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

Mary A. Bedell, Neal G. Copeland and Nancy A. Jenkins

Mammalian Genetics Laboratory, ABL-Base Research Program, NCI-Frederick Cancer Research and Development Center, Frederick, Maryland 21702-1201

Manuscript received November 14, 1995
Accepted for publication December 4, 1995

ABSTRACT

The Steel (SI) 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 cRNA 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 (SI) 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 path away 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 SI 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 Wmutations (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. 1996; 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 SI 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 SI 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
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 Sau3A digestion and insertion into eZAP vector (Stratagene, Inc., La Jolla, CA). An oligo-dT primed cDNA library of C57BL/6j brain RNA cloned into eZAP 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 (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 MGFlO 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. 5' RACE products of all six tissues (not shown) 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. 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 (Figure 1B), 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/TiNC/TAG) are located at positions 1547, 1828 and 2369 (Figure 2). In addition, the 3' UTR contains numerous motifs (ATTITA) 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
Alternatively, initiation of translation from the second nation codon overlaps the initiator methionine of Mgf. It is known that the three ATGs are in a sequence context that is most favorable for translation initiation. Observations suggest a mechanism for translational regulation of Mgf expression, but there is no experimental evidence for such translational control. To gain some information on the functional significance of the 3' UTR of Mgf, we have compared the sequence of this region of Mgf mRNA isolated from five different species (Figure 3). Although the complete 3' 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. Significant, 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 EcoRI produced four fragments (Figure 4), which are possibly found downstream when used as probes on Southern blots of mouse genomic DNA. Hybridization of the EcoRI-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 two flanking regions 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 EcoRI-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 RNAseq analysis.

**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 (---). 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 translation 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. 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.

<table>
<thead>
<tr>
<th>Species</th>
<th>5' UTR Sequence</th>
<th>Coding Region Sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mouse</td>
<td>gGGCGCCTGCT</td>
<td>CACCAAAUCACT TGG</td>
</tr>
<tr>
<td>Pig</td>
<td>CCGCAAGAGG</td>
<td>AAGCAAAACT TGG</td>
</tr>
<tr>
<td>Human</td>
<td>CCGCAAGAGG</td>
<td>AAGCAAAACT TGG</td>
</tr>
<tr>
<td>Chicken</td>
<td>CCGCAAGAGG</td>
<td>AAGCAAAACT TGG</td>
</tr>
<tr>
<td>Consensus</td>
<td>-GCC-CCC-</td>
<td>T-CGG-AAGC AG-GA</td>
</tr>
</tbody>
</table>

**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 analysis 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 RNAseq analysis.
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 EcoRI fragments (A–D) is shown with the solid line. The EcoRI-B, C, and D fragments (EcoB, EcoC and EcoD) are unique. The EcoRI-D fragment (EcoD) was subcloned and sequenced. The transcriptional start site is indicated (•). The 3′ end of EcoD is at position 179 (EcoD I). 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 BssHII and SmaI are underlined. A motif, at position 1091, that has a single mismatch from the consensus 3′ splice site is double underlined. Restriction enzyme sites for BssHII and SmaI are underlined.

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 (Fig- ure 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 AT- TAA motifs, a motif that is known to affect the stability of mRNAs of many growth factors and cytokines (Shaw and Kamin 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 TFIIID (reviewed by Sawa- doga 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 (II)-6-induced transcription of the gene encoding human C-reactive protein (Majello et al. 1990). In stromal cell cultures, II-6 treatment has been shown to abrogate the stimulatory effect of II-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; Pack er 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 nucle- otide 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 Spl+ and Spl− 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.

We are grateful to Douglas E. Williams and Stewart D. Lyman for the MGFr1 cDNA, Marilyn Powers for synthesis of oligonucleo- tides and automated DNA sequencing, and Erikur Stengrimsson and Carolyn M. Hlustad for reading the manuscript. M.A.B. was supported by fellowships from the Foundation for Advanced Cancer Studies and the National Institute for General Medical Sciences, Na- tional Institutes for Health. This research was supported by the Na- tional Cancer Institute, Department of Health and Human Services, under contract with ABL. The contents of this publication do not necessarily reflect the views or policies of the Department of Health and Human Services, nor does mention of trade names, commercial products, or organizations imply endorsement by the U.S. Govern- ment.

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

LITERATURE CITED


Bedell, M. A., C. L. Brannan, E. P. Evans, N. G. Copeland, N. A. Jenkins et al., 1995 DNA rearrangements located over 100 kb 5' of the Steel (S1) coding region in Steel-panda and Steel-contrasted mice deregulate Sl expression and cause female sterility by disrup- ting ovarian follicle development. Genes Dev. 9: 455–470.


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 

Flanagan, J. G., and P. Leder, 1990 The kit ligand: a cell surface 

Geissler, E. N., M. A. Ryan and D. E. Housman, 1988 The domi-
nant-white spotting (W) locus of the mouse encodes the c-kit 

Ghosh, D. 1991 New developments of a transcription factors data-

Huang, E., K. Nocka, D. R. Beier, T.-Y. Chu, J. Buck et al., 1990 
The hematopoietic growth factor KL is encoded at the W locus. 

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. Khanchi, K. Lo, B. Zenzie-Gregory and S. T. Smail, 
1994 DNA sequence requirements for transcriptional initiator 

Kadonaga, J. T., K. A. Jones and R. Tjian, 1986 Promoter-specific 
activation of RNA polymerase II transcription by SP1. Trends 

Keshet, E., S. D. Liman, 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 

Kozak, M., 1987 An analysis of 5′-noncoding sequences from 699 

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. Gilberto, 1990 Consis-
tutive and IL-6-induced nuclear factors that interact with the 
growth hormone secretagogue receptor promoter. EMBO J. 9: 
457–465.

Manova, K., K. Nocka, P. Besmer and R. F. Bachvarova, 1990 Ga-
ronal expression of c-kit encoded at the W locus of the mouse. 

Martin, F. H., S. V. Suggs, K. E. Langley, H. S. Lu, J. Ting et al., 
1999 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 hematopoietic growth factor encoded by the SI 

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

Motto, B., D. Van Der Roo, J. Rossant, A. Reith and A. Bernstein. 
1991 Contiguous patterns of c-kit and steel expression: analysis 

Nocka, K., S. Majumder, B. Charot, P. Ray, M. Cervons 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 im-

Orr-Urtreger, A., A. Avivi, Y. Zimmer, D. Givol, Y. Yarden et al., 
1990 Developmental expression of c-kit, a prote-ontocogene en-

The ligand of the c-kit receptor promotes oocyte growth. Dev. 

Palacios, R., and S.-J. Nishikawa, 1992 Developmentally regulated 
cell surface expression and function of c-kit receptor during lympho-
ctic 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 signal transduction: TPA-inducible factor jun/AP-1 activates 

Sawadogo, M., and A. Sentanac, 1990 RNA polymerase B II and 

Shaw, G., and R. Kamen, 1986 A conserved AU sequence from the 3′ 
untranslated region of GM-CSF mRNA mediates selective 

Silver, W. K., 1979a Steel, flexed-tail, alpocht and varitint-waddler, 
pp. 242–267 in The Coat Colors of Mice: A Model for Mammalian 

Silver, W. K., 1979b Dominant spotting, patch, and rump-white, 

Williams, D. E., J. Eisenman, A. Baird, C. Rauchel, K. Van Ness et al., 
1990 Identification of a ligand for the c-kit proto-oncogene. 

Zhang, Z., and R. V. Anthony, 1994 Porcine stem cell factor/c-kit 
ligand: its molecular cloning and localization within the uterus. 


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 SI locus of 
the mouse and is the ligand for the c-kit tyrosine kinase recep-

Communicating editor: R. E. Ganschow