Isolation, Structural Characterization, and Chromosomal Mapping of the Mouse Vascular Adhesion Protein-1 Gene and Promoter

Petri Bono, Marko Salmi, David J. Smith, Ilona Leppänen, Nina Horelli-Kuitunen, Aarno Palotie and Sirpa Jalkanen

*J Immunol* 1998; 161:2953-2960; ;
http://www.jimmunol.org/content/161/6/2953

---

**References**
This article cites **51 articles**, 26 of which you can access for free at: http://www.jimmunol.org/content/161/6/2953.full#ref-list-1

**Subscription**
Information about subscribing to *The Journal of Immunology* is online at: http://jimmunol.org/subscription

**Permissions**
Submit copyright permission requests at: http://www.aai.org/About/Publications/JI/copyright.html

**Email Alerts**
Receive free email-alerts when new articles cite this article. Sign up at: http://jimmunol.org/alerts
Isolation, Structural Characterization, and Chromosomal Mapping of the Mouse Vascular Adhesion Protein-1 Gene and Promoter 1,2

Petri Bono,3* Marko Salmi,* David J. Smith,† Ilona Leppänen,* Nina Horelli-Kuutunen,‡ Aarno Palotie,‡ and Sirpa Jalkanen*

Vascular adhesion protein-1 (VAP-1) is an endothelial cell adhesion molecule which mediates lymphocyte binding to endothelial cells. The cloning of a mouse VAP-1 (mVAP-1) cDNA revealed that mVAP-1 is a novel 110/220 kDa transmembrane molecule with significant identity to copper-containing amine oxidases. In this work the nucleotide sequence and primary structure of the mVAP-1 gene was determined and the promoter region was structurally characterized. The isolated approximately 14.4-kb mVAP-1 gene consists of 4 exons and 3 introns. Primer extension analysis and S′ rapid amplification of cDNA ends revealed multiple transcription initiation sites in different tissues suggesting that the mVAP-1 transcription is differently regulated in different tissues. Analysis of the sequence immediately upstream of the detected transcription initiation sites showed no canonical TATA or CCAAT elements, but putative regulatory elements were found close to the detected transcription start sites. The cloning of the mVAP-1 gene reveals the first insight into the genomic organization of murine amine oxidases and will, by targeted disruption of the gene, allow us to understand better the importance of VAP-1 in leukocyte trafficking and monoamine oxidase activity for the function of the immune system. The Journal of Immunology, 1998, 161: 2953–2960.

Materials and Methods

Isolation and analysis of an mVAP-1 genomic clone

Approximately 5 × 10⁷ recombinant λ phage clones from a 129 SVJ mouse genomic library in the Lambda Fix II vector (Stratagene, La Jolla, CA) were screened with an approximately 3,100-bp mVAP-1 cDNA fragment. This probe covered nucleotides 1 to 3,102 in the mVAP-1 cDNA sequence (6), and it was generated by digesting the previously cloned mVAP-1 cDNA in pUC19 plasmid with EcoRI and XhoI. The isolated fragment was labeled with [α-32P]dCTP at about 3,000 Ci/mmol (American International, Aylesbury, U.K.) in a random priming reaction (Amersham Multiprime DNA labeling kit). Approximately 100,000 plaques/plate were transferred to Hybond N nylon filters (Amerham), and duplicate filters were hybridized at 65°C in 5× SSC, 5× Denhardt’s reagent, 0.5% SDS, and 0.5 mg/ml denatured sheared salmon sperm DNA. After an overnight hybridization, the filters were washed at 65°C, twice for 30 min each time in 0.1× SSC and 0.1% SDS and autoradiographed with intensifying screens. One positive clone was identified and secondarily

*MediCity Research Laboratories, University of Turku, †National Public Health Institute, and ‡BioTie Therapies, BioCity, Turku, Finland; and Laboratory Department of Helsinki University Hospital and Department of Clinical Chemistry, University of Helsinki, Helsinki, Finland

Received for publication February 17, 1998. Accepted for publication May 15, 1998.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

1 This work was supported by the Finnish Academy, the Finnish Cancer Union, the Finnish Cultural Foundation, the Finnish Medical Foundation, the Emil Aaltonen Foundation, and the Sigrid Juselius Foundation.

2 The sequence of the mVAP-1 gene has been submitted to GenBank (accession number AF078705).

3 Address correspondence and reprint requests to Dr. Petri Bono, MediCity Research Laboratories, University of Turku, Tykistokatu 6, 20520 Turku, Finland. E-mail address: petbon@utu.fi

4 Abbreviations used in this paper: VAP-1, vascular adhesion protein-1; mVAP-1, mouse vascular adhesion protein-1; hVAP-1, human vascular adhesion protein-1; RACE, rapid amplification of complementary deoxyribonucleic acid ends; FISH, fluorescence in situ hybridization.
CLONING AND CHARACTERIZATION OF mVAP-1 GENE

RAW TEXT END
clones were sequenced on both strands by the dideoxy chain termination method (10) using a Sequenase version 2.0 kit (U.S. Biochemical, Cleveland, OH) or the sequencing service facilities of the Department of Medical Genetics, University of Turku (Turku, Finland). Standard molecular biology techniques were used in plaque hybridization, λ phage purification, Escherichia coli transformation, and plasmid and phage DNA purifications (11).Sequence analysis was performed using the Wisconsin Package version 8.1-UNIX of the Genetics Computer Group (GCG, Madison, WI). Putative binding sites for transcription factors were identified using Find-patterns and a Tsites Genetics Computer Group GCG file.

Southern blot analysis
Ten micrograms of genomic DNA from 129 SJV mice tail biopsies was digested with the restriction enzymes AvrII, BssHII, EcoRI, KpnI, NotI, SacI, SfiI, and SpeI; separated on agarose; and blotted onto Hybond N (Amersham, Little Chalfont, UK). The resulting 1282-bp fragment PB1 (corresponding to nucleotides 5901–7183 in the mVAP-1 gene sequence; see Figs. 1 and 2) was used to determine the sizes of the hybridizing bands. The hybridization and washing conditions were similar to those used in the primary screening of the genomic library. The probe was generated by digesting the approximately 9.3-kb mVAP-1 genomic subclone with XbaI, extracting the resulting 1282-bp fragment PB1 (corresponding to nucleotides 5901–7183 in the mVAP-1 gene sequence; see Figs. 1 and 2) from an agarose gel, and labeling the fragment with an Amersham multiprime DNA labeling kit.

5′RACE
A 5′RACE system version 2.0 for rapid amplification of cDNA ends (Life Technologies) was used according to the manufacturer’s instructions with some modifications. Total RNA from BALB/c mouse adipose tissue, heart, gut, and liver was prepared using a Utraspex kit (Biotechex, Houston, TX). One microgram of the RNA was used in a cDNA synthesis reaction, using either a 42-nucleotide antisense primer PBEXT1 (beginning at the +52 position in the mVAP-1 gene sequence; see Figs. 1 and 2) from an agarose gel, and labeling the fragment with an Amersham multiprime DNA labeling kit. The translated protein sequence is shown as single letter codes for amino acids.

ten micrograms of genomic DNA from 129 SJV mice tail biopsies was digested with the restriction enzymes AvrII, BssHII, EcoRI, KpnI, NotI, SacI, SfiI, and SpeI; separated on agarose; and blotted onto Hybond N (Amersham, Little Chalfont, UK). The resulting 1282-bp fragment PB1 (corresponding to nucleotides 5901–7183 in the mVAP-1 gene sequence; see Figs. 1 and 2) was used to determine the sizes of the hybridizing bands. The hybridization and washing conditions were similar to those used in the primary screening of the genomic library. The probe was generated by digesting the approximately 9.3-kb mVAP-1 genomic subclone with XbaI, extracting the resulting 1282-bp fragment PB1 (corresponding to nucleotides 5901–7183 in the mVAP-1 gene sequence; see Figs. 1 and 2) from an agarose gel, and labeling the fragment with an Amersham multiprime DNA labeling kit.

Ten micrograms of genomic DNA from 129 SJV mice tail biopsies was digested with the restriction enzymes AvrII, BssHII, EcoRI, KpnI, NotI, SacI, SfiI, and SpeI; separated on agarose; and blotted onto Hybond N (Amersham, Little Chalfont, UK). The resulting 1282-bp fragment PB1 (corresponding to nucleotides 5901–7183 in the mVAP-1 gene sequence; see Figs. 1 and 2) was used to determine the sizes of the hybridizing bands. The hybridization and washing conditions were similar to those used in the primary screening of the genomic library. The probe was generated by digesting the approximately 9.3-kb mVAP-1 genomic subclone with XbaI, extracting the resulting 1282-bp fragment PB1 (corresponding to nucleotides 5901–7183 in the mVAP-1 gene sequence; see Figs. 1 and 2) from an agarose gel, and labeling the fragment with an Amersham multiprime DNA labeling kit.

5′RACE
A 5′RACE system version 2.0 for rapid amplification of cDNA ends (Life Technologies) was used according to the manufacturer’s instructions with some modifications. Total RNA from BALB/c mouse adipose tissue, heart, gut, and liver was prepared using a Utraspex kit (Biotechex, Houston, TX). One microgram of the RNA was used in a cDNA synthesis reaction, using either a 42-nucleotide antisense primer PBEXT1 (beginning at the +52 position in the mVAP-1 gene sequence; see Figs. 1 and 2) from an agarose gel, and labeling the fragment with an Amersham multiprime DNA labeling kit.

Ten micrograms of genomic DNA from 129 SJV mice tail biopsies was digested with the restriction enzymes AvrII, BssHII, EcoRI, KpnI, NotI, SacI, SfiI, and SpeI; separated on agarose; and blotted onto Hybond N (Amersham, Little Chalfont, UK). The resulting 1282-bp fragment PB1 (corresponding to nucleotides 5901–7183 in the mVAP-1 gene sequence; see Figs. 1 and 2) was used to determine the sizes of the hybridizing bands. The hybridization and washing conditions were similar to those used in the primary screening of the genomic library. The probe was generated by digesting the approximately 9.3-kb mVAP-1 genomic subclone with XbaI, extracting the resulting 1282-bp fragment PB1 (corresponding to nucleotides 5901–7183 in the mVAP-1 gene sequence; see Figs. 1 and 2) from an agarose gel, and labeling the fragment with an Amersham multiprime DNA labeling kit.

5′RACE
A 5′RACE system version 2.0 for rapid amplification of cDNA ends (Life Technologies) was used according to the manufacturer’s instructions with some modifications. Total RNA from BALB/c mouse adipose tissue, heart, gut, and liver was prepared using a Utraspex kit (Biotechex, Houston, TX). One microgram of the RNA was used in a cDNA synthesis reaction, using either a 42-nucleotide antisense primer PBEXT1 (beginning at the +52 position in the mVAP-1 gene sequence; see Figs. 1 and 2) from an agarose gel, and labeling the fragment with an Amersham multiprime DNA labeling kit.

Ten micrograms of genomic DNA from 129 SJV mice tail biopsies was digested with the restriction enzymes AvrII, BssHII, EcoRI, KpnI, NotI, SacI, SfiI, and SpeI; separated on agarose; and blotted onto Hybond N (Amersham, Little Chalfont, UK). The resulting 1282-bp fragment PB1 (corresponding to nucleotides 5901–7183 in the mVAP-1 gene sequence; see Figs. 1 and 2) was used to determine the sizes of the hybridizing bands. The hybridization and washing conditions were similar to those used in the primary screening of the genomic library. The probe was generated by digesting the approximately 9.3-kb mVAP-1 genomic subclone with XbaI, extracting the resulting 1282-bp fragment PB1 (corresponding to nucleotides 5901–7183 in the mVAP-1 gene sequence; see Figs. 1 and 2) from an agarose gel, and labeling the fragment with an Amersham multiprime DNA labeling kit.

5′RACE
A 5′RACE system version 2.0 for rapid amplification of cDNA ends (Life Technologies) was used according to the manufacturer’s instructions with some modifications. Total RNA from BALB/c mouse adipose tissue, heart, gut, and liver was prepared using a Utraspex kit (Biotechex, Houston, TX). One microgram of the RNA was used in a cDNA synthesis reaction, using either a 42-nucleotide antisense primer PBEXT1 (beginning at the +52 position in the mVAP-1 gene sequence; see Figs. 1 and 2) from an agarose gel, and labeling the fragment with an Amersham multiprime DNA labeling kit.
min. DNA fibers for fiber-FISH were prepared from agarose-embedded mouse cells of fetal origin (see above) as described previously (16).

The FISH procedure was conducted in 50% formamide and 10% dextran sulfate in 2× SSC as described previously (17–19). A multicolor image analysis of metaphase chromosomes was performed as described previously (16). A 3.1-kb SalI-BglII fragment from the mVAP-1 gene, PB3, was used as a 5’ probe, and a 9.3-kb SalI fragment, PB4, was used as a 5’ probe in separate hybridization reactions (see Fig. 2 for locations of the probes). A reference probe for mouse chromosome 4 (Col15a1, 4B1-3) was used to insure localization of the mVAP-1 gene (20).

Results

Organization of the mouse VAP-1 gene

To isolate the gene encoding mVAP-1 and to determine its nucleotide sequence and organization, a mouse heart genomic λ library was screened with an mVAP-1 cDNA probe. One phage with an approximately 14.4-kb insert was isolated; this insert was subcloned, and its nucleotide sequence was determined. Since the isolated clone included all the nucleotides present in the previously isolated mVAP-1 cDNA, we concluded that we had isolated a gene encoding mVAP-1. The isolated clone also included 3593 bp of sequence that is 3’ of the previously isolated mVAP-1 cDNA (6). The sequence of the amino acid-coding regions of the gene was identical with the previously determined sequence except for one nucleotide change, which did not lead to an amino acid change (nucleotide 22 in the genomic sequence is A instead of the previously reported G in the cDNA sequence). The database comparisons of the 5’ and 3’ untranslated sequences against the most recent releases from SwissProt and GenEMBL did not show any homology to previously characterized mouse genes, indicating that a novel mouse gene had been isolated. The sequence of the entire gene (Fig. 1) has been submitted to GenBank (accession number AF078705).

The major features of the 14,356-bp mVAP-1 gene are summarized in Figure 2. The gene is composed of four exons separated by three introns, the sizes of which are 3179 bp (intron I), 346 bp (intron II), and 906 bp (intron III). Table I summarizes the lengths and locations of the exons and introns as well as the nucleotide sequences surrounding the splice donor and acceptor sites. The nucleotide sequences at the 5’ donor and 3’ acceptor sites of all introns conform to the GT...AG rule (21, 22). Exon 1 encodes amino acids 1 to 533 of the mVAP-1 protein. Exons 2 and 3 encode amino acids 534 to 628 and 629 to 672, respectively, and are separated by a short 346-bp intron. The last exon 4 contains the remainder of the coding region (673–765) and the untranslated 3’ sequence, including the consensus AATAAA polyadenylation signal (23), which is located 1740 bp after the stop codon. After the first consensus polyadenylation signal no alternative polyadenylation sites could be found in the 3’ sequence as has been reported for some genes with a long 3’ untranslated sequence (24) and, for example, in the human L-selectin gene (25). This is in agreement with our previous Northern blot analysis of mVAP-1 in which only a single, approximately 4.4-kb mRNA was detected from different mouse tissues (6).

Southern blot analysis of mouse genomic DNA

A genomic Southern blot analysis in which 129SVJ mouse genomic DNA was digested with several different restriction enzymes and, after blotting of the gel, probed with an mVAP-1 fragment PB1 (see Fig. 2 for location of the probe) resulted in the detection of single bands of the expected sizes (Fig. 3) with enzymes that cut several times within the mVAP-1 gene but not within the probe. When the genomic DNA was digested with rare cutting enzymes (e.g., BssHII and NotI), single bands were still

Table I. Intron-exon boundaries of the mVAP-1 gene

<table>
<thead>
<tr>
<th>Exon</th>
<th>Sequence of the Exon/Intron Junction</th>
<th>Intron</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>5’ Donor</td>
<td>3’ Acceptor</td>
</tr>
<tr>
<td>1</td>
<td>TGGCAGgtgaga</td>
<td>acacagGGCTGA</td>
</tr>
<tr>
<td>2</td>
<td>GGGGAggtgagttt</td>
<td>ttctagGTATCA</td>
</tr>
<tr>
<td>3</td>
<td>GGAGAAGtgagtc</td>
<td>tgctagGACTTG</td>
</tr>
<tr>
<td>4</td>
<td>3982</td>
<td></td>
</tr>
</tbody>
</table>
detected, suggesting that mVAP-1 is present as a single copy in the mouse genome.

Mouse VAP-1 has multiple transcription initiation sites

To locate the transcription initiation sites of the mVAP-1 gene, both primer extension analysis and 5’RACE PCR were performed. Three different antisense oligonucleotides complementary to the 5’ region of the mVAP-1 mRNA were used in reactions to prime the reverse transcription of mouse liver and gut smooth muscle mRNA. As shown in Figure 4, A and B, specific bands unique to mouse gut smooth muscle mRNA corresponding to positions −393 and −471 and bands unique to liver mRNA corresponding to positions −288 and −476 (Fig. 4, B and C) were detected. The control reactions from bEnd.3, an mVAP-1-negative mouse endothelial cell line (data not shown), mRNA were negative. To further locate the 5’ end of the mVAP-1 mRNA transcript, 5’RACE PCR products were also generated from mouse heart, gut, liver, and adipose tissue mRNA. After amplification the resulting PCR products were run on an agarose gel, blotted, and probed with an mVAP-1-specific 5’ fragment, PB2, as a probe (see Fig. 2 for location of the probe). After verification of the products by Southern hybridization, the positively hybridizing PCR products from each tissue were subcloned for sequencing (PCR products from adipose tissue and heart are shown as an example in Fig. 4D). The sequence analysis of these 5’RACE PCR products revealed first the anchor-specific primer and thereafter the 5’ end of the mVAP-1 mRNA transcript, which, together with the Southern blot, confirmed that the amplified products were specific for mVAP-1. Thus, 5’RACE PCR revealed two additional transcription start sites corresponding to position −463 in mouse heart and gut and to position −411 in adipose tissue and liver mRNA.

The locations of the transcription initiation sites detected by primer extension analysis and 5’RACE PCR are in agreement with the 5’ sequence of the mVAP-1 cDNA isolated from a BALB/c mouse heart cDNA library (6) except for one primer extension product from liver mRNA in which the length of the detected 5’ end of mVAP-1 mRNA was shorter than the previously reported 5’ untranslated sequence for the cloned mVAP-1 cDNA. These results indicate that different sized mVAP-1 mRNA transcripts exist in different tissues. The location of the transcription initiation sites are shown in Figure 1.

Characterization of the 5’-flanking region of mVAP-1

The examination of the 5’ sequence (nucleotides −748 to 1) of the mVAP-1 gene revealed no consensus TATA or CCAAT boxes in front of the transcription initiation sites. Several potential binding sites for transcription factors were found in the mVAP-1 promoter region (Fig. 1). These include an NF-κB binding site (26) at position −2406 with respect to the translation initiation codon, two
the mVAP-1 gene.

9

human diamine oxidase gene structure reveals that the locations of

mouse amine oxidase characterized at the molecular level, with the

comparison of the gene structure of mVAP-1, which is the first

ing monoamine oxidase gene has been unknown. Interestingly, the

genomic organization of any human or mouse copper-contain-

mine oxidases also have activity against diamines. Thus, to date

have activity only against monoamines (6), whereas human dia-

amine oxidases belong to a different subgroup of amine oxidases

The mVAP-1 gene encodes an adhesion molecule. Thus, exon 1 seems to be the most impor-

tant exon for mVAP-1, although it is not yet known whether the

adhesive properties of VAP-1 and the enzymatic activity against

monoamines are connected or separate phenomena.

Spl sites (GC boxes) at −142 and −472 (27), an AP-2 site at

−251 (28), an AP-3 site at −167 and a CCAAT site at −236 (29),

three PEA3 (30) sites at −368, −404 and −500, a GCF site at

−469 (31), and a GATA site at −681 (32). To date it is not known

which of these sites are used or if there are other sites that would

be important for the initiation and regulation of mVAP-1 mRNA

transcription.

**Localization of the mVAP-1 gene to chromosomes 4 and 11**

To determine the localization of the mVAP-1 gene, a 5′-end-spe-
cific mVAP-1 fragment, PB3, and a 3′-end-specific fragment of
the mVAP-1 gene, PB4, were hybridized on mouse metaphase
chromosomes. The identification of the mouse chromosomes was
based on a banding pattern resembling G bands (33). Fifty meta-
phase spreads were analyzed, and the mVAP-1 gene was assigned
with both probes to mouse chromosomes 4D3-E1 and 11B2-5
(Fig. 5).

**Discussion**

In this work we have isolated the gene encoding mouse VAP-1,
determined its nucleotide sequence and structural organization,
and shown its chromosomal localization in the mouse genome.
The isolated gene is a novel single copy gene composed of four exons
separated by three introns, and the exons of this gene encode a
VAP-1 protein identical to that encoded by the previously cloned
mVAP-1 cDNA. Although neither the structure of the isolated
gen nor the nucleotide sequence has homology to any currently
known mouse cell adhesion molecules, the mVAP-1 gene encodes a
protein with significant identity to a family of enzymes called the
copper-containing amine oxidases, which are a group of enzymes
that catalyze the oxidative deamination of different amines (8, 34).
The enzyme activity of different amine oxidases has been inten-
sively studied, but despite this, the real physiologic role of these
enzymes is still not known (8).

The genomic structure of an amine oxidase encoding a human
monoamine oxidase has been reported previously (35). However,
the diamine oxidases belong to a different subgroup of amine oxidases
than mVAP-1, since mVAP-1 and hVAP-1 have been shown to
have activity only against monoamines (6), whereas human dia-
mine oxidases also have activity against diamines. Thus, to date
the genomic organization of any human or mouse copper-contain-
ing monoamine oxidase gene has been unknown. Interestingly,
the comparison of the gene structure of mVAP-1, which is the first
mouse amine oxidase characterized at the molecular level, with the
human diamine oxidase gene structure reveals that the locations of
the intron/exon boundaries of mVAP-1 and human diamine oxida-

gene are identical within the amino acid-coding regions of the
genes. Despite the different length of the introns and the fact that
the 5′- and 3′-noncoding regions of the genes are distinct, the clear
conservation of the intron locations indicate that these genes may
share a common evolutionary history, although the substrate speci-
ficity between different species and subgroups of amine oxidases
(mVAP-1 vs a diamine oxidase) has diverged substantially (36).
Thus, the evolutionary conservation between mVAP-1 and a hu-
man diamine oxidase gene suggests an important biologic role for
these enzymes.

Although the overall identity between different cloned proteins
of the copper-containing amine oxidase family varies from 37 to
95%, the enzymatically active residues are completely conserved,
as shown in Table II. The intron/exon structure of the mVAP-1
gene reveals that the enzymatically active residues in the encoded
protein (a conserved tyrosine that is converted to topaquinone) (37,
38) and two of the three important histidines that act as copper
ligands (39) in these enzymes are located in the first exon. Exon 1
also includes 8 of the 12 potential glycosylation sites in the
mVAP-1 protein core, which is of interest, since hVAP-1 is an
endothelial sialglycoprotein that cannot mediate lymphocyte
binding to endothelium if the sialic acids decorating its protein
core are removed (2). Since mVAP-1 has been shown to contain
even more potential glycosylation sites than hVAP-1, the correct
glycosylation is probably essential also for function of mVAP-1 as
an adhesion molecule. Thus, exon 1 seems to be the most impor-
tant exon for mVAP-1, although it is not yet known whether the
adhesive properties of VAP-1 and the enzymatic activity against
monoamines are connected or separate phenomena.

The initiation of transcription in the mVAP-1 gene can occur at
multiple sites within a rather long region, since no single major
transcription initiation site could be detected. In 5′RACE PCR and
primer extension experiments six different transcription initiation
sites were found between nucleotides −288 and −476 (relative to
the start ATG). No consensus TATA box could be found in the
immediate proximity (25–30 bp) of any of these transcription ini-
tiation sites, although we noticed the presence of a TATA box
further in the 5′ region at position −725. To date we do not have
any evidence that this TATA box would be functional, but this
possibility cannot be ruled out. The mVAP-1 promoter region is
not GC rich (the GC content of the nucleotides −748 to 1 in the
mVAP-1 untranslated region is 48%), suggesting that mVAP-1
does not belong to housekeeping genes, which are usually very
GC-rich genes with no TATA or CCAAT boxes in their promoter

![FIGURE 5. FISH images of mouse metaphase chromosomes hybridized with genomic probes specific for the mVAP-1 gene. A hybridization signal is detected on both chromosomes 4 and 11. A. A color image of the mouse chromosome 4 (A, right) and an ideogram (A, left) showing specific hybridization signals on 4D3-E1. B. A color image of mouse chromosome 11 (B, left) and an ideogram (B, right) showing specific hybridization signals on 11B2-5. C. A fiber-FISH image of mouse genomic DNA fibers. Probes specific for the 5′ end (3.1 kb in size, green) and for the 3′ end (9.3 kb in size, red) illustrate the mVAP-1 gene.](http://www.jimmunol.org/ by guest on April 14, 2017)
region and which are widely expressed in different tissues and cell types (40, 41). A number of TATA-less genes have been reported to contain initiator elements (Inr) for the determination of the transcription initiation, but the mVAP-1 promoter region also lacks this consensus sequence, 5′-P,CAP-P,P,P,P,-3′ (42), around the detected transcription initiation sites. Thus, the mechanism and regulation of the mVAP-1 mRNA transcription remain unknown to date.

Several consensus sequences for cis-acting elements are found in the immediate proximity of the detected transcription initiation sites (Fig. 1). Especially noteworthy is an NF-κB binding site located close to the transcription initiation sites, since it has been shown that the transcriptional activator NF-κB is required for IL-1β- and TNF-α-mediated induction of other adhesion molecules, such as VCAM-1, E-selectin, and ICAM-1, at the sites of inflammation (43, 44). Since the expression of VAP-1 has been previously shown to be organ-selectively inducible by TNF-α and IL-1 in an organ culture system (45), the existence of this NF-κB site in the mVAP-1 promoter further supports the previous finding of VAP-1 as an inflammation-inducible adhesion molecule (4).

According to primer extension and 5′RACE PCR experiments, mVAP-1 mRNA transcription seems to initiate at several different locations. None of the initiation sites determined by these experiments was closely spaced, as has been reported, for example, with the genes encoding E-selectin, L-selectin, or platelet/endothelial cell adhesion molecule-1 (25, 46, 47). On the other hand, the existence of several clearly distinct transcription initiation sites is not exceptional for cell adhesion molecules, since, for example, the human P-selectin promoter has 12 different transcription initiation sites (48). The existence of several mRNA transcription initiation sites in different tissues may indicate that there are also tissue-specific differences in the regulation of mVAP-1 transcription, e.g., in endothelial and smooth muscle cells. Tissue-specific transcriptional control mechanisms have been reported, for example, with VCAM-1, the transcriptional control mechanism of which is differentially controlled in muscle cells and endothelium (49).

FISH analysis was performed with two different mVAP-1-specific probes (3.1 kb from the 5′ end and 9.3 kb from the 3′ end of the gene) to localize the mVAP-1 gene in the mouse genome. Interestingly, both probes localized to two distinct mouse chromosomes (4D3-E1 and 11B2-5), which is in accordance with our preliminary evidence that human VAP-1 is located in the human chromosome 17 (A.-M. Kujari, manuscript in preparation), since the syntenic regions of mouse chromosome 11 are the human chromosomes 5, 17, and X (50). However, since the result from the genomic Southern blot (also with rare restriction site cutters) suggested that mVAP-1 would be present as a single copy in the mouse genome, we repeated the Southern blot with the same 9.3-kb mVAP-1 fragment as that used in the FISH. The result remained unchanged (data not shown), which can be interpreted as indicating that FISH is probably a more sensitive procedure for detecting homologues, and the probe is cross-hybridizing to a related gene located in the mouse chromosome 4. Thus, we favor the idea that mVAP-1 is a single copy gene in mouse chromosome 11. However, a sequence with significant homology is found in chromosome 4, but the nature of this related gene is unknown to date.

The cloning of the mVAP-1 gene provides a valuable tool for the in vivo analysis of the importance of mVAP-1 for lymphocyte recirculation. Targeted disruption of this gene not only may reveal new clues as to the true biologic role of copper-containing monoamine oxidases but may also provide a new insight into the function and importance of VAP-1 for the function of the immune system in normal and various inflammatory processes.

Acknowledgments

We are grateful to Prof. E. Vuorio, Dr. L. Sistonen, and Dr. P. Jaakkola for valuable comments regarding the manuscript. We also thank M. Pohjansalo and T. Kanasuo for excellent technical assistance, and A. Sovikoski-Georgieva for secretarial help.

References


