Structure of the Murine Tissue Factor Gene

Chromosome Location and Conservation of Regulatory Elements in the Promoter

Nigel Mackman, Susan Imes, William H. Maske, Benjamin Taylor, Aldons J. Lusis, and Thomas A. Drake

Tissue factor (TF) is a transmembrane glycoprotein that mediates cellular initiation of the coagulation serine protease cascades. Moreover, expression of TF in human atherosclerotic plaques is likely to play a significant role in the thrombotic complications associated with plaque rupture. In this study the complete murine TF gene, Cf-3, was isolated from mouse NIH 3T3 cells and was found to consist of six exons spanning about 11 kilobase pairs (kbp) of DNA. A major transcriptional start site was located 24 bp downstream of a TATA box. Cf-3 was mapped to chromosome 3 by analysis of an intersubspecies test cross. Conserved transcription factor-binding sites were identified by comparison of 5' flanking regions of the murine and human TF genes. A region of the TF promoter required for constitutive expression exhibited 85% identity in DNA sequence and included two conserved binding sites for Spl. Furthermore, two AP-1 sites and an NF-κB site were conserved in a 56-bp region necessary for transcriptional activation in response to bacterial lipopolysaccharide. These highly conserved regions of the TF promoter, which contain several binding sites for well-characterized transcription factors, are likely to be functionally important in the complex pattern of TF gene expression observed in a variety of cell types. (Arteriosclerosis and Thrombosis 1992;12:474–483)

KEY WORDS • blood coagulation • chromosomal localization • gene regulation • genomic organization

Recent homology searches have revealed that tissue factor (TF) belongs to the cytokine receptor family, which is characterized by a homologous unit of approximately 200 residues in the extracellular domain. These proteins may share a common global architecture of binding domains based on the conservation of two pairs of cysteine residues. TF initiates the coagulation serine protease cascades by forming a complex with circulating factors VII/VIIa. Under normal circumstances TF is not expressed within the vasculature. However, TF expression can be rapidly induced in cultured peripheral blood monocytes and human umbilical vein endothelial cells in response to a variety of agonists, including bacterial lipopolysaccharide (LPS) and the inflammatory cytokines tumor necrosis factor-α (TNF-α) and interleukin-1 (IL-1). Activation of the coagulation protease cascades by aberrant expression of TF may be responsible for thrombotic episodes in patients with a variety of clinical disorders, including septic shock and various forms of cancer. In addition, the expression of TF in the necrotic core of human atherosclerotic plaques suggests that this protein may play a significant role in the initiation of coagulation that is associated with plaque rupture. More recently, oxidized low density lipoprotein (ox-LDL) has been shown to induce TF expression in vascular endothelial cells. The generation of ox-LDL in vivo is thought to be associated with the development of atherosclerotic vascular disease and may be a local mediator promoting thrombosis in atherosclerotic lesions.

In mouse tissues TF mRNA exhibited a widespread distribution that was especially abundant in brain, lung, kidney, and heart. The TF gene has been classified as an "immediate early" gene because TF mRNA is rapidly induced, in the absence of protein synthesis, in murine BALB/c 3T3 cells and AKR-2B fibroblasts in response to either serum or purified growth factors. Moreover, the murine TF gene is induced in response to estrogen in the immature rat uterus. Other studies have demonstrated that TF mRNA and functional TF protein can be induced in COS-7 cells and human fibroblasts by serum. Cell type–specific expression of TF is also observed in human tissues. These data suggest that TF may participate in biological processes other than hemostasis, including cell proliferation and inflammatory responses such as wound healing. For example, TF expression in these environments will...
FIGURE 1. Diagram showing organization of the mouse tissue factor gene. \(\lambda gMTF2282\) was isolated from a genomic library derived from mouse NIH 3T3 cells and contained an insert of approximately 17 kilobase pairs. This figure shows the position of exons 1-6. Restriction sites are as follows: A, AvaI; B, BamHI; E, EcoRI; H, HindIII; K, KpnI; P, PstI; S, SacI; SmaI. PstI site marked by the asterisk is polymorphic, and the SacI site in parentheses is present in the cDNA but is not present in the gene. Direction and extent of the DNA sequence determinations are indicated by arrows.

generate thrombin, which can subsequently mediate the activation of a variety of cell types.21

Previously, we have established the organization of the human TF gene together with its complete DNA sequence.22 A G+C-rich region of the TF promoter contained four GC boxes corresponding to binding sites for the transcription factor Sp1.23,24 In addition, cis-acting regions involved in constitutive and serum-induced expression of the TF promoter were identified using COS-7 cells.18 Our more recent studies have revealed that induction of TF mRNA in THP-1 monocytic cells is regulated at both transcriptional and posttranscriptional levels in response to LPS.25 Moreover, we have identified a 56-base pair (bp) LPS response element, containing two AP-1 sites and an NF-κB site, that mediates transcriptional activation of the human TF promoter in response to LPS.26

In the present study, we report the isolation of the complete murine TF gene, \(Cf-3\), from NIH 3T3 cells. This gene was mapped to chromosome 3 and is organized into six exons separated by five introns. Comparison of the 5' flanking region of the mouse and human TF genes allowed identification of conserved transcription factor-binding sites. For instance, two Sp1 binding sites were conserved in a region involved in constitutive expression, and there was conservation of two AP-1 sites and an NF-κB site within the 56-bp LPS response element. Interactions between transcription factors bound to these sites are likely to account for the complex pattern of TF gene expression observed in a variety of cell types.

Methods

cDNA Probes
cDNA was synthesized from mouse heart RNA, derived from a CB6 strain (BALB/c×C57BL/6; a gift from Dr. M. Sawdey), using a First Strand Synthesis kit (Stratagene, La Jolla, Calif.). Briefly, 7.5 \(\mu\)g total RNA was annealed with 300 ng of a 30-mer oligonucleotide primer, 5'-TCAATTCCCAATCACCTTTATTATATAA-3', complementary to a region of the mouse TF cDNA between 1,765 and 1,794. Next, the sample was incubated at 37°C for 1 hour in the presence of reverse transcriptase (Stratagene) before this cDNA was used as a template for a polymerase chain reaction (PCR). Denatured DNA was amplified by Taq DNA polymerase in the presence of specific 5' and 3' primers using a GeneAmp kit (Perkin-Elmer Cetus, Norwalk, Conn.). The 22-mer 5' oligonucleotide primer, 5'-ATCGCTCCTGTAGCGTAGCCAA-3', was complementary to the mouse TF cDNA between 40 and 61, and the 3' primer is the same 30-mer as described above. The reaction mixture was overlayered with mineral oil, and 20 cycles were carried out in a programmable heat block (TwinBlock System, Ericomp, San Diego, Calif.) set to denature at 94°C for 1 minute, anneal at 55°C for 1 minute, and extend at 72°C for 1 minute. Finally, the reaction was incubated at 72°C for 10 minutes and at 28°C for 20 hours. The 1,754-bp PCR product was cloned into the unique \(Sma\) I site of pUC18 to create pcMTF2253.

The position of the exons was determined by Southern blotting, using the following probes derived from...
pcMTF2253: a 187-bp HindIII–EcoRV fragment, which spans exon 1; an 818-bp EcoRV–Sac I fragment, which spans exons 2–6; and a 750-bp Sac I fragment, which contains the majority of the 3′ untranslated region of exon 6. A subclone of the entire coding region was generated, pcMTF2261, which contains a 1,005-bp insert encompassing nucleotides 39 to 1,044 of the cDNA. DNA probes were labeled with [α-32P]ATP (> 6,000 Ci/mmol, Amersham Corp., Arlington Heights, Ill.) using a Random Primed Labeling kit (Boehringer Mannheim, Indianapolis, Ind.).

Isolation of the Murine Tissue Factor Gene

A mouse NIH 3T3 genomic library in the λFIX II vector (Stratagene) was screened using the complete 1,754-bp insert of pcMTF2253 as a probe. One positive clone containing an ~17-kbp insert, AgMTF2282, was isolated from 1 × 10^6 plaques. Hybridization and wash conditions were as described previously.\(^{22}\)

Southern Blot Analysis

Genomic DNA from BALB/c mice was purchased from Clontech, Palo Alto, Calif. All other genomic DNA was isolated as described previously.\(^{22}\) Hybridization and wash conditions were as described above.

DNA Sequencing

DNA fragments were subcloned into pUC vectors\(^{27}\) for subsequent nucleotide sequencing by the dideoxy method\(^{28}\) using [α-32P]dATP (1,000 Ci/mmol, Amersham Corp.) and Sequenase II (United States Biochemical Corp., Cleveland, Ohio). Sequencing data were assembled using Staden’s database system.\(^{29}\) The average character of the final sequence was determined by 3.8 independent gel readings. The DNA sequence of the 1,100-bp 5′ flanking region was determined from both strands using the strategy shown in Figure 1. A unique EcoRV site was employed to determine the sequence of exon 2. For exons 3–5, pairs of primers were designed to determine the nucleotide sequence of the coding region and intron–exon boundaries (see Figure 1). The primers were complementary to the following regions of the cDNA: exon 3–5′ (323–339), exon 3–3′ (477–493), exon 4–5′ (534–550), exon 4–3′ (644–660), exon 5–5′ (717–733), and exon 5–3′ (821–837). The DNA sequence of exon 6 was determined using primers complementary to the following regions: 6–5′ (894–910), 6–3′ (1,019–1,035), 6–1 (1,131–1,147), 6–2 (1,381–1,397), and 6–3 (1,584–1,600). In addition, the DNA sequence of the final 300 bp of exon 6 was determined on the opposite strand by sequencing the 3′ end of the cDNA in pcMTF2253.

Primer Extension

The sequence of the 21-mer oligonucleotide primer, 5′-GCCTTTCTCTGGAGATGCTGC-3′, is complementary to nucleotides 191–221 of the cDNA sequence (Figure 2). The primer was phosphorylated with [γ-32P]ATP (>5,000 Ci/mmol, Amersham Corp.) using T\(_\alpha\) polynucleotide kinase (Boehringer Mannheim). Primer extension was performed as described previously.\(^{22}\) The hybridization reaction contained 4 μg poly(A+) RNA from mouse brain (Clontech) and 1×10^6 cpm of oligonucleotide primer.

Chromosomal Mapping

The murine gene for TF (Cf-3) was mapped using Southern analysis of genomic DNA samples from an intersubspecific test cross as previously reported.\(^{30,31}\) This test cross used two genetically distinct strains as parental types: MEV mice, which were developed as a linkage testing stock and which contain multiple ecotropic murine leukemia virus proviruses that serve as genetic markers, and CAST/Ei, which is an inbred strain developed from Mus castaneus, originating in Thailand. Southern blots probed with a 32P-labeled 1,005-bp mouse TF cDNA, derived from pcMTF2261 (see above), identified a Pst I polymorphism within Cf-3 that was used to distinguish alleles specific for each parental strain. F\(_1\) progeny of these strains were mated with one of two other inbred strains, BXD-32 or SWR/J, resulting in 95 progeny that made up the test cross used for the linkage analysis. The presence of either the MEV (M) allele or the CAST/Ei (C) allele in the progeny was used to determine the linkage of Cf-3 with the previously typed loci. The pattern of hybridizing bands for SWR/J was identical to that of the parental strain MEV, and a unique ~7.5-kbp band was used to distinguish the BXD-32 allele. Confidence intervals (95%) for recombination frequencies (r) were calculated using tables of the binomial distribution.

Results

Isolation of the Murine Tissue Factor Gene

The complete murine TF gene, Cf-3, was isolated on a single clone by screening a mouse NIH 3T3 genomic library with a mouse TF cDNA probe (Figure 1). A partial restriction map of the ~17-kbp insert was established from both the original clone, AgMTF2282, and subcloned fragments ligated into plasmid vectors (Figure 1). The approximate position of the exons was initially determined by Southern blotting using different portions of the cDNA as probes. A Pst I restriction fragment length polymorphism (RFLP) was identified during analysis of the chromosomal location of Cf-3 (see Figure 4). The size of the 6.6-kbp and 5.4-kbp DNA fragments indicated that the Pst I site located within intron 3 was polymorphic (Figure 1). More-
Factor Gene

with the reported average size of 137 bp for exons of the remaining five exons is 171 bp, which is consistent with an extensive 3' untranslated region. The average length of the cytoplasmic domains of the TF protein, as well as the contains 937 bp and encodes the transmembrane and

tissue in the mouse (haploid) genome.

Structural Organization of the Murine Tissue Factor Gene

The DNA sequence of all the exons, intron–exon boundaries, and 1,100 bp of the 5' flanking sequence was determined (Figure 2). The location and size of the six exons are shown in Figure 1 and Table 1. Exon 6 contains 937 bp and encodes the transmembrane and cytoplasmic domains of the TF protein, as well as the extensive 3' untranslated region. The average length of the remaining five exons is 171 bp, which is consistent with the reported average size of 137 bp for exons of higher eukaryotes.\(^3\) Sizes of the five introns were comparable to those of the human TF gene,\(^2\) and intron types were identical except for intron 3, which was type II in \(C_{42}^3\) and type I in the human TF gene. All splice acceptor and donor sequences (Table 2) agree with the GT-AG rule\(^24\) and conform to the consensus proposed by Mount.\(^25\) As expected, these sequences showed a high degree of conservation with the acceptor and donor sites of the human TF gene (Table 2).

Properties of the Tissue Factor Transcript

The first site of transcription was mapped by primer extension using mouse brain poly(A\(^+)\) RNA (Figure 3). The major start site was an adenine residue identified in Figure 2 as position +1, indicating that the complete 5' untranslated region of TF mRNA is 106 bp long.

An AU-rich domain in the last 167 bp of the 3' untranslated region of the TF transcript contains seven AUUUUA sequences that specify rapid turnover of mRNAs encoding transiently expressed cytokines.\(^26\) This AU-rich domain in the human, mouse, and rabbit TF mRNAs exhibited an average of 89% identity in nucleotide sequence,\(^32,27\) suggesting a highly conserved function. There is a single polyadenylation signal, AATAAA,\(^26\) in exon 6 of the murine TF gene.

Nucleotide Differences Between the Mouse Tissue Factor Gene and the cDNA

In the promoter region an additional cytosine nucleotide was found at position -10 (Figure 2) when this sequence was compared with that previously reported between the TATA box and the cap site.\(^13\) In addition, several differences were noted between the genomic sequence and the mouse TF cDNA sequence.\(^13\) In the coding region at position 526 the codon CTC of the TF gene was present in place of CTT found in the cDNA. This nucleotide difference has been noted in a cDNA isolated from AKR-2B murine fibroblasts and does not alter the amino acid composition of the protein.\(^18\) In the portion of exon 6 corresponding to the 3' untranslated region of the TF transcript, we observed three single-nucleotide differences from the cDNA sequence: position 1,044 G→T, which abolishes the Sac I site; position 1,087 G→A; and at position 1,161 the cytosine nucleo-

### Table 1. Location and Size of Exons and Introns in the Mouse Tissue Factor Gene

<table>
<thead>
<tr>
<th>Exon</th>
<th>Length (bp)</th>
<th>Amino acids</th>
<th>Intron Length* (bp)</th>
<th>Type†</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>194</td>
<td>29</td>
<td>1</td>
<td>~800</td>
</tr>
<tr>
<td>2</td>
<td>106</td>
<td>36</td>
<td>2</td>
<td>~4,400</td>
</tr>
<tr>
<td>3</td>
<td>218</td>
<td>72</td>
<td>3</td>
<td>~1,600</td>
</tr>
<tr>
<td>4</td>
<td>179</td>
<td>60</td>
<td>4</td>
<td>~1,000</td>
</tr>
<tr>
<td>5</td>
<td>160</td>
<td>53</td>
<td>5</td>
<td>~1,700</td>
</tr>
<tr>
<td>6</td>
<td>937</td>
<td>44</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*bp, base pairs.

†Numbering of residues corresponds to that in Figure 2.

‡Intron type is according to Sharp\(^32\): "0" indicates a splice between codons, "I" indicates a splice after the first nucleotide of a codon, and "II" indicates that the splice occurs after the second nucleotide.

### Table 2. Splice Junction Sequences

<table>
<thead>
<tr>
<th>Intron</th>
<th>Exon</th>
<th>Intron</th>
<th>Exon</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Human TCAG</td>
<td>GTGAGT...........CTCTGTGTTTAAAG</td>
<td>GCAA</td>
</tr>
<tr>
<td></td>
<td>Mouse GCAG</td>
<td>GTGAGT...........TCGCTTTATTGTCAG</td>
<td>GCTA</td>
</tr>
<tr>
<td>2</td>
<td>Human TAAG</td>
<td>GAAAGC...........CCCTTCCTTCTTAG</td>
<td>CATG</td>
</tr>
<tr>
<td></td>
<td>Mouse TAAG</td>
<td>GAAGGC...........CCCTTCCTCCTTCTTAC</td>
<td>TGGT</td>
</tr>
<tr>
<td>3</td>
<td>Human GAGA</td>
<td>GTAGAT...........TTTTCCTTGTTCAC</td>
<td>CAAA</td>
</tr>
<tr>
<td></td>
<td>Mouse GACA</td>
<td>GTAGAT...........TTTTTTTCACTTTCAC</td>
<td>CAAA</td>
</tr>
<tr>
<td>4</td>
<td>Human AAGA</td>
<td>GTGAGC...........TCTATCTTTTTACAG</td>
<td>AAAA</td>
</tr>
<tr>
<td></td>
<td>Mouse AAGA</td>
<td>GTGAGC...........TCTATCTTTTTACAG</td>
<td>AAAA</td>
</tr>
<tr>
<td>5</td>
<td>Human AGAG</td>
<td>GTGAGT...........CCCTTAATTTTTTAC</td>
<td>AATA</td>
</tr>
<tr>
<td></td>
<td>Mouse GGAG</td>
<td>GTGAGT...........CCCTTAGTTTTTTGCAC</td>
<td>AAAC</td>
</tr>
<tr>
<td>C</td>
<td>A</td>
<td>TTTTTTTTTT T</td>
<td></td>
</tr>
</tbody>
</table>

Consensus* | | AG | GT AGT...........N AG | G |
| A       | G    | CCCCCCCCCCCCC C |      |

*Consensus sequence from Mount.\(^25\)
Finally, we noted an unusual DNA rearrangement in the AT-rich domain of the 3' untranslated region between 1,639 and 1,689 (Table 3). The positions of two DNA blocks of 18 and 33 bp present in the TF gene were reversed when compared with the published cDNA sequence. Confirmation of our DNA sequence was obtained by determining the DNA sequence of the 3' end of pcMTF2253, an independently isolated cDNA encoding mouse TF derived from CB6 mice (see above); the sequence of this cDNA was identical to that of the gene.

**Chromosomal Mapping**

Genomic Southern blots were performed using DNA from CAST/Ei and MEV strains digested with the following restriction enzymes: EcoRI, HindIII, Pst I, Sac I, and Pvu II. The pattern of bands hybridizing to a mouse TF cDNA probe was used to identify RFLPs. Pst I yielded patterns that distinguished alleles from the two parental strains: a unique 5.4-kbp band was observed for MEV, whereas CAST/Ei could be identified by a unique 6.6-kbp band. Genomic DNA from 95 test cross mice was digested with Pst I and scored for the presence of either the MEV (M) allele or the CAST/Ei (C) allele. A representative portion of this analysis is shown in Figure 4. These results were compared with the distribution of 90 previously typed loci located on 15 of the 19 autosomes. Close linkage was found between Cf-3 and two loci on chromosome 3, salivary α-amylase (Amy-1) and an ecotropic provirus (Emv-27) (Table 4). There was one recombination between Cf-3 and Emv-27, and none between Cf-3 and Amy-1. The estimated map distances in centimorgans (with 95% confidence intervals) are Amy-1 0.00 (0.00-3.24) — Cf-3 1.09 (0.03-5.8) — Emv-27.

**Identification of Potential cis-Acting Regulatory Elements**

Comparison of 1,100 bp of the 5' flanking sequence of Cf-3 with a similar region flanking the human TF gene (F3) allowed identification of conserved regions of the two promoters that may serve regulatory roles. The DNA sequence of a proximal region (−266 to +14) of the mouse TF promoter exhibited 79% identity with an equivalent region of the human TF promoter (Figure 5), whereas a distal region (−1,050 to −267) was only 46% conserved. Therefore, the majority of functionally conserved elements of the TF promoter appear to be located within close proximity to the start site of transcription, consistent with the results of our functional studies. Both the mouse and human TF promoters contained a TATA box located 24 and 23 bp, respectively, upstream of the transcriptional start site (Figure 5). The mouse TF promoter contained a G+C-rich region that extended approximately 700 bp upstream of the cap site (Figure 2). A similar region in the human TF promoter was shown to fit the criteria of a CpG island.

Functional studies using the human TF promoter transfected into both COS-7 and THP-1 cells have defined several regions that are required for constitutive expression and response to serum or LPS (Figure 6). Comparison of the DNA sequence in these regions with that present in the mouse TF promoter identified several transcription factor—binding sites that may control expression of the TF gene. First, deletion of a region (−153 to −68) abolished promoter activity, and

**Table 3. DNA Rearrangement Within Exon 6**

<table>
<thead>
<tr>
<th>Genomic</th>
<th>1,633</th>
<th>ATTAA</th>
<th>GCTACTTGAATAATTTTAA</th>
<th>TGGTTAACATGATATATTACGAAAATTTA</th>
<th>AAGGTTTT</th>
</tr>
</thead>
<tbody>
<tr>
<td>cDNA</td>
<td>ATTAA</td>
<td>TGCTTAAGATATATATATTACGAAAATTTA</td>
<td>GCTACTTGAATAATTTTAA</td>
<td>AAGGTTTT</td>
<td></td>
</tr>
</tbody>
</table>

The 18-base pair (bp) block, marked with dashed lines, and the 33-bp block have exchanged in this region when the genomic DNA sequence is compared with that of the published cDNA. Numbering of nucleotides is taken from Figure 2, and ATTAA motifs are underlined.
### TABLE 4. Linkage of Cf-3 (Mouse Tissue Factor Gene) With Amy-1 and Emv-27 on Mouse Chromosome 3

<table>
<thead>
<tr>
<th>Genotype</th>
<th>No. of progeny</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amy-1</td>
<td>Cf-3</td>
</tr>
<tr>
<td>M</td>
<td>M</td>
</tr>
<tr>
<td>M</td>
<td>*</td>
</tr>
<tr>
<td>C</td>
<td>C</td>
</tr>
<tr>
<td>C</td>
<td>*</td>
</tr>
<tr>
<td>*</td>
<td>M</td>
</tr>
<tr>
<td>Total</td>
<td></td>
</tr>
</tbody>
</table>

Alleles inherited from the hybrid parent (CAST/Ei×MEV) F3, respectively.

*Inability to determine allele origin; r, recombination frequency; cM, centimorgans; CI, confidence interval.

### Discussion

In this study we isolated the complete murine TF gene from NIH 3T3 cells. The murine TF gene exhibited the same organization as the human TF gene and was divided into six exons. The DNA sequence of the murine TF gene was almost identical to that of the TF cDNA sequence isolated from BALB/c 3T3 cells, apart from an unusual rearrangement in the 3' untranslated region of exon 6. The region itself (nucleotides 1,639-1,689) and the flanking region contained three ATTATA sequences, which led us to speculate that this DNA rearrangement may have arisen either by some form of transposition or by a recombinational event involving the repeated ATTATA sequences, possibly during the synthesis of the cDNA.

A polymorphic Pst I site within intron 3 was used to distinguish alleles during the chromosomal mapping of the TF gene. Cf-3 was located on chromosome 3 and was closely linked to the gene for salivary a-amylase, Amy-1. Among other genes linked closely with Amy-1, the gene for macrophage colony-stimulating factor (Csfm) is of interest because Csfm mRNA expression in endothelial cells is induced by some of the same stimuli that induce TF mRNA, which include several cytokines, LPS, and ox-LDL.

The human homologue of Csfm is located on chromosome 5. The human genes for tissue factor (F3) and salivary a-amylase (Amy-1) have been localized by in situ hybridization on closely adjacent regions of human chromosome 1, which are 1p22–p21 and 1p21, respectively. Our results bring to at least nine the number of genes in the region of human chromosome 1p that map to mouse chromosome 3. A recent study using pulsed-field gel electrophoresis defined a synteric segment with conserved linkage, i.e., gene order conserved, for five of these genes. Additional studies using this approach will be needed to determine whether there is conserved

Sp1. The position of these sites is consistent with that of functional Sp1 sites, and their conservation strongly suggests that Sp1 may play a role in expression of the TF promoter in at least two distinct cell types.

A second overlapping region (−111 to +14) was shown to be necessary for serum-induced expression of the TF promoter in COS-7 cells. This region did not contain the well-defined serum-response element that is found upstream of the c-fos gene. However, we observed two highly conserved areas that spanned one of the Sp1 sites described above and a putative binding site for early growth response−1 (EGF-1) or Zif268 (Figure 5).

Finally, we have reported that a functionally important 56-bp region (−227 to −172) of the human TF promoter exhibited 85% sequence identity to a corresponding region of the mouse promoter, including complete conservation of two AP-1 binding sites and an NF-κB site. This 56-bp region conferred LPS responsiveness to a heterologous promoter in monocytic cells (Figure 6). Moreover, the AP-1 and NF-κB binding sites were shown to be required for transcriptional activation of the TF promoter in response to LPS.

Conservation of the AP-1 and NF-κB sites supports our conclusion that these cis-acting elements are major regulatory components of the TF promoter.

**FIGURE 5. Diagram showing nucleotide sequence comparison between human and mouse tissue factor (TF) promoters.** Adenine residue representing the start site of transcription for the mouse TF gene is marked ('*'), and TATA boxes are underlined. The nucleotide sequences matching consensus binding sites for transcription factors Sp1, AP-1, and NF-κB are indicated by a line above or below the sequence. EGR-1 site is marked by a dashed line. Sequences were aligned using the GAP program from the University of Wisconsin Genetics Computer Group. Nucleotide sequence for the human TF promoter is from Reference 22.
order of Cf-3 with other genes, particularly Amy-1. Genes on the long arm of human chromosome 1 have mapped principally to mouse chromosome 1, including blood coagulation factor V. Other blood coagulation factor genes mapped in the mouse are those for factors VIII and IX, which are on the X chromosome as they are in humans.53

Comparison of the nucleotide sequence of the murine and human TF promoters allows identification of conserved regions that may serve regulatory roles. Both promoters are spanned by a G+C-rich region that fits the criteria of a CpG island, although the role of this region in the regulation of the TF gene is currently unknown. However, in vertebrates most of the genome is methylated, and nonmethylated sequences are reduced to these short CpG islands, many of which include the 5' end of a gene. This has led to the speculation that methylation of these regions may control gene expression. Alternatively, CpG islands may function as gene markers for ubiquitous nuclear factors, which could maintain the DNA in a state that is accessible to the transcriptional machinery. Sp1 binding sites are G+C rich and often occur within CpG islands. Two Sp1 sites are conserved in the murine and human TF promoters and may play a role in the regulation of gene expression. Previously, it has been shown that Sp1 stimulates transcription at TATA-containing promoters via coactivators, which are molecular adaptors between trans-activators and the general transcription initiation machinery. The rate of TF gene transcription may also be increased by phosphorylation of Sp1, which has been proposed to regulate the function of the glutamine-rich activation domain of Sp1.

The TF gene is an immediate early gene because it can be rapidly induced in response to serum or purified growth factors. Therefore, it is likely that the TF promoter contains binding sites for transcription factors that mediate serum induction of responsive genes. We have identified a putative binding site for EGR-1 in a region previously shown to be responsive to serum.

This zinc finger–containing protein, whose mRNA is rapidly induced by serum, growth factors, and other mitogenic stimuli, is thought to play a role in regulating the genetic program induced by extracellular signals. Therefore, EGR-1 may in part mediate the induction of the TF gene in response to serum or growth factors. In addition, the TF gene can be induced in monocytes and endothelial cells in response to a variety of agonists. Recently, we have identified a 56-bp LPS response element that contains both AP-1 and NF-κB sites. Significantly, the transcription factors AP-1 and NF-κB can be activated by a variety of agonists, including the cytokines TNF-α and IL-1. Therefore, this 56-bp region may also be involved in the induction of the TF gene in response to these inflammatory cytokines.

The definitive analyses of gene expression and function must be performed in a living animal. At present, the mouse is the mammal most amenable to genetic analysis and manipulation, and there are reasonable mouse models of important human diseases, such as septic shock and atherosclerosis, in which TF plays a role. Isolation and characterization of the mouse TF gene are essential for such studies. Our finding of extensive conservation of transcription factor–binding sites between the mouse and human TF genes suggests that gene expression and regulation are comparable in the two species, supporting the relevance of pursuing fundamental questions concerning the role of TF in normal physiology and in disease pathogenesis in the mouse. Establishing gene location and polymorphisms facilitates genetic studies in the mouse for determining whether TF may be a candidate gene for atherosclerosis or other polygenic disorders. Plasmids expressing β-galactosidase activity from the mouse TF promoter can be used to generate transgenic mice, thereby allowing analysis of the role of NF-κB and other transcription factor–binding domains in vivo and facilitating studies of gene expression in disease pathogenesis. Finally, through disruption of the endogenous gene using gene targeting, a crucial analysis of the role of TF in hemo-
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stasis, development, and other biological processes is now possible.

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References


Structure of the murine tissue factor gene. Chromosome location and conservation of regulatory elements in the promoter.

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