The Mouse Gene for Vascular Endothelial Growth Factor

GENOMIC STRUCTURE, DEFINITION OF THE TRANSCRIPTIONAL UNIT, AND CHARACTERIZATION OF TRANSCRIPTIONAL AND POST-TRANSCRIPTIONAL REGULATORY SEQUENCES

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We describe the genomic organization and functional characterization of the mouse gene encoding vascular endothelial growth factor (VEGF), a polypeptide implicated in embryonic vascular development and postnatal angiogenesis. The coding region for mouse VEGF is interrupted by seven introns and encompasses approximately 14 kilobases. Organization of exons suggests that, similar to the human VEGF gene, alternative splicing generates the 120-, 164-, and 188-amino acid isoforms, but does not predict a fourth VEGF isoform corresponding to human VEGF-S. Approximately 1.2 kilobases of 5’-flanking region have been sequenced, and primer extension analysis identified a single major transcription initiation site, notably lacking TATA or CCAT consensus sequences. The 5’-flanking region is sufficient to promote a 7-fold induction of basal transcription. The genomic region encoding the 3’-untranslated region was determined by Northern and nuclease mapping analysis. Investigation of mRNA sequences responsible for the rapid turnover of VEGF mRNA (mRNA half-life, <1 h) (Shima, D. T., Deutsch, U., and D’Amore, P. A. (1995) FEBS Lett. 370, 203–208) revealed that the 3’-untranslated region was sufficient to trigger the rapid turnover of a normally long-lived reporter mRNA in vitro. These data and reagents will allow the molecular and genetic analysis of mechanisms that control the developmental and pathological expression of VEGF.

The mediators of neovascularization comprise a diverse collection of growth stimulators and inhibitors that have been designated because of their abilities to affect angiogenesis in vivo and/or endothelial cell proliferation in vitro (for review, see Ref. 1). Vascular endothelial growth factor (VEGF) was initially identified based on its ability to stimulate vascular permeability (called VPF, for vascular permeability factor) and was subsequently demonstrated to be an endothelial cell-specific mitogen and angiogenic factor (2, 3). In vivo, VEGF expression has been correlated with embryonic, physiological, and pathological blood vessel growth (4–6). VEGF’s role as a mediator of angiogenesis has been confirmed in two distinct pathologies; VEGF has been demonstrated to be a necessary component of experimental tumor angiogenesis and tumor growth in rodents (7, 8), and, more recently, it has been shown to be causative in the development of ocular angiogenesis secondary to retinal ischemia (9, 10).

The spatial and temporal expression patterns of VEGF and its tyrosine kinase receptors, flt-1 and flk-1/KDR, during periods of blood vessel growth have also led investigators to suggest a paracrine role for VEGF during the development of the embryonic vasculature (11, 12). The VEGF receptor flk-1 is expressed in regions of the early mesoderm, which are presumed to give rise to angioblasts, and is currently the earliest known molecular marker for the endothelial cell lineage. During later stages of embryogenesis, flt-1 and flk-1 receptor mRNA are restricted to the endothelium of vascular cords and blood islands, with VEGF mRNA expressed in adjacent embryonic tissues (13). Proof of a role for VEGF in vessel development comes from recent studies in which VEGF receptors were deleted by targeted disruption. Mice embryos, in which the flk-1 receptor was deleted by targeted disruption, lacked blood islands and died between days 8.5 and 9.5. In these embryos, no organized blood vessels were observed and hematopoiesis was dramatically reduced (14). Mice, in which flt-1 was mutated by targeted disruption, were able to form endothelial cells but unable to assemble them into normal vascular channels and thus died at mid-somite stages (15).

From these and other observations, VEGF emerges as a mediator of vasculogenic and angiogenic events associated with a wide range of biological events (16). Consistent with this concept, the local and systemic signals responsible for orchestrating the growth and regression of new blood vessels must ultimately regulate VEGF gene expression. Numerous effectors of VEGF gene expression have been identified, including cAMP, steroid hormones, protein kinase C agonists, polypeptide growth factors, oxygen, free radicals, glucose, cobalt, and iron. The potential mechanisms through which these agents modulate gene expression are varied, and include transcriptional regulation through AP-1, AP-2, steroid hormone receptors, p53, and NFκB, as well as post-transcriptional control of mRNA stability (17–21).

To begin an analysis of the relevant mechanisms controlling the developmental and pathological expression of VEGF and to develop reagents for defining the role of VEGF in embryonic development using mouse molecular genetics, we have isolated and characterized the mouse VEGF gene. The structure of the

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† The nucleotide sequence(s) reported in this paper has been submitted to the GenBank™/EMBL Data Bank with accession number(s) U41383.

‡ The abbreviations used are: VEGF, vascular endothelial growth factor; kb, kilobase(s); PIPES, 1,4-piperazinediethanesulfonic acid; bp, base pair(s); UTR, untranslated region; LTR, long terminal repeat.

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gene was determined by restriction mapping, sequencing of intron-exon junctions, definition of the transcription initiation and termination sites, and analysis of the sequence representing the VEGF proximal promoter. Using these structural data, we have assayed the mouse VEGF gene for cis-regions responsible for different aspects of gene regulation and to this end describe gene segments sufficient to promote basal transcriptional activity and post-transcriptional regulation of the VEGF gene.

MATERIALS AND METHODS

Gene Isolation and Physical Mapping—A 129 strain mouse cosmid genomic library prepared from liver (Stratagene) was screened with random primer 32P-labeled probes corresponding to the mouse VEGF cDNA coding region (kindly provided by Dr. Kevin Claffey, Beth Israel Hospital, Boston). Approximately 5 x 10^9 colonies were screened on nylon filters (Hybond N, Amersham) by hybridizing the VEGF probe overnight at 65 °C in 500 μm phosphate, pH 7.2, 7% SDS, 1% bovine serum albumin, 1 mM EDTA, and 100 μg/ml sheared, denatured salmon sperm DNA. Several positive clones were identified in this initial screen. After two rounds of rescreening and colony purification, cosmid DNA was isolated and analyzed by restriction digestion and Southern blot to identify useful DNA fragments for subcloning. Restriction-digested cosmid insert DNAs were subcloned into pBSII (Stratagene) for further analysis. Nucleotide sequencing of subclones indicated that VEGF cosmid clones encompassed the 5'-flanking of the VEGF gene (beginning near exon 4) and extended 30–40 kb in the 3'-direction.

To isolate additional clones encompassing the 5'-end of VEGF, a 280-bp cDNA probe template spanning exons 1–3 was generated, using the polymerase chain reaction, and used to rescreen 5 x 10^9 colonies. No additional VEGF clones were identified. As an alternative, the exon 1–3 probe was used to screen a 129 mouse genomic library in the lambda vector EMBL3 (kindly provided by Dr. Richard Moss, Brigham and Women's Hospital, Boston). The library was screened on charged nylon membranes (GeneScreen Plus, DuPont NEN) by hybridization at 42 °C with 50% formamide, 5 x Denhardt's solution, 0.5% SDS, and 100 μg/ml sheared, denatured salmon sperm DNA. Two positive clones were identified from screening 1 x 10^6 plaques. After two rounds of plaque purification, phage DNA was isolated and analyzed further. Restriction digestion and Southern blot analysis of genomic clones indicated that they encompassed the 5'-end of the VEGF gene; one clone, designated lambda 8, overlapped with cos15, a cosmid clone that terminates in the intron upstream of the exon 4 sequence. Sequence data, restriction maps, and Southern blot analysis of the mouse VEGF gene were compiled from 9- and 7.5-kb EcoRI subclones that encompass the VEGF coding region (see Fig. 1).

DNA Sequencing—Genomic fragments cloned into pBSII were sequenced with vector and gene-specific primers using the Sequenase 2.0 kit (USB, Cleveland, OH). Lipoprotein Lipase (Life Technologies, Inc.) was kindly provided by Dr. Greg Robinson, Hybridon Inc., Worcester, MA (see Materials and Methods). The mouse VEGF cDNA sequences were analyzed, based on alignment of the mouse VEGF cDNA sequence, with the published human VEGF exon structure (22). Nucleotide sequence analysis was performed with MacDNA's 3.4 software (Hitachi).

Southern Blot Analysis—Genomic DNA was prepared from 129 mouse spleen using standard protocols (23). Restriction enzyme-digested DNA (10 μg) was electrophoresed in 0.7% TBE-agarose gels overnight. Following electrophoresis, DNA was depurinated in 0.25 M HCl, denatured, and transferred to charged nylon (GeneScreen Plus) in 0.4 M NaOH. Membranes were rinsed in 2 x SSPE, dried, and hybridized as described for phage library screening. The probe was a 700-bp Smal DNA fragment that was ligated into pBSII. nuclease protection assay was performed as described (28) and used to normalize luciferase assay values.

Assays— Luciferase activity in cell extracts was assayed 48 h post-transfection according to standard protocols (27) using a Biocan luminometer (Wallace).

neat VEGF Fusion Constructs, Generation of Stable Cell Lines, and mRNA Stability Assays—A 4.4-kb Smal fragment, encompassing 2 kb of the VEGF 3'-UTR, including poly(A) signals, and 2.4 kb of 3'-flanking genomic DNA was ligated into the Smal site downstream of a neo resistance gene (neo) open reading frame in the eukaryotic expression plasmid LTR-neo (kindly provided by Dr. Michael Cole, Princeton University, Princeton, N.J.). LTR-neo transcripts terminate with SV40 UTR and poly(A) sequences (29). LTR-neo and LTR-VEGF constructs were individually transfected by electroporation into C127, a mammary carcinoma mouse cell line. The poly(A) addition site was cotransfected with test plasmids to allow selection of stable transfectants with hygromycin (200 μg/ml). Stable colonies (50–100) were pooled and expanded for use in mRNA stability assays.

Actinomycin D chase assays and Northern blot analysis of total RNA were performed as described (20). Briefly, confluent cells were incubated in culture media containing actinomycin D (5 μg/ml) and subsequence transfected for 0–8 h in standard culture conditions. Total RNA was extracted by the modified acid-phenol method using RNAzol B (Tel-test) and analyzed by Northern blot for LTR-neo, LTR-VEGF, and
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The organization of the coding region and the UTRs (22). The organization of introns, with exons 1 and 8 containing relatively short segments, is indicated. The VEGF open reading frame is indicated by black shading.

RESULTS

Isolation and Preliminary Characterization of the Mouse VEGF Gene—To isolate the VEGF gene, a probe representing the mouse VEGF164 open reading frame was used to screen a cosmid genomic DNA library prepared from strain 129 mouse liver. From an initial screen of 5 x 10^6 colonies, several positive clones were identified and analyzed further. Sequencing of the termini of cosmid inserts indicated that all of the clones originated within the middle of the VEGF coding region and proceeded approximately 30-40 kb in the 3' direction. Rescreening the cosmid library with the same probe, or a probe corresponding to the 5'-half of the VEGF open reading frame, did not identify cosmids harboring the 5'-end of the VEGF gene. Because the 5'-end of VEGF did not appear to be represented in the cosmid library, a 129 mouse genomic DNA library was constructed using the EMBl3 lambda phage vector. Screening with a probe spanning exons 1-3 to screen 1 x 10^7 plaques, two clones spanning the 5'-end of the VEGF gene were identified. One of these clones, designated lambda 8, also overlapped with regions represented in a VEGF cosmid clone (cos15); therefore, these two clones were used for structural analysis of the VEGF gene. The relation of the phage and cosmid clones to the mouse VEGF gene are shown schematically in Fig. 1.

A restriction map for the two overlapping clones was assembled by single, double, and partial digestions with EcoRI, BamHI, AccI, HindIII, and SmaI restriction enzymes (Fig. 1). The locations of exons relative to the restriction map were established by nucleotide sequencing the restriction sites proximal to exons and Southern blot analysis of cloned DNA with exon-specific probes (data not shown). Restriction enzyme analysis of genomic DNA by Southern blot was used to confirm mapping data and verified that mouse VEGF was encoded as a single copy gene (Fig. 2). The overlapping genomic clones define a contiguous stretch of 45 kb of DNA, of which approximately 14 kb represents the mouse VEGF coding region.

EXON-INTRON NUCLEOTIDE SEQUENCE AND ORGANIZATION—To determine the genomic organization of the mouse VEGF gene, nucleotide sequences of the coding regions and intron-exon borders were determined (Fig. 3) and aligned with published mouse VEGF cDNA sequences (4). Similar to the human gene, the coding region of mouse VEGF is interrupted by seven introns, with exons 1 and 8 containing relatively short segments of the coding region and the UTRs (22). The organization of transcriptional initiation for VEGF mRNA in the C127I mouse mammary epithelial cell line. Low levels of VEGF mRNA were detectable in the C127I cells grown under typical culture conditions, but were dramatically induced by exposing the cells to a low oxygen environment (Fig. 5).
Primer extension using poly(A)$^{1}$ RNA was used as a negative control. After reverse transcription and denaturing gel electrophoresis, a single abundant primer extension product of 123 bp was detected in the mRNA from hypoxic cell cultures (Fig. 5B). As would be expected, significantly lower levels of reaction product were seen in mRNA from normoxic cultures or in poly(A)$^{2}$ RNA from normoxic and hypoxic cultures. The precise location of transcription initiation was determined by comparison of the migration of the extension product with a sequencing gel ladder generated using the same primer and is designated $^{11}$. Nucleotide sequence is shown for regions upstream of the mouse VEGF open reading frame including the 1.0-kb 5'-UTR and 1.2 kb of the proximal promoter region (Fig. 3).

To determine if the sequences upstream of the transcription initiation site are sufficient to direct transcription, a 1.6-kb fragment, including 1.2 kb of 5'-flanking region and 0.4 kb of 5'-UTR, was fused in both orientations to a promoterless luciferase transcription reporter gene and examined for the ability to mediate basal transcription. In addition, 5'-deletions in the putative promoter region were also monitored for their effect on reporter activity.

The murine astrocytoma cell line, C6, was transiently transfected with reporter constructs, and cell extracts were assayed for luciferase activity 48 h post-transfection. VEGF sequences fused to the reporter in the appropriate transcriptional orientation resulted in a 7-fold increase in luciferase activity when compared to a promoterless luciferase construct (Fig. 6). In contrast, VEGF sequences fused in the opposite transcriptional orientation did not induce a significant level of reporter activity. Deletion of 445 or 770 bp from the 5'-end of

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**Fig. 3.** Nucleotide sequence for mouse VEGF intron-exon borders and the sequence surrounding the mouse VEGF transcription initiation site. Intron and 5'UTR sequence is shown in lower case letters, coding sequence is shown in upper case letters, and the VEGF translation start and stop codons are boxed. An arrow indicates the initiation site of RNA synthesis and is designated $^{11}$. Consensus binding sites for relevant transcription factors are marked as follows: AP-1, thin line; AP-2, heavy hatched line; NFkB, broken line; Sp1, heavy line.

**Fig. 4.** Comparison of mouse exon-intron 6 with the corresponding region of human VEGF. The nucleotide insertion in the human sequence that creates a continuous open reading frame in VEGF$^{206}$ is marked. The translation stop codon present in the homologous region of mouse VEGF genomic DNA is designated with an asterisk. Predicted amino acids are indicated below the nucleotide sequence using a single letter format.

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**Fig. 5.** Primer extension analysis of the VEGF transcription start site. A, Northern blot analysis of total RNA from normoxic and hypoxic cultures of C1271 demonstrating the differences instead by state VEGF mRNA levels. B, primer extension products were separated by electrophoresis on a denaturing polyacrylamide gel along with a DNA sequencing ladder generated with the same oligonucleotide used for primer extension. Poly(A)$^{+}$ and poly(A)$^{-}$ RNA from normoxic (N) or hypoxic (H) cultures of C1271 mouse mammary cells were used in the reactions. A single primer extension product was detected in the poly(A)$^{+}$ RNA, indicating the location of the transcriptional start site (the complementary nucleotide is shown by an asterisk).
Identification of the VEGF Transcription Termination Region—The region of transcriptional termination for VEGF was mapped by RNA hybridization and nuclease protection analyses. First, to approximate the junction of the VEGF 3′-UTR and the 3′-flanking region, genomic probes spanning the putative 3′-region of the VEGF gene were used in a slot blot hybridization analysis of mouse VEGF RNA (Fig. 7A). Independent hybridization of all three probes to the mouse RNA resulted in detectable, though variable, signal (Fig. 7B). No signal was detected in hybridizations to a yeast tRNA negative control. Relative to probes A and B, probe C weakly hybridized to immobilized mouse RNA. From the dramatic decrease in hybridization signal seen for probe C, it was predicted that this region of DNA spanned the junction of VEGF transcriptional termination and the non-transcribed 3′-flanking region.

Nuclease protection analysis of mRNA from C127I cells was used to obtain more precise information on the 3′-end of VEGF transcripts. A 2.2-kb radiolabeled antisense riboprobe was generated from a genomic DNA template and hybridized to total RNA from hypoxic and normoxic cultures of C127I and a yeast tRNA negative control. Following nuclease digestion and electrophoretic separation of nuclease products, a 510–520-nucleotide digestion-resistant product was present solely in total RNA from hypoxic C127I cultures.

Identification of VEGF mRNA Destabilizing Sequences—We have previously demonstrated that VEGF mRNA from cells grown under normoxic conditions is highly unstable, with a half-life of <1 h (20). Whereas the average half-life of cellular mRNAs is approximately 8 h, the mRNAs for many growth factors and oncoproteins (e.g. granulocyte-macrophage colony stimulating factor, c-fos, c-myc) are unstable, with half-lives ranging from 20 to 60 min. The signals responsible for destabilization of these mRNAs are most frequently located in the 3′-UTR (29, 32).

To investigate if a determinant of mRNA destabilization is present in the VEGF 3′-UTR, genomic sequences corresponding to this region, including the putative polyadenylation signal, were fused to a neomycin (neo) reporter mRNA (designated LTR-VEGF). The VEGF 3′-UTR replaces SV40 DNA sequences that normally terminate the neo transcripts. The neoSV40 mRNA fusion (designated LTR-neo) is normally quite stable, with a half-life of >8 h. The addition of destabilization sequences from the 3′ UTR of granulocyte-macrophage colony stimulating factor, c-fos, and c-myc to LTR-neo mRNA has been shown to direct its rapid decay (29).

Actinomycin D chase studies were used to compare the rate of decay for LTR-VEGF mRNA to that of LTR-neo mRNA (control) in the C127I mammary epithelial cell line. As expected, the control LTR-neo mRNA remained stable throughout an 8-h period in the absence of transcription (Fig. 8). In contrast, LTR-VEGF fusion mRNA behaved similar to the endogenous VEGF mRNA, with both VEGF and LTR-VEGF transfectants undergoing rapid decay with half-lives of less than 1 h. Levels of endogenous β-actin mRNA, a transcript with an average half-life, were relatively stable under each experimental condition over the time course of the experiment (33).

DISCUSSION

VEGF has been implicated as a multi-functional effector of vascular cell function. In addition to its well documented angiogenic properties, VEGF is also a potent stimulator of leuko-
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**Fig. 8.** The VEGF 3′-UTR contains a region that promotes destabilization of a normally stable fusion mRNA. LTR-neo and LTR-VEGF transfectants (schematic of fusion mRNA constructs shown; see "Materials and Methods") were used to analyze neo fusion mRNA, VEGF mRNA, and β-actin mRNA decay using an actinomycin D chase protocol. Time (h) after the addition of actinomycin D is marked above each lane.

Cyte migration, vascular permeability, and procoagulant functions in endothelium (34, 35). Moreover, the presence of significant VEGF mRNA and protein in various tissues of the adult suggests an additional role for VEGF in the maintenance of normal vascular cell integrity and/or behavior (4). How VEGF gene expression is regulated during the transition from periods of vascular quiescence to active vascular growth, remodeling, and repair is not understood. To begin to investigate the structure-function relationships critical for the regulation of VEGF expression, we have characterized the mouse VEGF gene.

Mapping the VEGF Transcriptional Unit—We have isolated genomic clones encompassing the VEGF gene, as well as 1.2 kb of 5′-flanking sequence and more than 25 kb of 3′-flanking sequence. Similar to the human gene, the mouse VEGF coding region is distributed across 8 exons, spanning 14 kb of genomic sequence. Exons 1 and 8 contain the translation start and stop codons, respectively, and the extensive UTR regions. Sequence data and primer extension analysis of the transcription initiation site, revealed the presence of a number of transcriptional activators, frequently Sp1, and an initiator element (Inr) that overlaps the region of RNA synthesis.

Sequence analysis of the 1.2-kb region, upstream of the transcriptional start site, revealed that the site of VEGF transcription termination was not previously defined for any of the VEGF genes; therefore, the size of the mouse 3′-UTR and approximate site of poly(A) addition were determined. Results from nuclease protection analysis revealed that the size of VEGF transcriptional termination is approximately 2.2 kb downstream from the end of the VEGF open reading frame. Including a 1.0-kb 5′-UTR, a coding region of 0.5–0.6 kb, and a 3′-UTR of 2.2 kb, the size of a VEGF transcript predicted by mapping data would be 3.7–3.9 kb, a size corresponding to the sizes of the most commonly observed VEGF transcripts.

Genomic Organization of VEGF Splice Variants—Details of the exon-intron structure for mouse VEGF suggest that alternative splicing generates the mRNAs that encode the 120-, 164-, and 188-amino acid isoforms of VEGF similar to the human gene (22). Yet, unlike human VEGF, the sequence structure of mouse exon 6 does not support the existence of a mouse equivalent to the human VEGF206 isoform. An in-frame stop codon is present in the mouse gene in the region corresponding to the human VEGF206 open reading frame. If synthesized and translated, mouse VEGF splicing variants that include this region would be predicted to produce a novel, secreted VEGF protein isoform of approximately 16 kDa. The existence of this mRNA or protein has not been described.

Since little is known about the physiological roles of the four VEGF isoforms, it is difficult to predict the functional significance of a divergence in isoform generation between humans and mice. The putative VEGF206 isoform was identified using the polymerase chain reaction to amplify VEGF-related cDNAs and consists of an alternative splice variant with an extended exon 6 region that results in a 17-amino acid insertion relative to the VEGF189 isoform. Analysis of VEGF synthesis, secretion, and bioactivity in vitro has revealed that an engineered VEGF206 protein shares similar biochemical and functional properties with VEGF189, suggesting that these two isoforms could provide redundant biological functions (31). To date, the biochemical and biological descriptions of VEGF206 have been sparse and have relied on data obtained from the fusion of the N-terminal region of VEGF165 to the partial VEGF206 cDNA clone; the expression of native VEGF206 mRNA or protein by tissue culture cells or in vivo has not been adequately described.

VEGF Promoter Analysis—Similar to other growth-related genes, including human VEGF, consensus TATA and CCAAT sequences for RNA polymerase II-initiated transcription are absent from the mouse VEGF core promoter region. Instead, GC-rich regions resembling consensus binding motifs for Sp1 are situated approximately 50–80 bp upstream of the predicted transcription start site in mouse VEGF. This organization is typical of the core region of many TATA-less promoters, which usually contain binding sites for sequence-specific transcriptional activators, frequently Sp1, and an initiator element (Inr) that overlaps the region of RNA synthesis.

The nucleotide sequence surrounding the VEGF mRNA initiation site, AGAAGCGCA (underline designates first transcribed nucleotide), does not conform to consensus Inr sequences; therefore, an investigation of VEGF transcriptional initiation is likely to shed light on a novel "TATA-less" mechanism for basal gene transcription.

Transient transfection assays indicate that a 1.2-kb segment of 5′-flanking region specifically directs the transcription of a reporter gene in VEGF-producing cells. Deletion of the 1.2-kb region, including the putative transcription initiation site, abolished promoter activity. Results from these functional analyses support transcript mapping and sequence analysis data that identify this DNA segment as the VEGF proximal promoter.

Transfection of C6 rat glioma with constructs deleting either 445 or 770 bp from the 5′-end of the 1.2-kb promoter region resulted in a similar 25% decrease in reporter activity, suggesting that cis-acting elements necessary for basal promoter activity in C6 cells reside in the 450-bp DNA segment deleted from the 5′-end of the promoter fragment. Yet, a relevant promoter activity resides within the first 450 bp upstream to the VEGF gene. Further studies will be required to identify and characterize the cis- and trans-acting components necessary for both basal and inducible regulation of VEGF gene transcription.

Sequence analysis of the 1.2-kb region, upstream of the transcription initiation site, revealed the presence of a number of potential cis-acting regulatory elements. Similar to the human VEGF gene, multiple consensus binding sites for AP-1 and AP-2 transactivating complexes are present. AP-1 activity has been shown to be stimulated by phorbol esters and growth factors, and both CAMP-dependent kinase and protein kinase C
pathways have been implicated in the activation of AP-2 (40, 41). Phorbol esters, peptide growth factors, and intracellular elevation of cAMP also induce steady-state VEGF mRNA, suggesting that the AP-1 and AP-2 consensus sites present in the VEGF promoter may mediate VEGF transcriptional activation in response to these effectors (17, 21). In addition, studies from a number of laboratories indicate that in some cells transcriptional activation plays a role in the up-regulation of VEGF mRNA by hypoxia (42–44). Further, the site of transcriptional initiation and numerous regions within the proximal promoter of mouse VEGF share significant similarity in both sequence and organization with the human homologue. The conserved initiation and numerous regions withintheproximalpromoteroftheVEGF 3'-UTR are primarily responsible for the control of mRNA stability. For the best-studied of these genes, oncogenes and cytokines regulated by post-transcriptional mechanisms controlling transcript stability suggest that certain cellular mRNAs are targeted for destruction. Experimental conditions that induce VEGF mRNA, such as phorbol ester treatment or hypoxia, are known to regulate mRNA stability (20, 32). Investigation of the cellular mechanisms controlling transcript stability suggest that certain mRNAs contain distinct structural elements for positive and negative regulation of mRNA stability. Specific destabilization sequences vary considerably but usually consist of AU-rich elements in the 3'-UTR of unstable mRNAs (46). Multiple AU-rich regions exist throughout the 3'-UTR of mouse VEGF (data not shown) and represent potential candidates for destabilizing sequences. Less is known about sequences that selectively or inducibly promote mRNA stability. For the transferrin receptor, a well studied model of inducible mRNA stability, the 3'-UTR contains a stem-loop sequence that interacts with inducible cellular factors to promote mRNA stability (47). During periods of iron starvation, RNA-protein interactions at the stem-loop sequence are dominant over the effects of a distinct region of AU-containing sequences, which otherwise trigger receptor-mediated mRNA degradation (48). Further investigation will be required to define sequences within the VEGF mRNA required for positive and negative regulation of post-transcriptional mRNA stability.

The findings reported here provide a framework for the comprehensive analysis of the regulation of VEGF expression and VEGF structure-function relationships. Such studies will be critical to understanding and eventually modulating the role of VEGF during physiological and pathological blood vessel growth.

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