be a *Hpa*II tiny fragment island, since the promoter is very G+C rich and includes a cluster of restriction enzyme sites for *Not*I, *Sac*II (two), and *Nar*I (two) (4, 25). In addition, no TATA or CAAT elements are appropriately located. This structure has been considered characteristic of promoters from genes that are expressed at low levels in all cells and are not carefully regulated (called housekeeping genes) (31, 36).

In this work, our goal is to resolve the apparent paradox of regulated levels of *Pim-1* mRNA in the context of a housekeeping-type G+C-rich promoter lacking TATA and CAAT elements. We provide evidence that the *Pim-1* G+C-rich promoter functions constitutively in test systems but endogenous *Pim-1* transcription is carefully regulated. This combination suggests that distant control elements (silencer or enhancer) modulate the transcriptional activity of the *Pim-1* housekeeping promoter.

MATERIALS AND METHODS

Cell culture. K562, Daudi, and Jurkat have been previously described (1, 32). To measure the half-life of *Pim-1* mRNA, dactinomycin was added to a final concentration of 0.005 mg/ml at time zero and cells were processed at 15 min, 30 min, 1 h, 2 h, and 3 h (11, 26, 49). In some experiments cycloheximide was used at a concentration of 0.01 mg/ml (11, 26, 49).

DNA fragments. The previously reported lambda 5 clone derived from the 380 cell line was used as a source of all subcloned genomic fragments (30). The 1.7-kilobase (kb) promoter region was sequenced by using M13 vectors and a chain termination approach (33). Part of this sequence has been previously reported, and one nucleotide that was incorrectly inserted in that prior sequence has been deleted (−374) (see Fig. 4). Northern (RNA) blots were probed with the *Xho*I-*Hin*dIII fragment from the pC1 *Pim-1* cDNA plasmid and then with the *Bgl*I-*Pst*I pHcGAPNR fragment from the glyceraldehyde-3-phosphate dehydrogenase (GADPH) cDNA (45).

For the reporter gene assays, the longest construct consisted of the region from *Hin*dIII to *Pst*I which was cloned into the *Hin*dIII site of the luciferase vector pSVOAL-AΔ5′ (shortened to pL in this paper) after adding a *Hin*dIII linker to a blunt *Pst*I site (12). Some deletions were generated by

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using exonuclease III, S1 nuclease digestion, *Hin*dIII linker addition, and cloning into the *Hin*dIII vector site (18). Other constructs were generated by directed cloning from known cloning sites followed by *Hin*dIII linker addition. The correctness and orientation of all deletions was documented by restriction mapping and double-stranded sequencing by using published methods (24).

Northern blotting. To isolate total RNA, cells were suspended in guanidium isothiocyanate lysis buffer (7). Samples were layered onto a 5.7 M cesium chloride cushion and centrifuged at 159,000 × *g* for 24 h at 20°C. The RNA pellet was suspended in water and precipitated twice with ethanol prior to use. In some experiments, poly(A)$^+$ RNA was directly isolated from Jurkat cells by using a published protocol (2). All RNA samples were quantitated carefully and separated on formaldehyde gels. Transfer to Hybond-N (Amersham Corp.) was performed by capillary action in 10× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate).

The prehybridization solution consisted of 50% formamide, 5× SSC, 0.5% sodium dodecyl sulfate (SDS), 1× Denhardt solution, and salmon sperm DNA. Hybridization was performed overnight at 42°C in a similar solution containing the radioactive probe.

Blots were washed to a final stringency of 0.1× SSC at 60°C. Autoradiography followed. All blots were reprobed with the pHcGAPNR probe to quantitate the amount of RNA loaded per lane.

Assay of run-on transcription. Nuclei were isolated from cell lines according to previously published methods (39). Cells (1×10^7 to 2×10^7) were washed twice in phosphate-buffered saline and were suspended in 3 ml of 0.3 M sucrose–10 mM Tris hydrochloride, pH 7.4–5 mM $MgCl_2$–0.2% Nonidet P-40. This was suspended above 2.5 ml of 0.8 M sucrose–10 mM Tris hydrochloride, pH 7.4–5 mM $MgCl_2$. The sample was centrifuged for 15 min at 1,500 × *g* and 4°C. The pellet was washed with 2 ml of 35% glycerol–50 mM Tris hydrochloride, pH 7.4–5 mM $MgCl_2$–0.1 mM EDTA and stored at −70°C in this buffer.

The transcription assay was done as previously described (17). A sample of nuclei (100 μl) was thawed, and 50 μl of 20 mM Tris hydrochloride, pH 8.0–10 mM $MgCl_2$–600 mM KCl was added. Portions (5 μl each) of 10 mM CTP, GTP, and ATP were added, and this procedure was followed by the addition of 10 μl of [^{32}P]UTP (100 μCi) and 25 μl of water. Transcription proceeded over 30 min at 30°C, in some instances in the presence of α-amanitin (1 μl of 0.5 mg/ml) (26). tRNA, $CaCl_2$, and DNase I (Promega Biotec) were added for a 5-min incubation. Proteinase K and SDS were added, and the sample was incubated for 1 h at 42°C. After three extractions, the RNA was precipitated, suspended in TE (10 mM Tris, pH 8–1 mM EDTA) and fractionated over a G-50 spun column (Boehringer Mannheim Biochemicals). Another DNase I digestion and proteinase K digestion and another set of extractions and precipitations followed. A 5-μl portion of 10% SDS and 50 μl of ice-cold 1 M NaOH were added to 200 μl of the RNA. After 10 min on ice, the sample was neutralized, precipitated, and suspended in 100 μl of 10 mM TES [*N*-tris(hydroxymethyl)methyl-2-aminoethanesulfonic acid], pH 7.4–0.2% SDS–10 mM EDTA.

Target filters were made by using linearized plasmids denatured with NaOH at 65°C for 30 min prior to the addition of ammonium acetate, pH 7. By using a slot blotter (Schleicher & Scheull, Inc.), 5 μg of plasmid per slot was applied to Hybond-N (Amersham). Filters were hybridized and washed exactly as for Northern blotting.

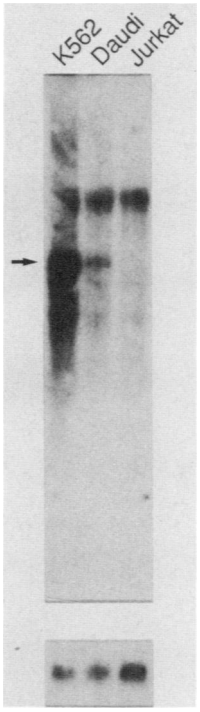

FIG. 1. *Pim-1* expression. A Northern blot of total RNA from K562, Daudi, and Jurkat was probed with the *Pim-1* probe (top panel) and reprobed with a GADPH probe to assess RNA loading (bottom panel). The arrow indicates the position of the *Pim-1* mRNA (approximately 2.8 kb). The signal in K562 is 20-fold higher than in Daudi, while the signal in Jurkat is undetectable in this experiment.

S1 analysis. The site of transcription initiation was defined by using S1 nuclease protection of both double-stranded and single-stranded probes (3). The double-stranded *Dde*I fragment extending about 55 base pairs 3′ of the transcription initiation cluster was kinased and suspended in 30 μl of 80% formamide–40 mM PIPES [piperazine-*N*,*N*′-bis(2-ethanesulfonic acid)] (pH 6.4)–400 mM NaCl–1 mM EDTA–20 to 100 μg of total cellular RNA. A control tube contained an equal amount of tRNA. Each sample was heated to 65°C for

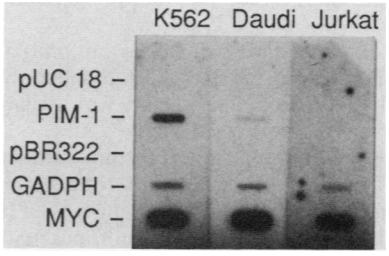

FIG. 2. Nuclear run-on transcription assay. Nuclear run-ons were performed to compare *Pim-1* transcription in K562, Daudi, and Jurkat cell lines. Identical filters were made by using the pUC18 (negative control), *Pim-1* C1, pBR322 (negative control), pGADPH (positive control), and pMyc (positive control) plasmids. Each filter was hybridized with labeled RNA from the appropriate cell line. Interpretation of the *Pim-1* signal was based on normalizing the GADPH signal for each line. As measured by densitometry, the K562 *Pim-1* signal is at least 10 times greater than the Daudi signal. The Jurkat signal is difficult to detect. This pattern directly correlates with the steady-state mRNA levels in the three lines.

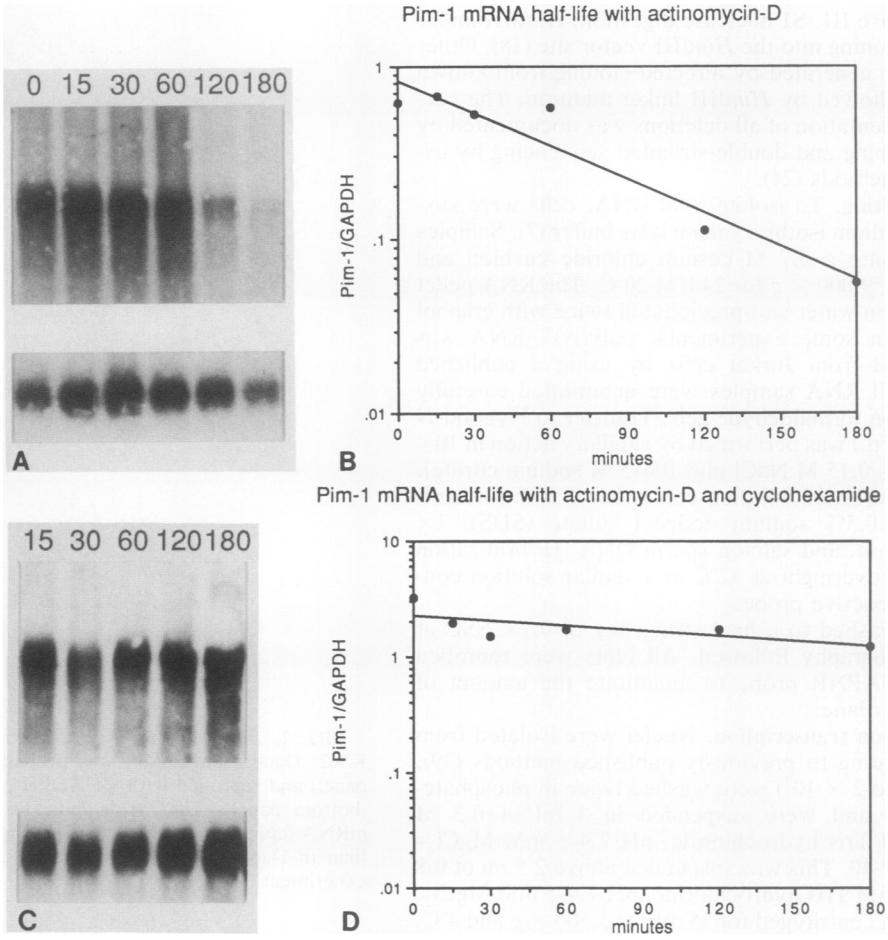

FIG. 3. Rate of *Pim-1* mRNA degradation. The half-life of *Pim-1* mRNA was analyzed in K562 by incubating with dactinomycin and isolating RNA at appropriate intervals (indicated in minutes). Northern blots were probed with *Pim-1* and reprobed with GADPH, as a control for loading (A). After analyzing the data by densitometry, the ratio of *Pim-1* to GADPH was plotted on a logarithmic scale and the half-life in this experiment was calculated to be 47 min (B). A similar analysis was performed with dactinomycin and cycloheximide (C and D). The half-life in this experiment was calculated to be 208 min when both agents were used together.

10 min and then placed at 30°C overnight. S1 nuclease (30 to 300 U) and salmon sperm DNA in 300 μl of nuclease buffer (0.56 M NaCl, 0.1 M sodium acetate, pH 5.2, 9 mM $ZnSO_4$) were added, and the sample was incubated at 30°C for 1 h. The reaction products were separated on a denaturing acrylamide gel.

To generate single-stranded probes, an end-labeled oligonucleotide was used to initiate chain elongation on an M13 template and the radioactive strand was isolated. The remainder of the procedure was unchanged.

Transfections. Cells were given 1 volume of fresh medium 1 day prior to transfection. Twenty-four hours later they were washed twice in HeBS (20 mM HEPES [*N*-2-hydroxymethylpiperazine-*N'*-2-ethanesulfonic acid], pH 7.05, 137 mM NaCl, 5 mM KCl, 0.7 mM Na_2HPO_4, 6 mM glucose) and were resuspended at 10^6/ml. Prior to transfer to the electroporation cuvette, 9×10^6 cells were mixed with 50 μl of plasmid DNA (at a concentration of 1 mg/ml, except for Rous sarcoma virus [RSV] luciferase, which was at a concentration of 0.2 mg/ml). The Biorad Gene Pulser with the capacitance extender set at 960 μF was set on the predetermined optimum voltage, and the cells were shocked. The time constant of the discharge was monitored to document that the machine functioned properly.

Subsequently, 9 ml of tissue culture medium was added and the cells were incubated at 37°C for 48 h. The cells were then washed with phosphate-buffered saline and were suspended in 0.1 ml of 0.1 M KH_2PO_4, pH 7.8, and 1 mM dithiothreitol (DTT). The samples were frozen and thawed four times to lyse the cells. The lysate was clarified by centrifugation.

Luciferase was quantitated by using a Monolight Luminometer (Luminescence Sciences, San Diego, Calif.). A 50-μl portion of lysate was added to 750 μl of assay buffer (25 mM glycylglycine, pH 7.8, 10 mM $MgCl_2$, 5 mM ATP). This was automatically mixed with 100 μl of 1 mM luciferin-K (Luminescence Sciences), and the light emission over 10 s was measured. All data were standardized for the amount of protein in the lysate, determined by using a colorimetric assay (Bio-Rad Laboratories).

Isolation of crude nuclear extracts. Nuclear extracts were isolated by using previously published methods (34). Approximately 10^8 cells were washed twice in phosphate-buffered saline without calcium or magnesium. Cells were washed and then suspended in buffer A (10 mM HEPES, pH 7.8, 15 mM KCl, 20 mM $MgCl_2$, 0.1 mM EDTA, 1 mM DTT, 1 mM phenylmethylsulfonyl fluoride, 1 μg of antipain [Sigma Chemical Co.] per ml, 0.3 μg of leupeptin [Sigma] per ml).

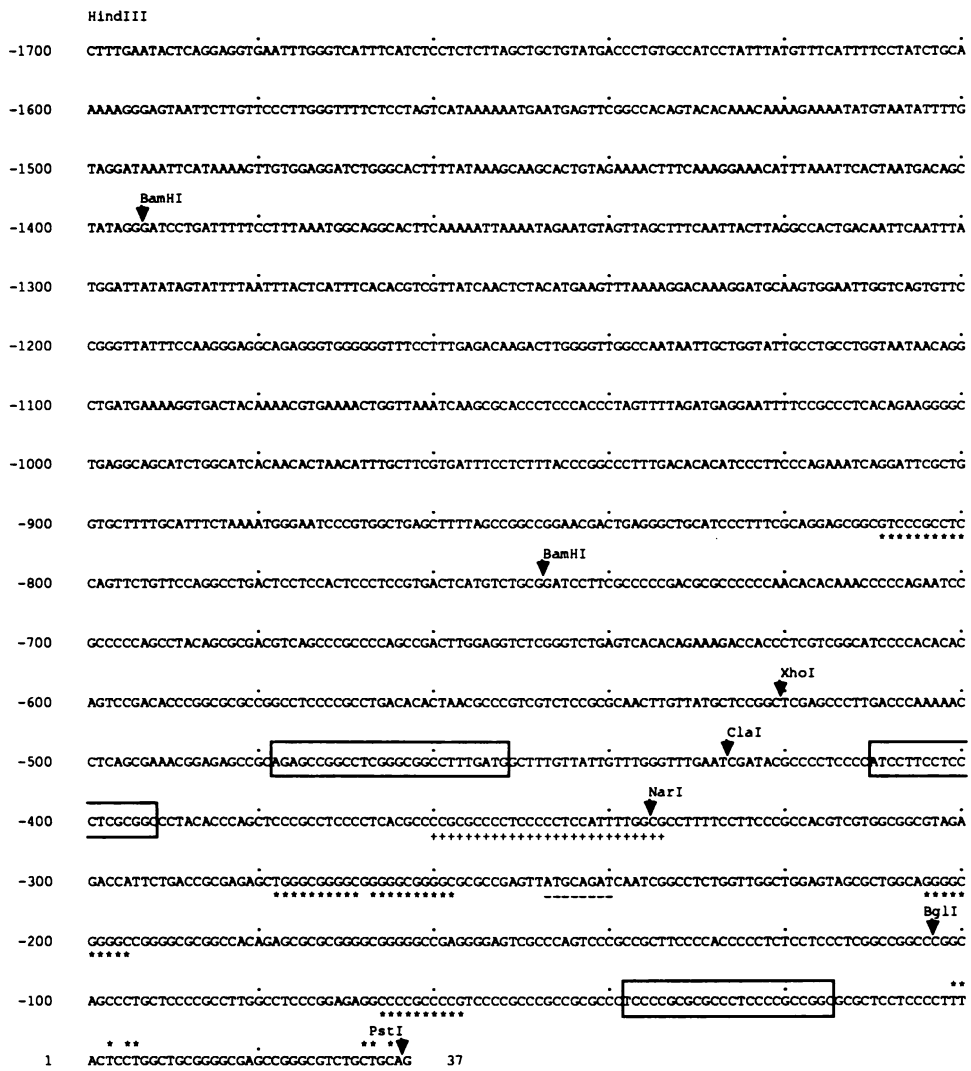

FIG. 4. *Pim-1* promoter sequence. 1.7 kilobases of the human *Pim-1* promoter is shown. Numbering is consistent with our previous publication, except that nucleotide −374 has been eliminated based on additional sequence data. Nucleotides are designated by numbers in relation to the previously defined approximate site of transcription initiation. The exact sites of transcription initiation have been defined in this work and fall into two clusters indicated by asterisks above nucleotides. The *Hin*dIII site (this recognition sequence starts at −1703, but only three downstream nucleotides are shown) and the *Pst*I site were used for the longest promoter constructs. Sites of transcription factor binding are indicated—Sp1 (underlined by asterisks), AP2 (boxes), and PPF-348 (pluses). The octamerlike sequence was shown to be nonfunctional in our analysis and is indicated by an underline.

After Dounce homogenization, nuclei were isolated by centrifugation at 2,200 × *g* and resuspended in buffer A; this procedure was followed by addition of $(NH_4)_2SO_4$ to 0.3 M. This solution was gently rocked at 4°C for 30 min prior to centrifugation at 100,000 × *g* for 1 h. After 0.2 g of $(NH_4)_2SO_4$ per ml was added to the supernatant, the sample was centrifuged again and the insoluble material was suspended in buffer B (50 mM HEPES, pH 7.8, 50 mM KCl, 0.1 mM EDTA, 1 mM DTT, 1 mM phenylmethylsulfonyl fluoride, 10% glycerol). This lysate was stored in aliquots at −70°C.

Assay of DNase I protection. Radioactive fragments were incubated on ice for 15 min with a portion of isolated transcription factor or crude lysate in 50 μl of 12.5 mM HEPES(K), pH 7.5–50 mM KCl–10% glycerol–0.005% Nonidet P-40–0.5 mM $ZnSO_4$–0.5 mM DTT (6). A 1-μg portion of poly(dI:dC) was included when crude lysates were used. An equal volume of 10 mM $MgCl_2$ and 5 mM $CaCl_2$ was added. After 1 min at room temperature, 2 μl of diluted DNase I (Worthington Diagnostics) was added. A 1-μl portion of 1 mg of salmon sperm DNA per ml was also added for the purified Sp1 experiments. After 1 min at room temperature, 90 μl of stop solution (20 mM EDTA, pH 8, 1% SDS, 0.2 M NaCl, 250 μg of carrier RNA per ml) was added. When studying crude lysates, a proteinase K digestion step was included at this point. Samples were extracted with phenol-chloroform and precipitated with ethanol. Samples were separated on 8% acrylamide gels with 50% urea. Affinity-purified Sp1 at a concentration of 5 to 20 μg/ml was generously provided by J. Kadonaga and R. Tijan (6). Affinity-purified AP2 produced in bacteria was provided by T. Williams and R. Tijan (48).

Mobility shift assay. The radiolabeled fragment was incubated for 30 min at room temperature with affinity-purified

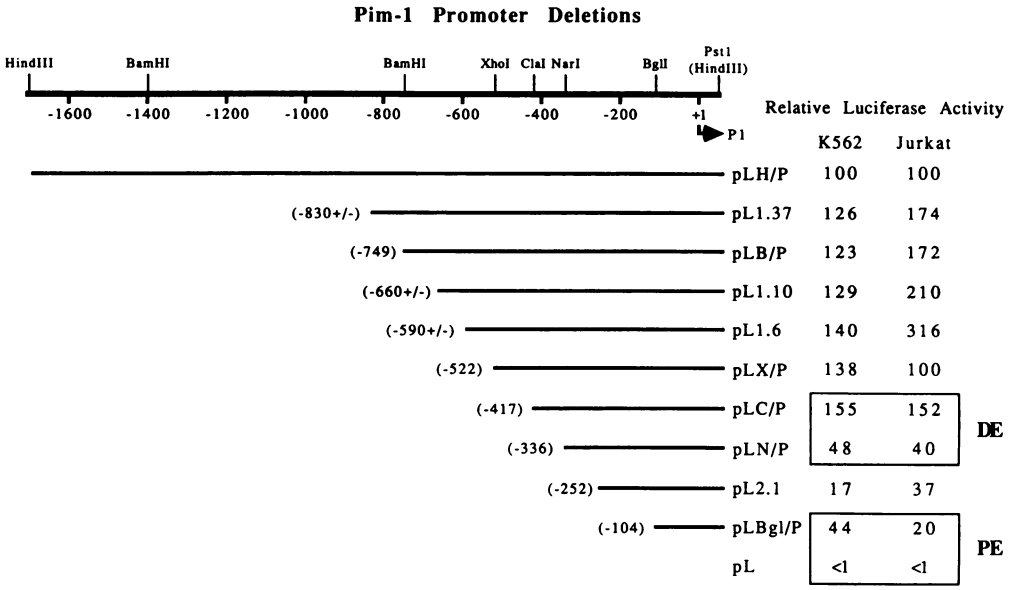

FIG. 5. Reporter gene assays. A functional study of the *Pim-1* promoter was made in the K562 and Jurkat cell lines. Deletions through the promoter were made, as indicated, to determine the areas most responsible for transcriptional control. The terminal nucleotide of the deletions is indicated in parentheses, with those denoted by +/− being estimated to within five base pairs. The relative luciferase activity is given as normalized to the full-length pLH/P signal. All data points represent average values for at least four determinations. Two intervals resulted in a 200% increase in signal in both cell lines and these were designated proximal element (PE) and distal element (DE). No major lineage-specific regions were detected in this study, except between pL1.6 and pLX/P in the Jurkat cell line. However, this region cannot explain the higher expression in K562.

fusion protein (kindly provided by L. Staudt) in 20 μl containing 10 mM Tris hydrochloride, pH 7.5, 50 mM NaCl, 5 mM DTT, 1 mM EDTA, 4% glycerol, 1 mg of bovine serum albumin per ml, and 0.1 mg of poly(dI:dC) per ml. Glycerol sample buffer was added, and the sample was separated on a 4% acrylamide gel with a running buffer of 50 mM Tris hydrochloride, pH 8.5–380 mM glycine–2 mM EDTA (22, 42).

RESULTS

In earlier work we demonstrated that steady-state levels of *Pim-1* mRNA differ significantly between cell lines. To focus on this issue, we have studied the K562, Daudi, and Jurkat cell lines, representing myeloid and B- and T-lymphocytic lineages, respectively, in more detail. When probing Northern blots performed with total RNA samples, one can estimate that the K562 signal is at least 50 times higher than that of Jurkat (which is often undetectable) and more than 20 times higher than the Daudi signal. A representative Northern blot is shown in Fig. 1.

To explore the variation in steady-state mRNA level between K562, Daudi, and Jurkat cells, we performed nuclear run-on assays, allowing us to quantitate the rate of *Pim-1* transcription. The data showed that the K562 signal was more than 10 times higher than the Daudi signal and more than 50 times that of Jurkat (Fig. 2). To document that transcription of *Pim-1* was catalyzed by RNA polymerase II, the experiment was repeated by using K562 with 2 μg of α-amanitin per ml to block RNA polymerase II transcription. The K562 signal was decreased greater than 90% (data not shown). These experiments showed that the amount of transcription as judged by the run-on assay directly correlated with the differences in steady-state level of mRNA.

To understand posttranscriptional processing of *Pim-1* mRNA, we measured the half-life of *Pim-1* mRNA in the K562 cell line. Cells were incubated in the presence of dactinomycin to block the initiation of new transcripts. RNA was isolated at regular intervals, and Northern blots were performed to determine the rate of mRNA degradation. A plot of signal strength indicated that *Pim-1* degradation followed first-order kinetics, with a half-life of approximately 47 min (Fig. 3A and B). This experiment was repeated with cycloheximide to determine the effect of protein synthesis inhibition on degradation. Cycloheximide was found to prolong the *Pim-1* mRNA half-life to more than 3 h (Fig. 3C and D).

The measurement of the half-life of *Pim-1* mRNA in Daudi and Jurkat allowed us to assess the contribution of posttranscriptional regulation to the steady-state RNA differences we observed. The half-life in Daudi was approximately 71 min (data not shown). To analyze Jurkat, we performed Northern blots by using poly(A)$^+$ RNA and derived a similar half-life of 35 min (data not shown). Therefore, posttranscriptional regulation appeared to contribute little to the observed differences in steady-state mRNA expression.

To further analyze the control of *Pim-1* transcription, we sequenced 1.7 kb of the promoter region (Fig. 4). This region could be clearly divided by frequency of nucleotide usage. The region from nucleotide −1703 to −874 was only 40% G+C; the region from nucleotide −873 to −1 was 71% G+C. We compared the mouse and human *Pim-1* promoters in an effort to help us localize highly conserved sequences that might represent crucial control regions (38; Genbank no. M13945). This comparison revealed 71% overall homology, with areas of higher homology scattered throughout the region.

By using S1 nuclease mapping, we detected two major clusters of transcriptional start sites. In each cluster, transcription appears to start over 5 to 10 base pairs, suggesting that the exact cap site is variable (Fig. 4, data not shown).

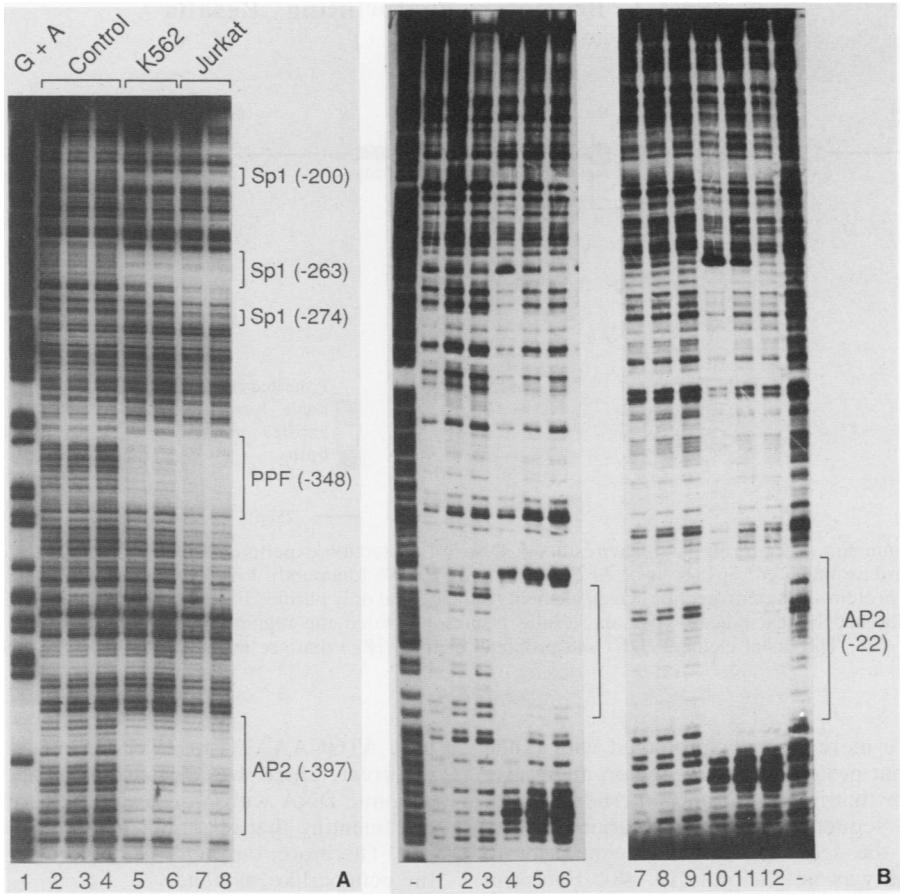

FIG. 6. DNase I protection studies. Representative protection studies are shown. (A) Crude lysates were studied with the *Bam*HI-*Nar*I fragment (labeled at the *Nar*I site). Lane 1, Maxam-Gilbert G+A sequence reaction (27); lanes 2 to 4, negative controls; lanes 5 and 6, the K562 pattern; and lanes 7 and 8, the Jurkat pattern. The AP2 (−397) and the PPF (−348) sites are clearly documented. Because they are higher, the three Sp1 sites are more difficult to see on this gel. These sites were seen more clearly with longer gel runs and other probes. (B) The *Cla*I-*Pst*I (*Hin*dIII) fragment was studied on both strands with purified AP2 protein. G+A sequence reactions were run on the outside lanes. Lanes 1 to 3 and 7 to 9 represent negative controls; lanes 4 to 6 and 10 to 12 represent the pattern with purified AP2. The region at −22 is clearly documented.

Functional assays of the *Pim-1* promoter were performed by using the cloned firefly luciferase gene. The 1.7-kb *Pim-1* promoter was cloned into the pL vector. The activity of this promoter was compared with that of the RSV long terminal repeat, a constitutive positive control, in the K562 and Jurkat cell lines. The RSV luciferase signal averaged 60,000 light units in K562 and 37,000 light units in Jurkat. The full-length *Pim-1* construct (pLH/P) averaged 15% of the RSV luciferase signal in K562 and 39% in Jurkat. These data suggest that the RSV long terminal repeat and the full-length *Pim-1* promoter function constitutively in both K562 and Jurkat.

The analysis of the deletions through the *Pim-1* promoter is shown in Fig. 5. In K562, the shortest construct, pLBgl/P, gave a signal that was 44% of the full-length fragment, indicating that the basic promoter elements resided in this short region that we call the proximal element. A distal element from position −427 to position −336 that augmented transcription three- to fourfold was also defined. Longer constructs had minimal additional effect.

Transfections with the Jurkat cell line showed a similar pattern (Fig. 5). When data were normalized to the signal for the full-length 1.7-kb construct, pLBgl/P yielded a 20% signal and the distal element showed a three- to fourfold augmentation. An additional region between nucleotides −590 and −522 that had a threefold effect in this line, but not in K562, was identified. Importantly, we could identify no localized segment in the 1.7-kb region that could explain 50-fold higher transcription in the K562 cell line, supporting the contention that the 1.7-kb *Pim-1* promoter functions with minimal tissue selectivity.

We were able to identify some of the transcription factors involved in controlling transcription from the constitutive *Pim-1* promoter. We examined the region between −600 and −1 by DNase I protection assays by using crude lysates of K562 and Jurkat (Fig. 6 and 7). Data from crude lysates were confirmed by data from isolated transcription factors whenever possible. In all cases, lysates from K562 and Jurkat showed similar patterns, confirming that no element in this region accounted for the tissue-selective expression differences between the cell lines.

In the 1.7-kb promoter, 5 consensus Sp1 boxes (G/T) (G/A)GGCG(G/T) (G/A) (G/A) (C/T) are found (6). The motif centered at –805 was not studied. The other four motifs (−274, −263, −200, and −62) were protected by crude lysates and purified Sp1 factor (Fig. 7). The box at −62 appeared to be of the highest affinity. It was of interest that the boxes at −274 and −263 were weakly protected with

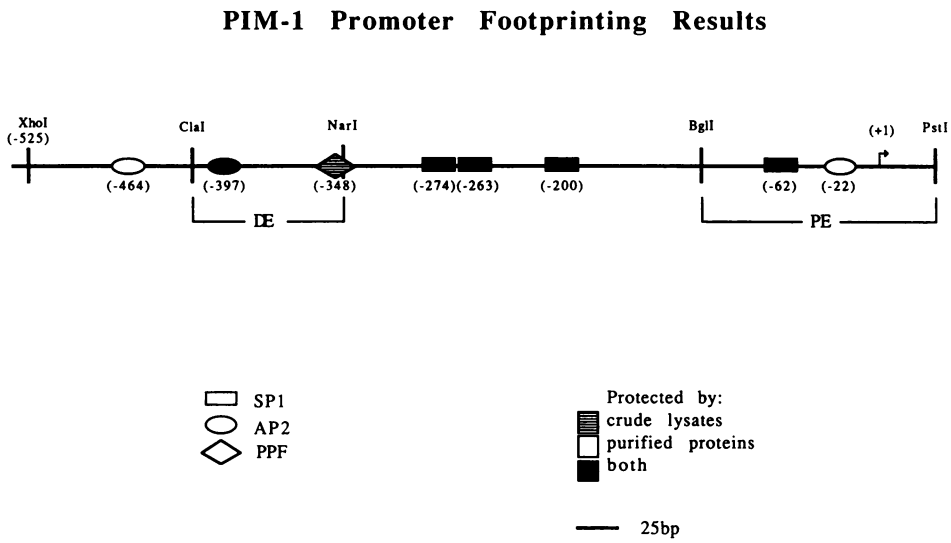

FIG. 7. Schematic summary of footprint data. The results of DNase I protection experiments for 525 nucleotides of the *Pim-1* promoter are summarized. Sites are as indicated: Sp1 (boxes), AP2 (ovals), and PPF-348 (diamond). Filled boxes indicate that both crude lysates and the appropriate purified protein showed protection. Open elements indicate that only purified proteins protected the region, perhaps indicating a lower-affinity site. Hatched boxes indicate that only crude lysates protected the region. No protected regions were found between nucleotides −600 and −525. The distal element (DE) and proximal element (PE) that are most important in the reporter gene assays are shown.

purified Sp1 but were more strongly protected with crude lysates, suggesting that protection in this region might also involve another factor that is present only in crude lysates.

The AP2 consensus sequence is somewhat variable; therefore, an analysis of the sequence in this region prior to footprint experiments was not helpful (19, 48). Footprint experiments identified three protected regions in the area studied (centered at −464, −397, and −22). The region at −397 seemed to be of the highest affinity because crude lysates and purified protein both showed a distinct footprint (Fig. 6A). The two other boxes were best demonstrated with purified protein only (Fig. 6B). It is difficult to identify the AP2 consensus sequence in these three protected regions; therefore, the extent of the protection is indicated in Fig. 4. It is also possible that there are two other AP2-binding sites in this promoter, but the footprints were intermittent and could not be convincingly demonstrated (−92 and −158). It is possible that these represent low-affinity AP2 sites.

A protected segment centered at −348 may represent the footprint of a new transcription factor (Fig. 6A and 7). This region was protected equally with both K562 and Jurkat lysates and not by the purified AP2 factor. The sequence in this region does not correspond to any known consensus. However, the region contains a CCA which can be the core recognition motif of the NF1/CTF family of factors (8, 14, 16, 35, 37). We cannot exclude the involvement of a member of this family in this footprint.

We carefully studied the octamerlike sequence at position −248 in the *Pim-1* promoter. We found no protection with crude lysates. We suspected that OTF-1 or -2 might bind this sequence (9, 42, 43). Therefore, purified OTF-2 produced in *Escherichia coli* was used in a gel retardation assay. While the OTF-2 preparation bound a fragment from a functional immunoglobulin gene promoter (153-base-pair *Eco*RI-*Pst*I fragment from p10Ava [28]), no binding to an appropriate *Pim-1* fragment (*Cla*I-*Nci*I) could be demonstrated (data not shown). We suspected that the *Pim-1* clone we isolated might have a point mutation in this region altering a wild-type ATGCAAATC to ATGCAGATC. This suspicion was incorrect, since we digested several samples of human genomic DNA with *Sau*3A and performed a Southern blot documenting that all samples studied had an intact *Sau*3A site. Therefore, our analysis could assign no significance to the octamerlike sequence.

DISCUSSION

Pim-1 is an important protein kinase that can act as an oncogene (5, 10, 13, 29, 30, 38, 47). In this work we have provided additional documentation of the highly regulated expression of *Pim-1*. The K562, Daudi, and Jurkat lines were chosen for detailed analysis because in our previous study, they exhibited levels of expression that were typical of the myeloid and B- and T-cell lineages. In this work, the difference in steady-state mRNA level was shown to be due to transcriptional rather than posttranscriptional regulation. Further, *Pim-1* transcription was shown to result from RNA polymerase II action.

At the sequence level, the 1.7-kb *Pim-1* promoter resembled a housekeeping or constitutive promoter and functional studies confirmed this. Similar overall levels of luciferase signal resulted from full-length constructs in either the K562 or Jurkat cell line. Furthermore, the deletion studies found no subregion of differential regulation that would explain higher K562 expression. Finally, DNase I protection studies found no region of differential protection that might indicate a factor specific to one line.

In the constitutive promoter region, we defined two elements responsible for transcription and designated them the proximal element and the distal element. We have determined that the proximal element binds the Sp1 and AP2 transcription factors. Similarly, the distal element binds the AP2 and a possible new transcription factor called PPF-348 (*Pim-1* promoter factor-348). We have documented that this factor is not Sp1 or AP2. However, we cannot exclude a member of the NF1/CTF family at this site, since the CCA

sequence could represent the core binding motif (8, 14, 16, 35, 37).

The findings of careful transcriptional regulation and a constitutive promoter in the *Pim-1* gene is best explained by a control element (enhancer-silencer) outside of the 1.7-kb region studied that interacts with the constitutive promoter. Several other less likely possibilities include a tissue-specific transcription termination in the 5′ untranslated part of the gene, the presence of a second promoter outside the 1.7 kb studied which directs selective expression, or a chromatin structure which blocks the access of the transcriptional machinery to the *Pim-1* promoter in some tissues. We anticipate that further work will identify a distant control element.

It is informative to compare the *Pim-1* promoter to other promoters. Recently, it has been suggested that RNA polymerase II promoters fall into three groups (41). Group I promoters (globin genes, for example) are expressed at high levels in limited settings and are not expressed outside those settings. These promoters have a TATA box, a CAAT box, and a discrete transcription start site (often at a single base pair), but are not G+C rich. Members of the second group (including the gene for Tdt) are similar to group I, but they lack a TATA box. Group III promoters are G+C rich, with transcription starts scattered over 10 base pairs or more and lack a TATA or CAAT box. This is the housekeeping or constitutive group, which includes *Pim-1* as well as hydroxymethylglutaryl-coenzyme A reductase, hypoxanthine phosphoribosyltransferase, 3-phosphoglycerate kinase, adenine phosphoribosyl-transferase, adenosine deaminase, epidermal growth factor receptor, N-*myc*, and H-*ras* (15, 20, 21, 23, 31, 36, 40, 46). While this group contains some genes performing housekeeping functions, it is important to note that several members are also involved in growth control and can function as oncogenes. In this regard, the term housekeeping must be used carefully or it may be misleading. In the case of the *Pim-1* gene, it is clear that this promoter structure does not preclude careful regulation at the level of transcription. The further molecular characterization of this important type of transcriptional control will be the focus of future work.

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