Characterization of the Human elk-1 Promoter

POTENTIAL ROLE OF A DOWNSTREAM INTRONIC SEQUENCE FOR elk-1 GENE EXPRESSION IN MONOCYTES*

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To characterize the human elk-1 promoter, we mapped the transcriptional start site and isolated elk-1-specific genomic phage clones that contained extensive upstream and downstream sequences. A TATA-like motif was identified immediately upstream of the transcriptional start site. Functional analyses of DNA fragments containing the TATA element and the identification of a DNase I-hypersensitive chromatin site (HS 1) in close proximity to the TATA box suggest that the identified TATA motif is important for elk-1 transcription in vivo. Sequences upstream and downstream from the TATA box were found to contribute to elk-1 promoter activity. A second hypersensitive site (HS 2) was identified within the first intron in pre-monocytic cells, which express Elk-1 only when differentiating to monocytes. In a variety of other cell types, which display a constitutive Elk-1 expression, HS 2 did not exist, suggesting that inducibility of elk-1 expression is associated with the presence of HS 2. Egr-1 and the serum response factor were found to interact specifically with the intronic sequence at +265 and +448, respectively. Because Egr-1 mRNA and protein levels were observed to increase significantly before induction of elk-1 expression, we propose that Egr-1 is important for the regulation of elk-1 transcription in differentiating monocytes.

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The Ets family of transcription factors is composed of proteins that share the Ets domain, a DNA binding domain that recognizes a GGAA/T-based sequence (for review, see Refs. 1–3). Ets proteins are involved in a variety of cellular activities, including proliferation and differentiation, and are suggested to contribute to the development of certain diseases (for review, see Ref. 4). Based on the homology within the Ets domain, Ets proteins are divided into several subclasses.

One subclass of Ets factors consists of the ternary complex factors (TCFs), which are able to form a ternary complex with the serum response factor (SRF) and the serum response element (SRE) (for review, see Refs. 5–7). TCFs include the Ets proteins Elk-1, Sap1a, Sap1b, Net/Erp/Sap2, and Netb (8). Although all TCFs can be activated by different mitogen-activated protein kinases such as ERK, JNK, and p38/RK (6, 8), there are differences in the efficiency by which these kinases are able to phosphorylate the different TCFs (9–14). For instance, in NIH3T3 cells, Elk-1 and Sap1a are similarly well activated by p38, but only Elk-1 is a good substrate for JNK. Because certain stimuli activate certain signaling pathways, each TCF may serve as an effector for a specific set of stimuli. One could speculate, therefore, that in some cell types, a particular stimulus may exert its effect on the cell through one, but not another TCF. In such a case, the cell's ability to respond to that stimulus would depend upon a sufficient expression level of this TCF, which, on the other hand, would allow this cell to control its sensitivity to certain stimuli by regulating the expression of this TCF.

By screening several cell lines for Elk-1 expression we found that, compared with a variety of non-monocytic cells, pre-monocytic cells expressed Elk-1 only at very low levels. However, Elk-1 expression could be induced when these cells were stimulated to undergo differentiation toward mature monocytes upon exposure to 12-O-tetradecanoylphorbol-13-acetate (TPA). To understand the mechanism underlying this monocyte-specific regulation of Elk-1 expression, we cloned and analyzed the human elk-1 promoter. We were able to determine the transcriptional start site of this promoter, and we further located a TATA-like element immediately upstream of the start site and identified an intronic Egr-1 binding site as a potential TPA-responsive element. The TATA box and the Egr-1 binding site were each found to be located within a hypersensitive area, HS 1 and HS 2, respectively. More importantly, HS 2 was observed to exist only in monocytic cells and not in cells showing a constitutive expression of elk-1. We assume that Elk-1 serves a specific function, e.g. as an effector of a particular stimulus, in differentiating monocytes, which cannot be replaced by another TCF such as Sap1a, whose expression was found to be unchanged upon treatment with TPA.

MATERIALS AND METHODS

Accession Number—The genomic sequence of the human elk-1 locus provided here (see Fig. 3) is deposited under Y11432 HSELK1 in the EMBL Nucleotide Sequence Data base. Upon scanning this sequence against the data base no significant relatedness to other sequences was observed.

Library Screening, Cloning, and Sequencing—For screening of a

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The nucleotide sequence(s) reported in this paper has been submitted to the GenBank™/EBI Data Bank with accession number(s) Y11432 HSELK1.

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The abbreviations used are: TCFs, ternary complex factor(s); SRF, serum response factor; SRE, serum response element; TPA, 12-O-tetradecanoylphorbol-13-acetate; HS, hypersensitive site; PIPES, 1,4-piperazinediethanesulfonic acid; bp, base pairs.

1 The potential role of a downstream intronic sequence for Elk-1 gene expression in monocytes.
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Fig. 1. Physical maps of the genomic clones (B21, B6, and B9) and the structural organization of the human elk-1 gene. The position of the first five exons is shown relative to the transcriptional start site. Selected restriction sites are indicated.

Human genomic λ library (CLONTECH), the 1.3-kilobase pair EcoRI/BamHI fragment and the 0.23-kilobase pair EcoRI/NarI fragment of the human elk-1 cDNA (15) were labeled with 32P using a random priming kit (Stratagene). After hybridizations in hybridization buffer (50% formamide, 4 × SSPE, 5 × Denhardt’s solution, 100 μg/ml sonicated denatured salmon sperm DNA, 1% SDS, 10% dextran sulfate) at 42 °C for 16 h, filters were washed once in 2 × SSC and 0.2% SDS for 5 min at room temperature and twice in 0.5 × SSC and 0.2% SDS for 30 min at 65 °C. Positive plaques were identified by exposure of the filters to x-ray films (Kodak) for 3 days. The phage DNA of four positive plaques was isolated using ultracentrifugation (140,000 g) and exhaustive organic extractions (16). After restriction analyses of the phage DNA, appropriate fragments were subcloned into pBluescript (Promega) for further analysis. Both strands were sequenced by the dideoxy chain termination method, performed after progressive deletion of the subcloned genomic fragments using exonuclease III (Nested Deletion Kit; Promega).

Sequence Analysis—For the identification of putative regulatory elements the programs PC/Gene (IntelliGenetic, Inc.) and Transfac (E. Wingender, GBF Braunschweig, Germany) were employed.

Cell Culture—The cell lines used were ML-1 (myeloblast-like cells (17)), U-937 (histiocytic lymphoma cells (18)), RK 13 (rabbit kidney epithelial-like cells; ATCC CCL37), CV-1 (African green monkey kidney-derived cells; ATCC CCL70), and TE65 (osteosarcoma cells; ATCC CRL 1543). U-937 and ML-1 cells were grown in RPMI 1640 medium (+Glutamax, Life Technologies, Inc.). Cell density was kept below 105/ml. RK 13 cells were grown in Eagle’s minimum essential medium containing Earle’s salts and 4.5 g/liter glucose (Life Technologies, Inc.). TE65 and CV-1 cells were grown in Dulbecco’s modified Eagle’s medium containing 4.5 g/liter glucose (Life Technologies, Inc.). All media contained 10% fetal calf serum (Biochrom), streptomycin (100 μg/ml), and penicillin (100 units/ml).

Isolation and Analysis of RNA—For Northern blot analyses, total RNA was prepared following the one-step protocol of Chomczynski and Sacchi (19). Electrophoresis of RNA and transfer onto nylon membranes (GeneScreen Plus, NEN Life Science Products) were performed essentially as described (20). After UV cross-linking, prehybridization and hybridization were performed at 42 °C overnight in hybridization solution (50% formamide, 4 × SSPE, 5 × Denhardt’s solution, 100 μg/ml sonicated denatured salmon sperm DNA, 1% SDS, 10% dextran sulfate). Filters were washed once in 2 × SSC and 0.2% SDS for 5 min at room temperature and twice in 0.2 × SSC and 0.2% SDS for 30 min at 65 °C. Radioactive signals were detected by exposing the filter to an x-ray film (Kodak) for 5–10 days or, for quantitation, to a PhosphorImager screen (Fuji) for overnight.

For RNase protection and primer extension analysis, poly(A)+-selected RNA from TE65 cells was used. After lysis and homogenization of cells in 4 mM guanidinium isothiocyanate (25 mM sodium acetate (pH 5.5), 0.1% 2-mercaptoethanol), total RNA was purified using CsCl density gradient centrifugation. After butanol/chloroform extraction and precipitation, the pellet was dissolved in water, and poly(A)+ RNA was isolated using the mRNA purification kit from Amersham Pharmacia Biotech.

Detection of DNase I-hypersensitive Sites—Cells were collected by centrifugation at 500 × g for 10 min at 4 °C and washed twice in ice-cold phosphate-buffered saline. The cell pellet was resuspended in approximately 5 volumes of RSB buffer (10 mM Tris-HCl (pH 7.4), 10 mM NaCl, 3 mM MgCl2) containing 0.02% Nonidet P-40 and held on ice for 10 min. The nuclei were collected by centrifugation at 700 × g for 10 min at 4 °C and washed twice in ice-cold RSB buffer without Nonidet P-40. 100 μl of the nuclei suspension, adjusted to an optical density of 40 (measured at 260 nm as a 1:100 dilution in 1 N NaOH), was incubated with various amounts of DNase I (Sigma) for 10 min at 37 °C. The reaction was terminated by adding 100 μl of stop buffer (20 mM Tris-HCl (pH 8), 10 mM EDTA, 1% (w/v) SDS, 600 mM NaCl, 400 μg/ml proteinase K). After careful mixing, the solution was rotated overnight at 37 °C. DNA was extracted using phenol/chloroform and treated with RNase A (0.1 mg/ml) and RNase T1 (1,500 units/ml) for 2 h at 37 °C. After another extraction with phenol/chloroform, DNA was precipitated by the addition of sodium acetate and isopropl alcohol. For Southern analysis (21) 25 μg of DNA was incubated with 100 units of the appropriate restriction enzyme at 37 °C for 16 h.

RNAse Protection Analysis—2 μg of poly(A)+-selected RNA was hybridized with 200,000 cpm of 32P-labeled probe (purified using a denaturing acrylamide gel) in 30 μl of hybridization buffer (80% formamide, 40 mM PIPES (pH 6.4), 400 mM NaCl, 1 mM EDTA) at 50 °C for 16 h, after heat denaturation at 85 °C for 10 min. Excess probe and unhybridized RNA were digested by the addition of 350 μl of RNase reaction buffer (10 mM Tris-HCl (pH 7.5), 300 mM NaCl, 5 mM EDTA, 40 μg/ml RNase A, 2 μg/ml RNase T1) and incubation at 30 °C for the indicated time. The reaction was terminated by the addition of 50 μg of proteinase K and SDS (final concentration 0.5%), incubation at 37 °C for 15 min, phenol extraction, and ethanol precipitation. The pellets were dissolved in 5 μl of 90% formamide and 10 mM EDTA loading buffer. Nucleic acids were separated on an 8 m urea, 8% acrylamide gel. Radioactive signals were detected by autoradiography using intensifying screens.

Primer Extension Analysis—After heat denaturation at 85 °C for 10 min, 2 μg of poly(A)+ RNA was hybridized with 200,000 cpm 32P-labeled oligonucleotide (0.5 ng) in 15 μl of hybridization buffer (10 mM Tris-HCl (pH 8.3), 150 mM KCl, 1 mM EDTA) at 42 °C for 16 h. The primer extension reaction was started by the addition of 45 μl of reverse transcriptase reaction buffer (23 mM Tris-HCl (pH 8.3), 50 mM KCl, 10 mM MgCl2, 5 mM dithiothreitol, 1 mM dNTP, 0.3 mM EDTA) containing 5 units of murine mammary tumor virus reverse transcriptase (RNase H minus, Promega). After incubation at 42 °C for 60 min, the RNA template was removed by adding 105 μl of RNase mix. After incubation at 37 °C for 15 min followed by phenol extraction, the DNA was precipitated with ethanol. The pellets were dissolved in 5 μl of 90% formamide and 10 mM EDTA loading buffer, and the DNA was separated on an 8 m urea, 8% acrylamide gel. Radioactive signals were detected by autoradiography using intensifying screens.

Preparation of Cell Extracts—Nuclear and cytosolic fractions of cells were prepared following the protocol of Andrews and Faller (22). Briefly, cells were collected by centrifugation at 500 × g for 10 min at 4 °C and washed twice in ice-cold phosphate-buffered saline. The cell pellets containing 5–15 × 106 cells were resuspended in 400 μl of buffer A (10 mM Hepes (pH 7.9), 1.5 mM MgCl2, 10 mM NaCl, 0.5 mM dithiothreitol, 1 mM Na2VO4, 10 mM NaF, 20 mM 2- phosphoglycerate, 1 mM p-nitrophenylphosphate, 2.8 μg/ml aprotinin, 0.2 mM phenylmethylsulfonyl fluoride, 0.5 μg/ml pepstatin, 0.5 mM benzamidine, 2.5 μg/ml leupeptin, 5 units/ml a2-macroglobulin) and kept on ice for 10 min. The cells were homogenized using a syringe. After centrifugation (500 × g)
for 10 min at 4 °C), the supernatant was frozen in liquid nitrogen as "cytosolic fraction." The nuclear pellet was resuspended in 50 μl of extraction buffer C (10 mM Hepes (pH 7.9), 25% glycerol, 420 mM NaCl, 1.5 mM MgCl₂, 0.2 mM EDTA, 0.5 mM dithiothreitol, 1 mM Na₃VO₄, 10 mM NaN₃, 20 mM 2-phosphoglycerate, 1 mM p-nitrophenylphosphate, 2.8 μg/ml aprotinin, 0.2 mM phenylmethylsulfonfyl fluoride, 0.5 μg/ml pepstatin, 0.5 mM benzamidine, 2.5 μg/ml leupeptin, 5 units/ml α₂-macroglobulin) and kept on ice for 30 min. After centrifugation (15,000 × g for 10 min at 4 °C) the supernatant was frozen in liquid nitrogen as nuclear extract.

Electromobility Shift Assays—Reaction mixtures contained the following components: 6.5 μl of binding buffer (10 mM Hepes (pH 7.9), 5 mM MgCl₂, 0.1 mM EDTA, 10 mM NaN₃), 1 μl of 10 × SED (20 mM spermidine, 25 mM EDTA, 135 mM dithiothreitol), 1 μl of bovine serum albumin (4 mg/ml), 1 μl of nuclear extract (5 μg), and 2 μg of DNA probe. The mixture was incubated at room temperature for 30 min in a 37°C water bath and then loaded on a 5% non-denaturing polyacrylamide gel.
albumin (20 mg/ml), 1 μl of salmon sperm DNA (0.5 μg/ml), 1–2 ng of 32P-labeled probe (30,000–50,000 cpm) and 1–2 μl of in vitro translated protein or of a nuclear extract (23).

Western Blotting—Nuclear extracts or cytosolic fractions were separated by SDS, 8% polyacrylamide gel electrophoresis before electrophoretic transfer onto a Hybond C Super membrane (Amersham Pharmacia Biotech). After blocking with 5% nonfat dry milk, the membranes were incubated with an anti-EGR-1 antiserum (mouse anti-p82-EGR-1, Santa Cruz) at a concentration of 0.5 μg/ml in TBS for 1 h at room temperature. The blots were subsequently incubated for 1 h at room temperature with an anti-mouse horseradish peroxidase-conjugated antibody prior to exposure to the ECL substrate (Amersham Pharmacia Biotech). For detection of the signals x-ray films (Kodak) were exposed for 10–30 s. All Western blotting reagents were from Amersham Pharmacia Biotech.

Flow Cytometry—Approximately 10⁵ cells were washed with ice-cold phosphate-buffered saline containing 2% fetal calf serum and incubated with an anti-EGR-1 antiserum (mouse anti-p82-EGR-1, Santa Cruz) at a concentration of 0.5 μg/ml in TBS for 1 h at room temperature. The cells were subsequently incubated for 1 h at room temperature with an anti-mouse horseradish peroxidase-conjugated antibody prior to exposure to the ECL substrate (Amersham Pharmacia Biotech). For detection of the signals x-ray films (Kodak) were exposed for 10–30 s. All Western blotting reagents were from Amersham Pharmacia Biotech.

Construction of Luciferase Reporter Plasmids—Using the subcloned genomic fragments as templates the indicated segments of the promoter region were prepared by polymerase chain reaction, using oligonucleotide primers specific for this region and containing appropriate restriction sites for insertion into the polylinker of the plasmid pKS/L (5) upstream of the luciferase gene.

RESULTS

Identification and Characterization of Human elk-1 Genomic Clones—We screened a human genomic λ library with DNA probes corresponding to the coding sequence and the 5'-untranslated region of human elk-1. The elk-1-specific segments of three phages (B6, B9, B21) were analyzed in detail. B6 and B9 were overlapping clones that contained the first three translated exons III–V of the elk-1 gene encoding amino acids 1–337.
The B21 clone included the 5'-untranslated regions (exons I and II), large parts of the second intron, and sequences upstream of exon I. Note that the last untranslated exon, exon II, is separated from the first translated exon, exon III, by a large, approximately 12-kilobase pair-long intron. Because of the length of this intron, the B21 clone did not share any sequence with B6 or B9. Using fluorescence in situ hybridization analyses, however, we have reported previously that B6 and B21 hybridize to the same locus on the X chromosome (24). This indicates that these sequences are genomically linked. This was confirmed recently by Mills and co-workers who isolated genomic clones that contained sequences of both B6 and B21. In the process of screening with the B6 or B21 sequence we also identified an elk-1 related pseudogene that we mapped to chromosome 14q32.3

Identification of the Transcriptional Start Site of the Human elk-1 Gene—The human osteosarcoma cell line TE85 constitutively expresses elk-1 at high levels. Poly(A)+ RNA was isolated from these cells and analyzed by the primer extension technique. Using an oligonucleotide corresponding to a stretch of nucleotides between positions +166 and +186 of the B21 sequence (see Fig. 3), a major transcriptional start site was identified (termed nucleotide position +1) (Fig. 2A, arrow). The smaller fragment in Fig. 2A may suggest an additional, minor transcriptional start site some 65 bp downstream of the major one. However, by using RNase protection assays, performed with the XhoI/SacI fragment as a probe (Fig. 2B), we could only confirm the existence of the major start site (data not shown). According to these data, the start site is located 39 nucleotides further upstream of the 5'-end of the previously published elk-1 cDNA sequence (15). It places the transcriptional start site approximately 25 bp downstream of a TATA-like sequence (Fig. 3).

The TATA-like Element in the elk-1 Gene Supports Transcription in Transient Transfection Experiments—Several DNA fragments of human the elk-1 gene including the TATA motif (Fig. 4A) were cloned upstream of a luciferase gene and tested for their abilities to support transcription in transient transfection experiments using RK 13 cells. Transfection with plasmid e-43, which contained the minimal fragment 243/163, resulted in a luciferase activity that was approximately 10-fold higher than that obtained with the control plasmid missing this fragment (Fig. 4B). This suggests that the −43/+63 fragment can function as a promoter. To test whether flanking sequences may affect promoter activity, we first gradually extended the elk-1 promoter 5’ up to nucleotide at position −936. Highest activity was found with the e-480 promoter construct, suggesting that enhancer element(s) were located between −43 and −480. This region contains potential binding sites for several transcription factors, including Sp1, Ap1, cAMP re-

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2 F. Mills, personal communication.
3 U. Lehmann and A. Nordheim, unpublished data.
4 R. A. Hipkind, unpublished data.
response element-binding protein, and the CCAAT-binding protein, and the CCAAT-binding protein (Fig. 3). Further extension to −711 slightly decreased transcription from the elk-1 promoter. The sequence between −711 and −936 was found to have a repressive effect on elk-1 promoter activity, suggesting that this region contains silencer elements(s). When 3′-flanking sequences down to +679 were added to the e-711 promoter fragment, elk-1 promoter activity was increased by 2-fold. This implies that this downstream sequence, which contains binding sites for Egr-1 and SRF/TCF (Fig. 3), may contribute to the regulation of elk-1 gene transcription. Similar data regarding the differential activities of the different promoter fragments were obtained when transfection experiments were performed with CV-1 cells (data not shown).

A DNase I-hypersensitive Site Is Located around the TATA-like Element in elk-1-expressing TE85 Cells—Transcriptionally active regions in the chromatin are often susceptible to DNase I treatment. To identify DNase I-hypersensitive site(s) within the human elk-1 promoter, we subjected nuclei isolated from TE85 cells, which express elk-1 constitutively, to DNase I digestion. After deproteinization and HindIII treatment, the genomic DNA was analyzed by Southern blot hybridization. Fig. 5A shows a typical DNase I cleavage pattern obtained from hybridizing the blotted DNA with the elk-1-specific probe A (Fig. 5B). Only one fragment (panel A, arrow pointing leftward) smaller than the full-length HindIII fragment (arrow pointing rightward) was visualized under these conditions. Note that even high concentrations of DNase I, which allowed complete digestion of the HindIII fragment, did not lead to the generation of any other distinct fragment. The size of the fragment resulting from DNase I treatment positions the DNase I-hypersensitive site (HS 1) to an area around the TATA-like element, in close proximity to the XhoI site shown in Fig. 5B. Results obtained from reprobing the blots with elk-1-specific DNA probe B (Fig. 5B) allowed the same conclusion as to the location of HS 1 (data not shown). As with probe A, no additional hypersensitive site could be identified with probe B. These probing strategies also allowed us to estimate HS 1 to extend over a region of approximately 150–200 bp.

A Second Constitutive DNase I-hypersensitive Site Downstream of the elk-1 TATA-like Element Was Identified in the Human Pre-monocytic Cell Line ML-1, Which Displays Inducible elk-1 Expression—In contrast to a variety of non-monocytic cell lines, the human pre-monocytic cell lines ML-1 and U-937 were found to synthesize very low basal levels of elk-1 mRNA (Fig. 6A and data not shown). Treatment with TPA triggered these cells to differentiate to monocytes (Fig. 6C). This was accompanied by the expression of monocytic markers such as CD14 and CD11C and by down-regulation of the expression of pre-monocytic proteins like MPO (Fig. 6A and B). In differentiating ML-1 (and U-937) cells, elk-1 mRNA levels were found to be strongly up-regulated (Fig. 6A), which resulted in enhanced Elk-1 DNA binding activity (data not shown). Because
TPA stimulation did not change the half-life of elk-1 mRNA (t1/2 = 5 h) (data not shown), it is likely that transcriptional activation was responsible for the up-regulation of elk-1. In contrast to elk-1, expression levels of other TCFs (Sap-1α and Net) remained unchanged (data not shown), suggesting that Elk-1 fulfills a specific function in differentiating monocytes. By analyzing ML-1 chromosomal DNA for elk-1-specific DNase I-hypersensitive sites, we found an additional hypersensitive site, HS 2, downstream of the TATA-like element within intron I (Fig. 7). We estimate HS 2 to extend over some 150–200 bp in length. HS 2 was also found in nonstimulated ML-1 cells (data not shown), demonstrating that HS 2 was present before the elk-1 gene was transcriptionally activated by TPA. In nonmonocytic Jurkat T cells, SKW 6.4 B cells and, as shown above, TE85 cells, which constitutively express Elk-1, HS 2 was not observed (data not shown). This suggests that a pre-established open chromatin structure within intron I represents a prerequisite for inducible elk-1 expression in monocytic cells.

HS 2 Contains Binding Elements for TPAstimulation, which results in the expression of SRF/Elk-1. To test this possibility, we first analyzed the abilities of Elk-1 and SRF/Elk-1 to bind to the putative Elk-1 binding site within intron I (lane 1) but failed to interact with a mutated version of this sequence (lane 3) and form a ternary complex with Elk-1 (cIII, lane 3).

We next compared the expression pattern of Egr-1 and SRF with that of Elk-1 in differentiating ML-1 cells. Egr-1 mRNA was detectable as early as 1 h of exposure to TPA (data not shown), preceding the rise in elk-1 expression. Typically for an immediate-early response gene (26, 28), mRNA expression of Egr-1 was found to be transient, starting to decline rapidly after an additional 2–3 h of TPA treatment (Fig. 8A). The Egr-1 protein level and DNA binding activity were found to be strongly increased after 2 h of exposure to TPA (Fig. 8B). The ability of Egr-1 to bind to the intronic Elk-1 binding site was found to decrease strongly subsequent to the initial 2-h TPA treatment (Fig. 8B, lane 2). Thus, Elk-1 may be involved in the initial TPA-dependent up-regulation of elk-1 expression but is unlikely to contribute to elk-1 transcription at a later stage of the differentiation process. In contrast to the Elk-1 expression, SRF mRNA levels were not found to increase significantly before 6 h of TPA treatment, a time at which elk-1 expression already reached its maximum (Fig. 8A). This suggests that SRF could not participate in the TPA-induced rapid increase in elk-1 transcription but may have been needed to maintain a high expression level of Elk-1 during monocytic differentiation. For this potential function, SRF might have required the newly produced Elk-1 and, in this way, may have initiated a positive feedback loop for Elk-1 expression.

**DISCUSSION**

In an effort to characterize the human elk-1 promoter, we identified a TATA-like element immediately upstream of a major transcriptional start site, as determined by primer extension analyses and RNase protection assays. We could show that a DNase I-hypersensitive site, termed HS 1, was surrounding the TATA-like sequence, suggesting that the TATA-containing region was accessible for DNA-binding factors. HS 1 extended over an approximate region of 150–200 bp and accordingly may have been generated by the removal of one nucleosome. The presence of HS 1 was independent of whether expression of elk-1 was constitutive, as in TE85 cells, or inducible, as in ML-1 cells. We could further demonstrate that a minimal elk-1 promoter, containing the TATA motif, was able to support transcription. It is therefore very likely that this TATA sequence functions as a TATA box for elk-1 transcription in vivo.

The downstream sequence, including exon I, most of exon II, and the intron separating these exons, was found to contribute to elk-1 promoter activity in transient transfections experiments. Interestingly, a second DNase I-hypersensitive site, HS 2, was found in the intronic sequence. HS 2 was constitutively present in the monocytic cell lines ML-1 and U-937, which display inducible elk-1 expression. In contrast, HS 2 was not found in non-monocytic cells lines TE85, Jurkat, and SKW 6.4, which expressed elk-1 at constant high levels. This may suggest that the potential for the inducibility of elk-1 expression is associated with the constitutive presence of HS 2 and the accessibility of this area for certain DNA-binding proteins. Two transcription factors, Egr-1 and SRF, could specifically bind to the HS 2 region and were found to be up-regulated in differentiating ML-1 cells along with elk-1. We have also preliminary data suggesting that both Egr-1 and SRF can increase elk-1 expression already reached its maximum. This may suggest that Elk-1 may be important...
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for initiation of elk-1 transcription at early stage of the differentiation process, to be replaced by other factors that allow long lasting up-regulation of elk-1 expression. Such a factor could be an Ets protein because a putative Ets binding site was found to be located immediately downstream of the Egr-1 site. Several Ets proteins, including PU.1, Ets1, and GABPβa, are expressed in monocytic cells (30–32). Of these, PU.1 was observed to be able to bind to the Ets binding site of the elk-1 promoter (data not shown) and was reported to be up-regulated during differentiation of pre-monocytic U-937 cells (32). U-937 was also found here to show an increased elk-1 expression upon TPA treatment (data not shown). Further investigations are required to elucidate the regulatory function of the intronic sequence containing HS 2 and the roles of Egr-1, Ets factors and SRF for elk-1 transcriptional regulation in monocytic cells.

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