Multiple Pathways for *Steel* Regulation Suggested by Genomic and Sequence Analysis of the Murine *Steel* Gene

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ABSTRACT

The *Steel* (*Sl*) locus encodes mast cell growth factor (Mgf) that is required for the development of germ cells, hematopoietic cells and melanocytes. Although the expression patterns of the *Mgf* gene are well characterized, little is known of the factors which regulate its expression. Here, we describe the cloning and sequence of the full-length transcription unit and the 5′ flanking region of the murine *Mgf* gene. The full-length *Mgf* mRNA consists of a short 5′ untranslated region (UTR), a 0.8-kb ORF and a long 3′ UTR. A single transcription initiation site is used in a number of mouse tissues and is located just downstream of binding sites for several known transcription factors. In the 5′ UTR, two ATGs were found upstream of the initiator methionine and are conserved among different species, suggesting that *Mgf* may be translationally regulated. At least two *Mgf* mRNAs are produced by alternative use of polyadenylation sites, but numerous other potential polyadenylation sites were found in the 3′ UTR. In addition, the 3′ UTR contains numerous sequence motifs that may regulate *Mgf* mRNA stability. These studies suggest multiple ways in which expression of *Mgf* may be regulated.

MUTATIONS at the *Dominant White Spotting* (*W*) and *Steel* (*Sl*) loci identify two genes essential for the development of several cell lineages: neural crest-derived melanocytes, germ cells, hematopoietic stem cells, mast cells and erythroid cells (reviewed by SILVERS 1979a,b). The products of both loci have been cloned and together comprise a pathway for intercellular communication for these diverse cell populations. The product of the *W* locus is a receptor tyrosine kinase, called Kit, that is closely related to the platelet-derived growth factor receptor family (CHABOT *et al.* 1988; GEISSLER *et al.* 1988). The *Sl* locus encodes mast cell growth factor [Mgf, also known as Kit ligand (KL), stem cell factor (SCF) and Steel factor (SLF)], the ligand for Kit (COPELAND *et al.* 1990; FLANAGAN and LEDER 1990; HUANG *et al.* 1990; WILLIAMS *et al.* 1990; ZSEBO *et al.* 1990).

While Kit is expressed on the surface of cells affected by *W* mutations (NOCKA *et al.* 1989; MANOVA *et al.* 1990; ORR-URTREGER 1990), Mgf is expressed by stromal cells that support the growth and differentiation of the affected cells (MATSUI *et al.* 1990; KESHET *et al.* 1991). This receptor-ligand interaction is thought to be required for guiding the migration and survival of certain migratory cell populations during embryogenesis. For example, Kit is expressed by primordial germ cells and neural crest-derived melanocytes that populate the genital ridges and skin, respectively, while Mgf is abundantly expressed in mesodermal cells along the pathway of migration and at the final destination of these cells. Several lines of evidence support the notion that the level of Mgf expression in both embryonic and adult tissues is critical to its function. All *Sl* mutant alleles exert semidominant phenotypes that have been attributed to haploinsufficiency (SILVERS 1979a). In the postnatal ovary, a threshold level of Mgf is thought to be required for initiation and maintenance of follicle growth (HUANG *et al.* 1993; BEDELL *et al.* 1995). In addition, expression of *Mgf* mRNA in the mature ovarian follicles may be regulated by hormones of the estrous cycle (MOTRO and BERNSTEIN 1993). Surprisingly, both Mgf and Kit are expressed in tissues not known to be affected in *Sl* or *W* mutants. In particular, these genes are expressed in a complementary pattern in the central nervous system of both embryos and adults (MATSUI *et al.* 1990; KESHET *et al.* 1991; MOTRO *et al.* 1991), suggesting that this signaling pathway may be involved in development and/or function of the nervous system. Although the spatial and temporal patterns of expression of these genes are now well documented, little is known of the elements that control expression of either gene.

Mgf cDNAs that range in size from 1 to 2 kb have been isolated from a variety of species, including mouse (ANDERSON *et al.* 1990; HUANG *et al.* 1990; ZSEBO *et al.* 1990), human and rat (MARTIN *et al.* 1990), pig (ZHANG and ANTHONY 1994), and chicken (ZHOU *et al.* 1993). The largest of these clones is a murine *Mgf* cDNA (MGF10, see Figure 1; ANDERSON *et al.* 1990) that contains a 0.8-kb open reading frame (ORF) with 1 kb of

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3′ untranslated region (UTR). However, the major *Mgf* transcript seen on Northern blots from tissues of all species is much larger and has been estimated to be 5.5–6.5 kb with mouse and chicken displaying minor transcripts that ranged in size from 3–4.6 kb (ANDERSON *et al.* 1990, 1991; HUANG *et al.* 1990; MARTIN *et al.* 1990; ZSEBO *et al.* 1990; ZHOU *et al.* 1993; ZHANG and ANTHONY 1994). These results indicate that a considerable portion of the *Mgf* mRNAs was not cloned in cDNA form. Furthermore, it was not known whether the *Mgf* transcripts of various size were produced by alternative sites of transcription initiation or termination or by alternative splicing.

Here we describe the full length *Mgf* transcription unit and 5′ flanking sequence. These studies identify multiple pathways that may be involved in *Sl* gene regulation. In a companion paper, we use these cDNA and genomic clones for deletion analysis of various homozygous lethal *Sl* alleles.

MATERIALS AND METHODS

Libraries, clones and sequencing: A genomic library of C57BL/6J DNA was prepared by partial *Sau*3A digestion and insertion into λDash vector (Stratagene, Inc., La Jolla, CA). An oligo-dT primed cDNA library of C57BL/6J brain RNA cloned into λZAP was purchased from Stratagene (La Jolla, CA). Portions of MGF10 (ANDERSON *et al.* 1990; see Figure 1) were used to screen the genomic and cDNA libraries. Positive clones were isolated, characterized and subcloned into pSK plasmid (Stratagene) using conventional methods. Sequencing was performed using either a dideoxy method (United States Biochemical, Cleveland, OH) or an automated DNA sequencing system (Applied Biosystems Inc., Foster City, CA). Computer analysis of the nucleotide sequence was performed using the Wisconsin Sequence Analysis package (Genetics Computer Group, Madison, WI).

Analysis of *Mgf* mRNA: Total RNA from various tissues of wild-type adult mice was prepared using RNAzol (Tel-Test, Inc., Friendswood, TX). PolyA+ RNA was prepared using a mRNA Purification Kit (Pharmacia Biotech, Piscataway, NJ). Northern blot analysis was performed as described previously (BEDELL *et al.* 1995) using probes shown in Figure 1. The 5′ ends of *Mgf* mRNA were identified by a RACE technique using an Amplifinder kit (Clontech Laboratories, Inc., Palo Alto, CA) essentially as specified by the manufacturer. First strand cDNA synthesis was primed with oligonucleotide A shown in Figure 2. After ligation of the Amplifinder anchor primer to the 5′ end of the cDNA, two rounds of PCR amplification were performed using the anchor primer and various nested primers from the *Mgf* coding region (see Figure 2). 5′ RACE products were electrophoresed in agarose gels, excised from the gel, purified using Gene-Clean (Bio 101, Inc., La Jolla, CA) and were either directly sequenced or subcloned into pSK for sequencing of individual clones.

RESULTS

Isolation of full-length *Mgf* cDNA clones: To obtain cDNA clones representing full-length murine *Mgf* transcripts, a wild-type mouse brain cDNA library was probed with a portion of the 3′ UTR of MGF10 (ANDERSON *et al.* 1990; see Figure 1A). Four overlapping cDNAs

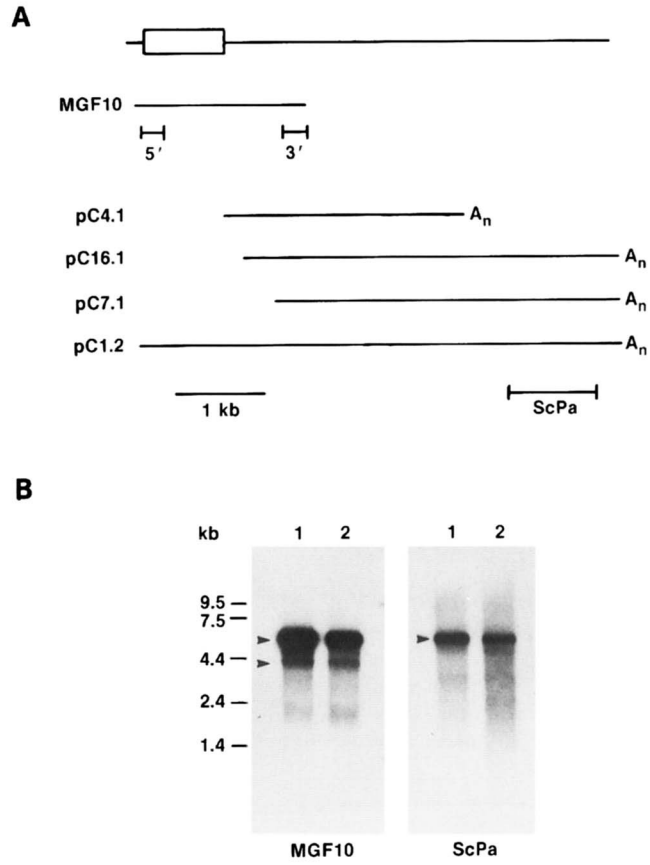


FIGURE 1.—(A) Alignment of *Mgf* cDNAs to full-length *Mgf* mRNA. The composite, full-length *Mgf* mRNA of 5.4 kb (see text) is shown at the top with the 813-nt coding region (□) and 5′ and 3′ UTRs of 197 and 4432 nt, respectively (———). Fragments of MGF10, a 2-kb DNA containing the ORF and ~1 kb of 3′ UTR (ANDERSON *et al.* 1990) were used to screen genomic and cDNA libraries; the former was screened with a 230-nt fragment (5′) while the latter was screened with a 280-nt fragment (3′). The four cDNAs isolated using the 3′ probe are shown below MGF10. PolyA tract (A_n). (B) Northern blot analysis of *Mgf* mRNA. PolyA+ mRNA of adult mouse brain (lane 1) and kidney (lane 2) was electrophoresed, blotted and hybridized with MGF10, a cDNA probe that contains the ORF and a portion of the 3′ UTR (left) or ScPa, a probe containing a distal portion of the 3′ UTR from pC1.2 (right). The position of RNA size markers are indicated to the left of the panel. Note that while MGF10 detects both the abundant 5.4 kb and minor 3.7 kb bands, only the former is detected by the ScPa probe.

(pC4.1, pC16.1, pC7.1 and pC1.2) were isolated, sequenced and aligned with the known *Mgf* cDNA sequence. A schematic of the full-length *Mgf* mRNA, representing the composite of both 5′ and 3′ flanking sequences (see below), is shown at the top of Figure 1 with the relative positions of the four newly isolated cDNAs shown below. The sequence of the full-length *Mgf* mRNA is shown in Figure 2. All four cDNAs terminate in polyA tracts. Three cDNAs (pC16.1, pC7.1 and pC1.2) have the same 3′ ends but vary in the extent of 5′ sequences, while the fourth cDNA (pC4.1) terminates 1.7 kb upstream of the ends of the other cDNAs

(Figure 1A). The 3′ UTR of the latter clone is 2732 nt in length while all other cDNAs contain 3′ UTRs of 4432 nt. None of the four cDNA clones contained novel 5′ UTR or coding sequences compared with that of *Mgf* cDNAs described previously.

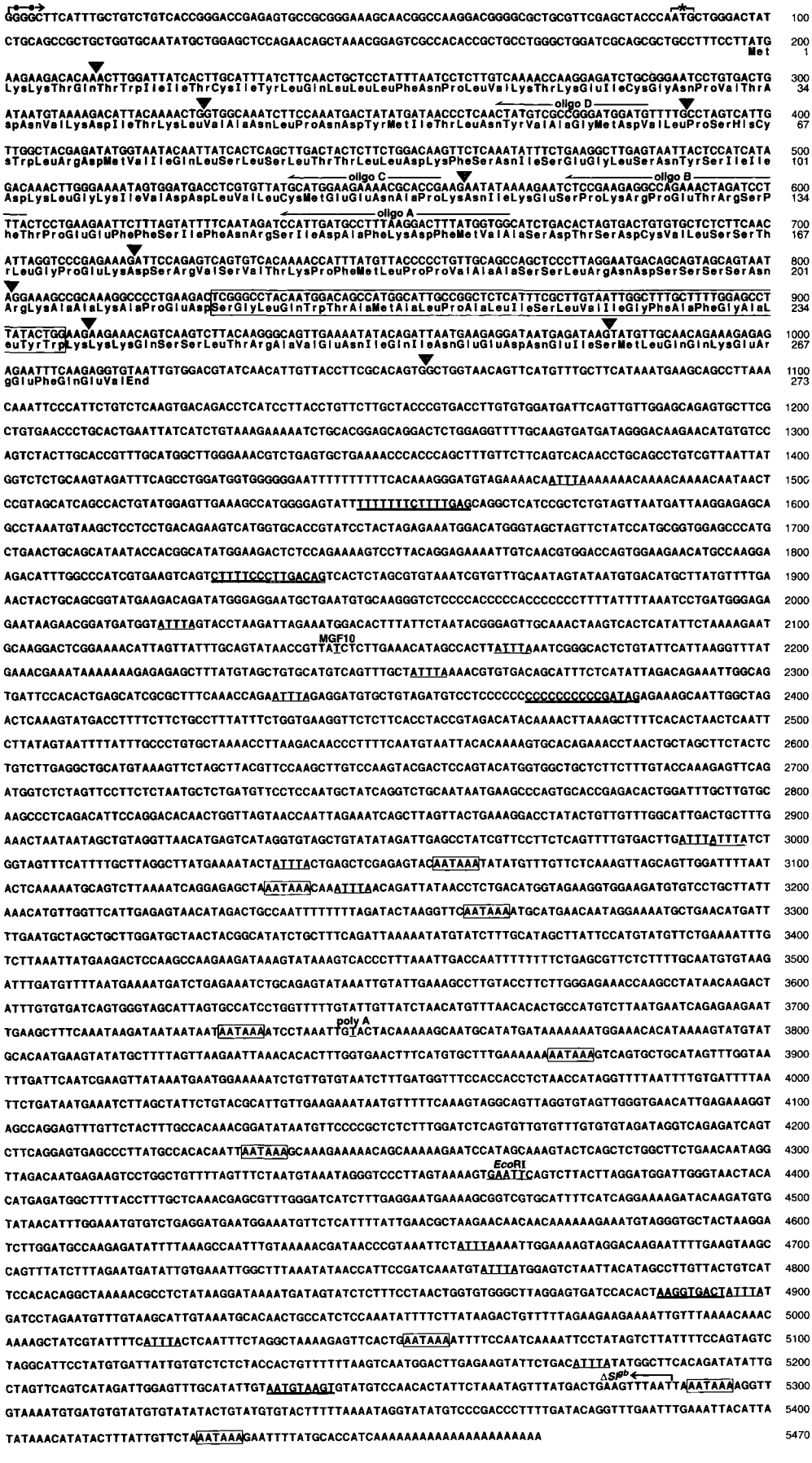
Restriction enzyme mapping, hybridization analysis and partial sequencing of a genomic clone encompassing the 3′ flanking region demonstrated that the 3′ termini of the cDNAs represent authentic polyadenylation sites. Alignment of the cDNAs to the composite *Mgf* mRNA (Figure 1A) indicates that the shorter 3′ UTR may represent the 3′ terminus of a small *Mgf* transcript while the longer 3′ UTR is included in a larger *Mgf* transcript. To test whether this alignment is correct, Northern blots of adult mouse tissues were hybridized with either MGF10 or with a probe from the 3′ terminus of pC1.2 (see Figure 1B). While MGF10 detects two *Mgf* transcripts of ~5.5 kb and ~4 kb, only the former transcript is detected by the 3′ UTR sequence from pC1.2 (Figure 1B). These results indicate that the two *Mgf* mRNAs are produced by alternative usage of polyadenylation sites. Other bands of minor abundance are apparent with both probes and may reflect either additional, alternative *Mgf* mRNAs or cross-hybridization to related sequences.

To define the 5′ end(s) of the *Mgf* mRNA, RACE was performed on cDNA using nested primers located in the 5′ end of the *Mgf* ORF. The *Mgf* primers chosen were downstream of the first four introns of *Mgf* mRNA (Figure 2) and should allow any alternative splice products in this region to be detected. 5′ RACE products of RNA from six tissues (brain, lung, kidney, heart, ovary and testes) of adult mice were analyzed. After one cycle of amplification using the RACE anchor primer and oligonucleotides B, C or D (Figure 2), the major band produced by each primer pair was identical in all six tissues (not shown). That these bands represent authentic *Mgf* sequences was confirmed by hybridization to an *Mgf* cDNA probe. Other, minor bands were observed but were not consistently produced during repetitive experiments. The major band from each tissue was then excised, purified and subjected to a second round of amplification using either oligonucleotide C or D and the PCR products derived from each tissue were directly sequenced. All of the RACE products were colinear except for the last three to four nucleotides at the 5′ end. However, the sequence in this region was too heterogeneous to define a specific start site. To circumvent this, the RACE products from all six tissues were subcloned into plasmid and five or six individual clones from each tissue were isolated and sequenced. Of 35 different RACE clones that were sequenced, all initiated at one of three Gs at position 1, 2 or 4 shown in Figure 2. The majority of the 5′ ends were at position 1 (23/35; 66%), with minor start sites at position 2 (7/35; 20%) and position 4 (5/35; 14%). These results identify the major start sites for *Mgf* transcription with no evidence for alternative start sites further upstream or alternative splicing within the first four coding exons of *Mgf*. The transcription start site identified by the RACE technique has been confirmed by RNase protection analysis (see below).

Because the 5′ ends of *Mgf* transcripts vary by only a few nucleotides, the presence of transcripts of different size observed on Northern blots appears to reflect differences in the length of the 3′ UTR. The longest *Mgf* mRNA would total ~5.4 kb and is comprised of a 5′ UTR of 197 nt, an ORF of 818 nt and a 3′ UTR of 4432 nt (Figure 2). This 5.4 kb mRNA most likely corresponds to the most abundant transcript observed on Northern blots of murine tissues (Figure 1B). A second *Mgf* mRNA of ~3.7 kb, with a 3′ UTR of only 2732 nt, could be produced by alternative use of the polyadenylation sites and differs from the 5.4 kb transcript only in the length of the 3′ UTR. This 3.7-kb mRNA likely represents the smaller, less abundant transcript (Figure 1B). The total amount of mouse genomic DNA spanned by the larger *Mgf* mRNA was estimated to be ~50 kb by Southern blot analysis using various cDNA probes.

Although the *Mgf* 3′ UTR contains a single intron (intron 9) at position 1056 (see Figure 2), the remainder of the 3′ UTR is colinear with the genomic clone. Scanning of the nucleotide sequence in the 3′ UTR revealed the presence of several sites that may represent alternative splicing sites: sequences that display a single mismatch from the consensus 5′ donor splice site (A/CAGGTA/GAGT) are located at nucleotide positions 4886 while three possible 3′ acceptor splice sites (C/T_{11}NC/TAG) are located at positions 1547, 1828 and 2369 (Figure 2). In addition, the 3′ UTR contains numerous motifs (ATTTA) that have been associated with decreased mRNA stability (Shaw and Kamen 1986; Brewer 1991). A total of 14 ATTTA motifs are found in the 3′ UTR of the 5.4-kb *Mgf* transcript with nine of these motifs in the 3′ UTR of the 3.7-kb transcript (Figure 2). In addition, nine different motifs that perfectly match the consensus polyadenylation sequence (AATAAA) are found in the *Mgf* 3′ UTR (Figure 2). Two of these are immediately upstream of the polyadenylation tracts of the *Mgf* cDNAs and are likely to represent the signals for termination of the 3.7- and 5.4-kb *Mgf* transcripts. However, three other AATAAA motifs are found upstream of the 3.7-kb polyadenylation site and four AATAAA motifs are found upstream of the 5.4-kb polyadenylation signal (Figure 2). This raises the possibility that additional 3′ termini may exist for *Mgf* mRNA.

Examination of the 5′ UTR sequence revealed that there are three ATGs located at positions 88, 123 and 198, with the latter encoding the initiator methionine for Mgf (Figure 2). With few exceptions, eukaryotic mRNAs initiate translation at the ATG closest to the 5′ end of the message (Kozak 1987). If translation were to initiate from the first ATG in *Mgf* mRNA, at position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oligo C
oligo B
oligo A
MGF10
poly A
EcoRI
ΔSIgb

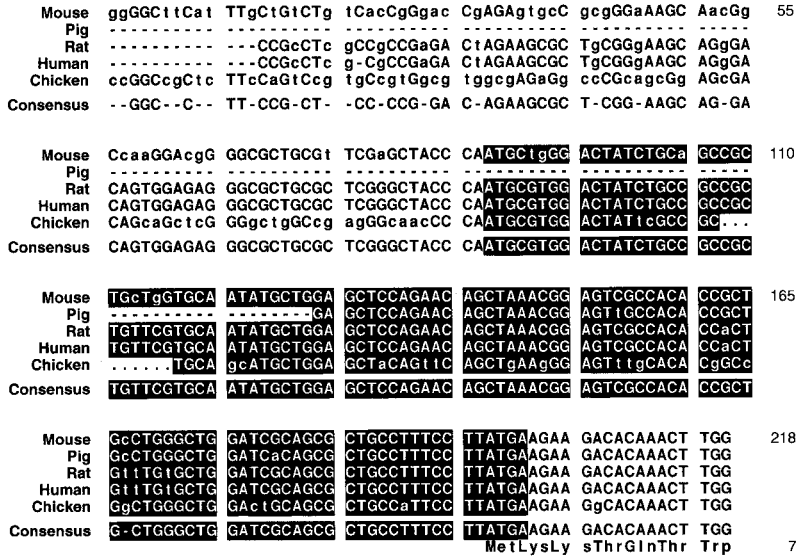

FIGURE 3.—The 5′ UTR of *Mgf* is highly conserved. The nucleotide sequence of 5′ UTRs from *Mgf* mRNAs of five different species (mouse, present study; pig, ZHANG and ANTHONY 1994; rat and human, MARTIN *et al.* 1990; chicken, ZHOU *et al.* 1993) were aligned. Sequence not available (– – –); gaps in alignment (. . .). Sequences that match the consensus for all species are in capital letters while sequences that do not match the consensus are in lower case. The numbering refers to mouse *Mgf* mRNA and begins at the transcriptional start site. The first seven amino acids of the mouse Mgf protein are shown below the consensus nucleotide sequence. A potential upstream reading frame, that would initiate at the first ATG in the 5′ UTR and terminate at a stop codon that overlaps the initiator methionine, is highlighted. Note the high degree of conservation within this upstream reading frame and that both the first ATG and the termination codon are conserved in different species.

88, a 37 aa polypeptide would be produced whose termination codon overlaps the initiator methionine of Mgf. Alternatively, initiation of translation from the second ATG would produce a 7 aa polypeptide. However, none of the three ATGs are in a sequence context that is known to be most favorable for translation initiation [(GCC)GCCA/GCCATGG, where residues at −3 and +4 are most critical; KOZAK (1987)]. Although these observations suggest a mechanism for translational regulation of *Mgf* expression, there is at present no experimental evidence for such translational control. To gain some information on the functional significance of the 5′ UTR of *Mgf*, we have compared the sequence of this region of *Mgf* mRNA isolated from five different species (Figure 3). Although the complete 5′ UTR of each of these species has not been published, alignment of the available sequences revealed from 82 to 94% identity within the 110-nt interval between the first ATG at position 88 of the mouse sequence and the initiator methionine. Significantly, both of the upstream ATGs and their respective termination codons are conserved in different species. This high degree of conservation between species suggests that there may in fact be some form of translational regulation of *Mgf* expression.

Isolation and characterization of the 5′ flanking region of *Mgf*: Although the above experiments identified the 5′ ends of *Mgf* mRNA, the location of these sequences relative to the coding region in genomic DNA was not known. To elucidate this, a genomic library of C57BL/6J DNA was screened with a probe encompassing the very 5′ end of MGF10 (ANDERSON *et al.* 1990, see Figure 1A) and a 17-kb clone was identified and subcloned into Bluescript (p14.1, Figure 4). Cleavage of p14.1 with *Eco*RI produced four fragments (Figure 4), three of which (B, C and D) were found to recognize unique fragments when used as probes on Southern blots of mouse genomic DNA. Hybridization of the *Eco*RI-B fragment (EcoB) to blots of mouse DNA prepared by pulsed field gel electrophoresis revealed the presence of an island of rare-cutting enzymes between the 5′ flanking region and the *Mgf* coding region (see BEDELL *et al.* 1996). This suggests the presence of a CpG island(s) that is frequently found upstream of genes (reviewed by BIRD 1987). Further analysis of p14.1 revealed that only the *Eco*RI-D (EcoD) fragment contains sequences that hybridize to the 5′ *Mgf* probe. The 1.7-kb EcoD fragment was subcloned and sequenced (Figure 4). The 3′ end of EcoD is located within the 5′ UTR of *Mgf*, at position 179, just upstream of the initiator methionine. The remainder of the 5′ UTR, including the transcription initiation site identified by RACE (shown as +1 in Figure 4), is colinear with the genomic sequence of the EcoD fragment. To confirm that the RACE products described above represent the site of transcription initiation, a portion of the 3′ region of EcoD was used as a probe in RNase

FIGURE 2.—Sequence of the full-length murine *Mgf* mRNA. The sequence of the coding region was taken from ANDERSON *et al.* (1990) while the sequence of the 5′ UTR and 3′ UTR were derived from the analysis of 5′ RACE products (see text) and cDNAs (see Figure 1A), respectively. The nucleotide sequence is numbered from the transcriptional start site identified by 5′ RACE (the three alternative sites at positions 1, 2 and 4 are indicated by ⌜•→) with oligonucleotides (oligo A–D) used for RACE analysis shown (——). The encoded amino acid residues are shown below the nucleotide sequence with numbering beginning at the initiator methionine at nucleotide position 197 and the transmembrane domain boxed. The first ATG in the 5′ UTR is indicated (⌜*⌝). The positions of introns are indicated (▼): introns 1–7 (MARTIN *et al.* 1990); intron 8 (BRANNAN *et al.* 1992); intron 9 (present study). The 3′ ends of MGF10 (ANDERSON *et al.* 1990) and pC4.1 (see Figure 1A) at positions 2144 and 3746 are shown above the sequence (MGF10 and polyA, respectively). The distal breakpoint of the Sl^{gb} deletion (see BEDELL *et al.* 1996) is at position 5287 (Δ Sl^{gb} ←⌝). The following sequence motifs in the 3′ UTR are shown; polyadenylation signals (AATAAA, boxed), mRNA instability (ATTTA, underlined) and potential splice sites (double underlined).

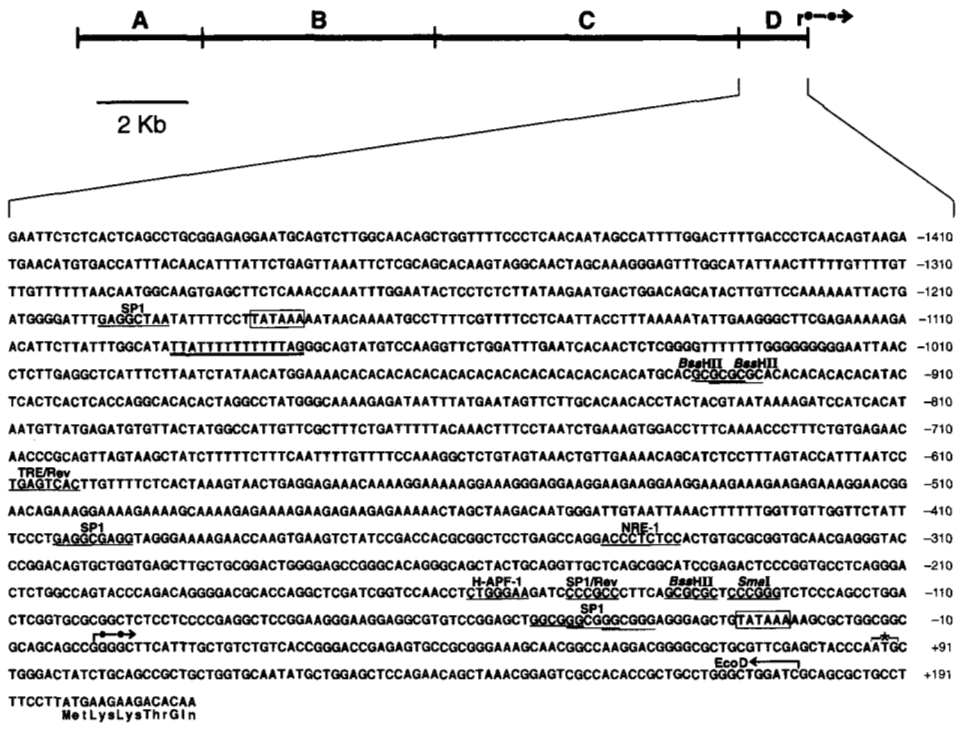

FIGURE 4.—Schematic and partial sequence of a genomic clone for the 5′ flanking region of Mgf. A 17-kb genomic clone (p14.1) containing four *Eco*RI fragments (A–D) is shown with the solid line. The *Eco*RI-B, C, and D fragments (EcoB, EcoC and EcoD) are unique. The *Eco*RI-D fragment (EcoD) was subcloned and sequenced. The transcriptional start site is indicated (┌●→). The 3′ end of EcoD is at position 179 (EcoD ←┐). The sequence is numbered with +1 as the site of transcription initiation and extends to the first intron of *Mgf* with the encoded amino acids shown below the nucleotide sequence. The first ATG in the 5′UTR is indicated (┌*┐). Potential transcription factor binding motifs are in boxes (TATAAA) (SAWADOGO and SENTENAC 1990) or are underlined: SP1 (KADONAGA *et al.* 1986); TRE/Rev (reverse of TRE) (LEE *et al.* 1987; SASSONE-CORSI *et al.* 1990); NRE-1 (BANIAHMAD *et al.* 1987); H-APF-1 (MAJELLO *et al.* 1990). A motif, at position 1091, that has a single mismatch from the consensus 3′ splice site is double underlined. Restriction enzyme sites for *Bss*HII and *Sma*I are underlined.

protection analysis of various adult tissues (not shown). These analyses were conducted with RNA from brain, lung, heart, kidney, ovary and testes and verified that *Mgf* transcription in all tissues initiates near the previously identified sites.

Analysis of the EcoD sequence in the vicinity of the *Mgf* transcription initiation site revealed a TATA box consensus sequence (TATAAA) at −28 nucleotides and a set of three overlapping GGCGGG motifs at nucleotides −51 to −38 (Figure 4). These motifs are the core binding sites for the transcription factors TFIID and SP1, respectively (see reviews by KADONAGA *et al.* 1986; SAWADOGO and SENTENAC 1990). The proximity of these motifs to the transcription initiation site indicates that they may constitute proximal elements of the *Mgf* promoter. Comparison of the EcoD sequence to the Transcription Factor Site database (7.3 release; see GHOSH 1991) revealed other sequence motifs further upstream of the transcriptional initiation site that are similar to the binding sites of known transcriptional regulatory proteins (see Figure 4), including hepatocyte-acute phase factor-1 (H-APF-1) (MAJELLO *et al.* 1990), negative regulatory element-box 1 (NRE-1) (BANIAHMAD *et al.* 1987), and the reverse of the TPA responsive element (TRE/Rev) (LEE *et al.* 1987; SASSONE-CORSI *et al.* 1990). Curiously, another consensus TATA box was found at position −1182 that is preceded by an SP1 binding motif. However, we have yet to find any evidence for transcription initiation in the vicinity of these upstream motifs. Although sequences resembling 5′ splice donor sequences are not present in EcoD, a near consensus 3′ splice acceptor sequence is present at position −1091 (Figure 4). Computer searches of the EMBL, GenBank and Eukaryotic Promoter databases did not reveal any sequence similarity to EcoD that was considered significant. Last, EcoD contains recognition sequences of two rare-cutting enzymes (*Bss*HII and *Sma*I) that may be part of a CpG island, a 90-nt region at position −972 that contains CA repeats and a 117-nt region at position −579 that contains repeats of varying pattern involving Gs and As.

DISCUSSION

In studies described here, we report the cloning of the full-length *Mgf* transcription unit as well as genomic sequences that extend 5′ to this transcribed region. Examination of the nucleotide sequences of the 5′ and 3′ flanking regions has revealed potential regulatory elements for *Mgf* expression. The full-length *Mgf* tran-

script is comprised of a short 5′ UTR, a 0.8-kb ORF and a long 3′ UTR that contains at least two polyadenylation sites. Differential use of polyadenylation signals appears to be the mechanism by which two *Mgf* transcripts of 3.7 and 5.4 kb, differing only in the length of the 3′ UTR, are generated. Because the larger transcript is much more abundant than the smaller transcript (Figure 1B, ANDERSON *et al.* 1990; HUANG *et al.* 1990; ZSEBO *et al.* 1990), these differences in the 3′ UTR may affect the relative stability of the two mRNAs. Examination of the sequence of the *Mgf* 3′ UTR reveals numerous ATTTA motifs, a motif that is known to affect the stability of mRNAs of many growth factors and cytokines (SHAW and KAMEN 1986; BREWER 1991). Regulation of mRNA stability may therefore be one mechanism for controlling the expression of the *Mgf* gene. The present studies also demonstrate that the 5′ UTR of *Mgf* contains two upstream ATGs that are conserved in different species and may provide a means for regulation of Mgf translation.

A major transcription initiation site was identified for *Mgf* mRNA that functions in all adult tissues tested and is located 28 nt downstream from a consensus TATA motif, the binding site for TFIID (reviewed by SAWADOGA and SENTENAC 1990). However, the sequence around the *Mgf* initiation site does not contain a consensus initiator sequence (JAVAHERY *et al.* 1994) that is thought to specify precise initiation. This may explain the heterogeneity observed in the 5′ ends of the *Mgf* mRNA, with transcription initiating at one of three Gs located at position +1, +2 and +4. The *Mgf* 5′ flanking sequence contains numerous potential binding sites for SP1, located from 38 to 51 nt upstream of the transcription initiation site. Although the functional significance of these and other motifs, such as the TRE, NRE and H-APF-1 motifs (Figure 4) identified in the *Mgf* 5′ flanking region remains to be determined, there is evidence for a potential role of one of these motifs in *Mgf* transcription. The H-APF-1 binding site is thought to be required for the interleukin (Il)-6-induced transcription of the gene encoding human C-reactive protein and is found upstream of many Il-6-responsive genes (MAJELLO *et al.* 1990). In stromal cell cultures, Il-6 treatment has been shown to abrogate the stimulatory effect of Il-7 on *Mgf* mRNA levels (PALACIOS and NISHIKAWA 1992). The mechanism by which *Mgf* expression is affected by these cytokines is not presently known but could perhaps be mediated through the H-APF-1 binding motif in the 5′ flanking region of *Mgf*. The only other factors that appear to be directly involved in regulation of *Mgf* mRNA expression act through the cyclic AMP pathway (ROSSI *et al.* 1993; PACKER *et al.* 1994). Although a cAMP-responsive element (TGACGTCA; see SASSONE-CORSI *et al.* 1990) was not found in the upstream genomic sequence of *Mgf*, it is of note that the cAMP-responsive element is very similar to the TPA-responsive element (TRE; TGACTCA) and in fact differs by only one nucleotide from the TRE/Rev motif in EcoD (TGAGTCA; see Figure 4). Molecular dissection of genomic fragments reported here should allow identification of *cis*-acting elements that control *Mgf* transcription. The regulation of *Mgf* transcription may, however, be complex as tissue specific effects on *Mgf* mRNA expression occur in Sl^{pan} and Sl^{con} mice as the result of rearrangements located 115 and 195 kb, respectively, upstream of the *Mgf* coding region in these mutants (BEDELL *et al.* 1995). Because we have found no evidence for alternative *Mgf* transcriptional start sites located far upstream of the *Mgf* coding region, the rearrangements may disrupt far-distant regulatory elements for *Mgf* expression. Alternatively, transcription of *Mgf* in some tissues may be susceptible to long-range position effects on chromatin structure.

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Note added in proof: The sequences have been deposited in GenBank under accession numbers U44724 and U44725.

LITERATURE CITED

ANDERSON, D. M., S. D. LYMAN, A. BAIRD, J. M. WIGNALL, J. EISENMAN *et al.*, 1990 Molecular cloning of mast cell growth factor, a hematopoietin that is active in both membrane bound and soluble forms. Cell **63:** 235–243.

ANDERSON, D. M., D. E. WILLIAMS, R. TUSHINSKI, S. GIMPEL, J. EISENMAN *et al.*, 1991 Alternate splicing of mRNAs encoding human mast cell growth factor and localization of the gene to chromosome 12q22-q24. Cell Growth Diff. **2:** 373–378.

BANIAHMAD, A., M. MULLER, C. STEINER and R. RENKAWITZ, 1987 Activity of two different silencer elements of the chicken lysozyme gene can be compensated by enhancer elements. EMBO J. **6:** 2297–2303.

BEDELL, M. A., C. I. BRANNAN, E. P. EVANS, N. G. COPELAND, N. A. JENKINS *et al.*, 1995 DNA rearrangements located over 100 kb 5′ of the *Steel* (*Sl*)-coding region in *Steel-panda* and *Steel-contrasted* mice deregulate *Sl* expression and cause female sterility by disrupting ovarian follicle development. Genes Dev. **9:** 455–470.

BEDELL, M. A., L. S. CLEVELAND, T. N. O'SULLIVAN, N. G. COPELAND and N. A. JENKINS, 1996 Deletion and interallelic complementation analysis of *Steel* mutant mice. Genetics **142:** 000–000.

BIRD, A. P. 1987 CpG islands as gene markers in the vertebrate nucleus. Trends Genet. **3:** 342–347.

BREWER, G. 1991 An A + U- rich element RNA-binding factor regulates c-*myc* mRNA stability *in vitro*. Mol. Cell. Biol. **11:** 2460–2466.

CHABOT, B., D. A. STEPHENSON, V. M. CHAPMAN, P. BESMER and A. BERNSTEIN, 1988 The proto-oncogene *c-kit* encoding a transmembrane tyrosine kinase receptor maps to the mouse *W* locus. Nature **335:** 88–89.

COPELAND, N. G., D. J. GILBERT, B. C. CHO, P. J. DONOVAN, N. A. JENKINS *et al.*, 1990 Mast cell growth factor maps near the *Steel*

locus on mouse chromosome 10 and is deleted in a number of *Steel* alleles. Cell **63:** 175–183.

FLANAGAN, J. G., and P. LEDER, 1990 The *kit* ligand: a cell surface molecule altered in *Steel* mutant fibroblasts. Cell **63:** 185–194.

GEISSLER, E. N., M. A. RYAN and D. E. HOUSMAN, 1988 The dominant-white spotting (*W*) locus of the mouse encodes the *c-kit* proto-oncogene. Cell **55:** 185–192.

GHOSH, D. 1991 New developments of a transcription factors database. Trends Biochem. Sci. **16:** 445–447.

HUANG, E., K. NOCKA, D. R. BEIER, T.-Y. CHU, J. BUCK *et al.*, 1990 The hematopoietic growth factor KL is encoded at the *Sl* locus and is the ligand of the *c-kit* receptor, the gene product of the *W* locus. Cell **63:** 225–233.

HUANG, E. J., K. MANOVA, A. I. PACKER, S. SANCHEZ, R. F. BACHVAROVA *et al.*, 1993 The murine *Steel* panda mutation affects kit ligand expression and growth of early ovarian follicles. Dev. Biol. **157:** 100–109.

JAVAHERY, R., A. KHACHI, K. LO, B. ZENZIE-GREGORY and S. T. SMALE, 1994 DNA sequence requirements for transcriptional initiator activity in mammalian cells. Mol. Cell. Biol. **14:** 116–127.

KADONAGA, J. T., K. A. JONES and R. TJIAN, 1986 Promoter-specific activation of RNA polymerase II transcription by SP1. Trends Biochem. Sci. **11:** 20–23.

KESHET, E., S. D. LYMAN, D. E. WILLIAMS, D. M. ANDERSON, N. A. JENKINS *et al.*, 1991 Embryonic RNA expression patterns of the *c-kit* receptor and its cognate ligand suggest multiple functional roles in mouse development. EMBO J. **10:** 2425–2435.

KOZAK, M., 1987 An analysis of 5′-noncoding sequences from 699 vertebrate messenger RNAs. Nucleic Acids Res. **15:** 8125–8148.

LEE. W., P. MITCHELL and R. TJIAN, 1987 Purified transcription factor AP-1 interacts with TPA-inducible enhancer elements. Cell **49:** 741–752.

MAJELLO, B., R. ARCONE, C. TONIATTI and G. CILIBERTO, 1990 Constitutive and IL-6- induced nuclear factors that interact with the human C-reactive protein promoter. EMBO J. **9:** 457–465.

MANOVA, K., K. NOCKA, P. BESMER and R. F. BACHVAROVA, 1990 Gonadal expression of c-*kit* encoded at the *W* locus of the mouse. Development **110:** 1057–1069.

MARTIN, F. H., S. V. SUGGS, K. E. LANGLEY, H. S. LU, J. TING *et al.*, 1990 Primary structure and functional expression of rat and human stem cell factor DNAs. Cell **63:** 203–211.

MATSUI, Y., K. M. ZSEBO and B. L. M. HOGAN, 1990 Embryonic expression of a haematopoietic growth factor encoded by the *Sl* locus and the ligand for *c-kit*. Nature **347:** 667–669.

MOTRO, B., and A. BERNSTEIN. 1993 Dynamic changes in ovarian c-*kit* and *Steel* expression during the estrous reproductive cycle. Devel. Dynamics **197:** 69–79.

MOTRO, B., D. VAN DER KOOY, J. ROSSANT, A. REITH and A. BERNSTEIN. 1991 Contiguous patterns of *c-kit* and *steel* expression: analysis of mutations at the *W* and *Sl* loci. Development **113:** 1207–1221.

NOCKA, K., S. MAJUMDER, B. CHABOT, P. RAY, M. CERVONE *et al.*, 1989 Expression of c-*kit* gene products in known cellular targets of *W* mutations in normal and *W* mutant mice-evidence for an impaired c-*kit* kinase in mutant mice. Genes Dev. **3:** 816–826.

ORR-URTREGER, A., A. AVIVI, Y. ZIMMER, D. GIVOL, Y. YARDEN *et al.*, 1990 Developmental expression of c-*kit*, a proto-oncogene encoded by the *W.* locus. Development **109:** 911–923.

PACKER, A. I., Y. C. HSU, P. BESMER and R. F. BACHVAROVA, 1994 The ligand of the c-*kit* receptor promotes oocyte growth. Devel. Biol. **161:** 194–205.

PALACIOS, R., and S.-I. NISHIKAWA, 1992 Developmentally regulated cell surface expression and function of c-*kit* receptor during lymphocyte ontogeny in the embryo and adult mice. Development **115:** 1133–1147.

ROSSI, P., S. DOLCI, C. ALBANESI, P. GRIMALDI, R. RICCA *et al.*, 1993 Follicle-stimulating hormone induction of steel factor (SLF) mRNA in mouse Sertoli cells and stimulation of DNA synthesis in spermatogonia by soluble SLF. Dev. Biol. **155:** 68–74.

SASSONE-CORSI, P., L. J. RANSONE and I. M. VERMA, 1990 Cross-talk in signal transduction: TPA-inducible factor *jun*/AP-1 activates cAMP-responsive enhancer elements. Oncogene **5:** 427–431.

SAWADOGO, M., and A. SENTANAC, 1990 RNA polymerase B (II) and general transcription factors. Annu. Rev. Biochem. **59:** 711–754.

SHAW, G., and R. KAMEN, 1986 A conserved AU sequence from the 3′ untranslated region of GM-CSF mRNA mediates selective mRNA degradation. Cell **46:** 659–667.

SILVERS, W. K., 1979a Steel, flexed-tail, splotch and varitint-waddler, pp. 242–267 in *The Coat Colors of Mice: A Model for Mammalian Gene Action and Interaction.* Springer Verlag, New York.

SILVERS, W. K., 1979b Dominant spotting, patch, and rump-white, pp. 206–241 in *The Coat Colors of Mice: A Model for Mammalian Gene Action and Interaction.* Springer-Verlag, New York.

WILLIAMS, D. E., J. EISENMAN, A. BAIRD, C. RAUCH, K. VAN NESS *et al.*, 1990 Identification of a ligand for the c-*kit* proto-oncogene. Cell **63:** 167–174.

ZHANG, Z., and R. V. ANTHONY, 1994 Porcine stem cell factor/*c-kit* ligand: its molecular cloning and localization within the uterus. Biol. Reprod. **50:** 95–102.

ZHOU, J.-H., M. OHTAKI and M. SAKURAI, 1993 Sequence of a cDNA encoding chicken stem cell factor. Gene **127:** 269–270.

ZSEBO, K. M., D. A. WILLIAMS, E. N. GEISSLER, V. C. BROUDY, F. H. MARTIN *et al.*, 1990 Stem cell factor is encoded at the *Sl* locus of the mouse and is the ligand for the *c-kit* tyrosine kinase receptor. Cell **63:** 213–224.

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