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LETTER

The Exon Structure of the Mouse *Sc1* Gene Is Very Similar to the Mouse *Sparc* Gene

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Sc1 and Sparc are two extracellular proteins sharing similarity in their carboxyl terminus, with 63% identity over a 232-amino-acid region. We have cloned and mapped the genomic locus of mouse *Sc1*. The mouse *Sc1* gene contains 11 exons spanning ~35 kb of DNA. The genomic structure (exon/intron boundaries) of *Sc1* exons 6 to exon 11 is identical to those of the similar portion of the *Sparc* gene. This suggests that Sc1 and Sparc originated from a common ancestral gene. Using fluorescence in situ hybridization analysis, *Sc1* was localized to band 5E4 of mouse chromosome 5.

[The sequence data described in this paper have been submitted to GenBank under accession nos. U64827 and U66157–U66166.]

Sc1 (originally isolated during a screen for synaptic complex components; Johnston et al. 1990) and Sparc (secreted protein, acidic, rich in cysteine; also known as osteonectin, because of the high levels found in bone tissue; Termine et al. 1981; Mason et al. 1986) are two extracellular matrix (ECM) associated proteins that share a high degree of identity. Sc1 is expressed highly in the nervous system and heart (Johnston et al. 1990; Mendis et al. 1994; McKinnon and Margolskee 1996) and at lower levels in other tissues, and Sparc, while high in bone, has a more general distribution (Sage et al. 1989; Mundlos et al. 1992; Mendis et al. 1994; Porte et al. 1995). Additionally, SC1 has been isolated from the high endothelial venules of the immune system (Girard and Springer 1995). Sc1 and Sparc are ~63% identical over their carboxy-terminal 230 amino acids, suggesting functional conservation of this region. The unique amino-terminal region of Sc1 (~400 amino acids) is unrelated to any presently described protein. Qr1 (a recently identified gene in quail), encodes a product of similar size to Sc1 and also shares carboxy-terminal homology to Sparc and Sc1 (Guermah et al. 1991).

The amino acid sequence of Sparc, particularly the region of similarity to Sc1 and Qr1, is conserved highly across a number of species, including mouse (McVey et al. 1988), bovine (Findlay et al. 1988), human (Swaroop et al. 1988; Villarreal et al. 1989), and *Xenopus* (Damjanovski et al. 1992). *Caenorhabditis elegans Sparc* has also been cloned, and is related closely to its vertebrate counterpart. This indicates that SPARC's function predates the occurrence of bone tissue (a vertebrate tissue rich in SPARC; Schwarzbauer and Spencer 1993). The actual in vivo function of Sparc is presently unknown, although a consistent observation is that Sparc appears to be involved in processes that modulate cell–ECM interactions (Lane and Sage 1994).

In this report we investigate the similarity of Sc1 and Sparc at the genomic level. This provides insights regarding potential conservation of function during evolution of SPARC-related proteins, such as Sc1.

RESULTS

The similarity of *Sc1* to *Sparc* at the cDNA level is apparent over a large stretch of their carboxy-terminal regions (Johnston et al. 1990; Lane and Sage 1994; Girard and Springer 1995). The murine *Sparc* genomic locus comprises ~26 kb, contains 10 exons that give rise to a message of 2.2 kb, and is located on chromosome 11 (McVey et al. 1988). Mapping and sequencing of the murine *Sc1* genomic locus revealed a single copy gene spanning ~35 kb of genomic DNA, located on chromosome 5 and containing 11 exons that show a high degree of similarity to the exon/intron structure of *Sparc*.

One distinguishing feature of the murine *Sc1*

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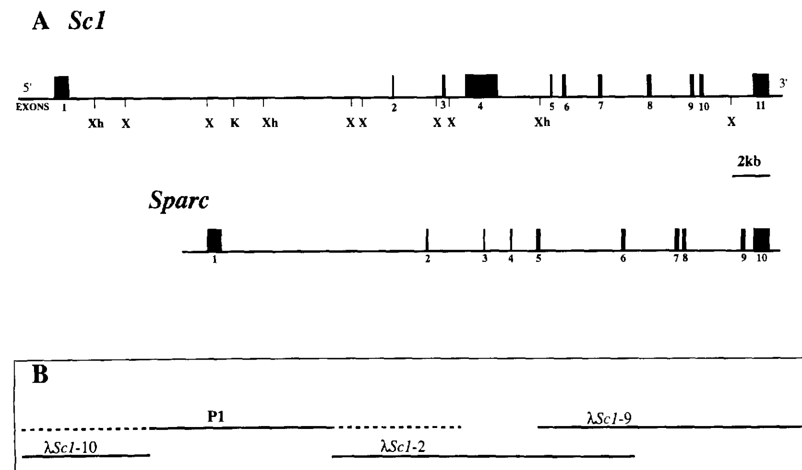

Figure 1 Map of the murine *Sc1* genomic locus. (*A*) The mouse *Sc1* locus contains 11 exons (indicated by solid boxes) spanning ~35 kb of DNA (scale bar, 2 kb). The restriction sites are (K) *Kpn*I; (Xh) *Xho*I; and (X) *Xba*I. The genomic map of mouse *Sparc* serves as a comparison to *Sc1*. The *Sc1* region of similarity with *Sparc* is exons 6–11 (corresponding to *Sparc* exons 5–10). The ATG start codon and the signal sequence are contained in exon 2 of both *Sparc* and *Sc1*. (*B*) The lambda fix II clones used to generate the map for *Sc1* are indicated as solid lines below the exon map. The P1 clone used to map exons 1 and 2 and the first intron is indicated as a solid and dashed line. The solid line shows the region of the *Sc1* locus not found in any λ clones; the dashed lines indicate the overlap with the λ clones.

locus is the large first intron. In fact, no *Sc1* λ clones were obtained from two independent screens of a mouse library that contained overlap between exon 1 and exon 2. Moreover, Southern blot analysis failed to show unambiguously a single DNA fragment containing both exon 1 and exon 2, following digestion of mouse genomic DNA with a variety of commercial restriction enzymes (data not shown). Single bands were obtained with rare cutters such as *Not*I and *Sal*I, although the limited genomic digestion that results from these enzymes makes interpretation of the Southern blot pattern unclear. Therefore, to map the large first intron we obtained P1 clones that contained exon 1 and exon 2 on a contiguous DNA fragment by screening with PCR primer pairs against exon 2, and then identified those clones that also contained exon 1 (using another primer set). Further mapping and sequencing was achieved by subcloning the P1 inserts as *Xho*I and *Xba*I fragments. Figure 1 shows a restriction map of the mouse *Sc1* locus. DNA sequencing of the *Xho*I and *Xba*I junctions in the original P1 clones was used to orient the fragments. Intron sizes were confirmed using long PCR on genomic DNA and the P1 clones, as well as Southern blot analysis (data not shown).

Figure 2 shows the complete sequence of all *Sc1* locus exons, and these correspond to the murine *Sc1* cDNA isolated from a mouse brain library (GenBank accession nos. U64827 and U66157–U66166). The region of *Sc1* that is similar to *Sparc* is contained in *Sc1* exons 6 to 11 (Fig. 3). The exon/intron boundaries of these *Sc1* exons correspond exactly to those of *Sparc*, although the intron lengths for *Sc1* differ in many cases in *Sparc* (Fig. 1). *Sc1* contains a relatively long first intron (16-kb) and a signal sequence for secretion (in exon 2), as does *Sparc*. However, *Sc1* has three additional unique exons: exons 3, 4, and 5. These three exons encode a substantial portion of the Sc1 protein; in fact, the large size of exon 4 (equivalent to 330 amino acids) suggests a functional module, as present in SPARC (see Discussion).

The putative promoter region upstream of *Sc1* exon 1 shows little homology to the promoter region of *Sparc*, consistent with the differences in tissue expression of the two genes. While transcription factor binding motifs can be identified using gene analysis software, the lack of

Figure 2 Sequence of the exons and intron boundaries of the *Sc1* gene. The complete sequence of the *Sc1* exons together with intron sizes is presented. The *Sc1* gene transcribes a message of 2730 bp. The dashed lines between exons indicate additional intron sequences. *Sc1* exons 6–11 have conserved exon/intron boundaries in comparison to exons 5–10 of murine *Sparc*. These exons show exactly the same interruption in coding sequence found in the analogous region present in *Sparc* (McVey et al. 1988). For example, the junctions between exon 9 and 10 and exon 10 and 11 of *Sc1* split a codon in a similar manner to the related exons in *Sparc* (*Sparc* exons 8 and 9, and exons 9 and 10 respectively). GenBank sequence accession nos. are U64827 (cDNA) and U66157-U66166 (exons 1–11).

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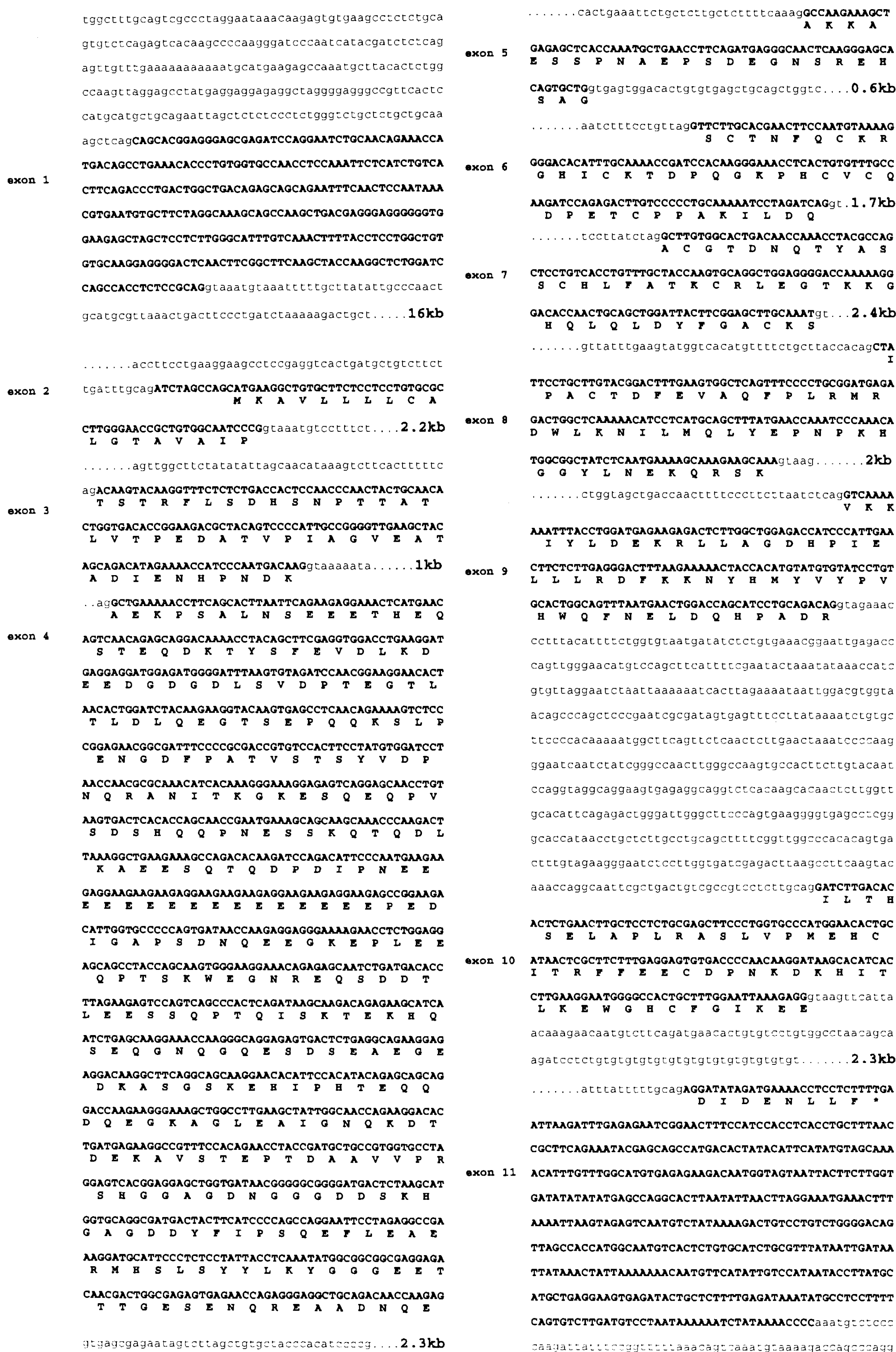

Figure 2 (*See facing page for legend.*)

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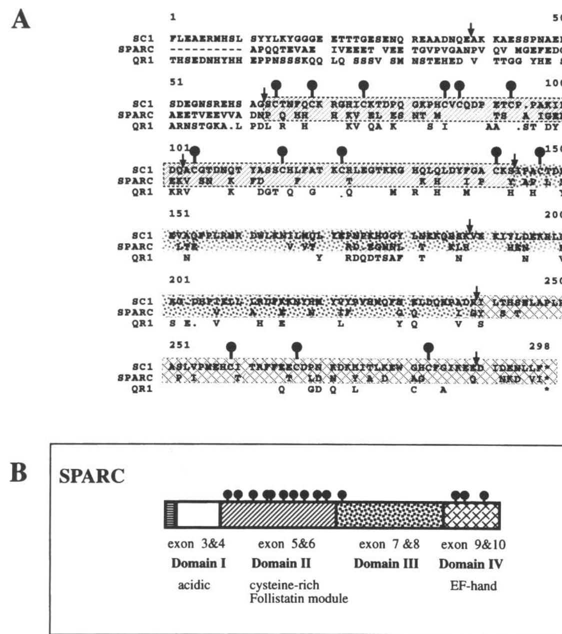

Figure 3 Comparison of the structural domains of Sc1, Sparc, and Qr1. (*A*) Alignment of Sc1, Sparc, and Qr1 demonstrates their similarity and modular nature. The proposed regions of homology in Sc1 and Qr1 are inferred based on their extensive similarity to specific structural domains present in Sparc. Only the last 298 amino acids are presented for mouse Sc1 (total length 650 amino acids) and quail Qr1 (676 amino acids) compared with the full-length (minus signal sequence) mouse Sparc. The amino terminus of all three proteins is acidic (pI values are: Sc1, 4; Sparc, 4.3; and Qr1, 5.1). The stippled and cross-hatched areas indicate regions of putative homology and correspond to the same regions described in *B*. The flags (⚲) represent conserved cysteine residues, and the arrows indicate the identical exon/intron boundaries present in Sc1 and Sparc. In Sparc and Qr1, only amino acids not identical to Sc1 are indicated; however, in many cases these amino acid differences are conserved changes (not indicated). (*B*) Diagram of the structural features of Sparc as they relate to the exon structure. The modular nature of Sparc is revealed by the assignment of protein domains to specific exons. Domains I and IV (which contains the EF hand motif) are Ca^{2+} binding sites. As above, the flags (⚲) indicated in domain II represent conserved cysteine residues, present in Sparc, Sc1, and Qr1. These cysteine residues are the basis for the structural similarity of the SPARC-gene family with Follistatin and Agrin (Patthy and Nikolics 1993).

any similarities with other promoter sequences (present in GenBank) from genes showing a similar tissue pattern of expression makes interpretation difficult. For example, Sc1 is known to be expressed in astrocytes in the adult rodent brain (Mendis et al. 1994; McKinnon and Margolskee 1996), but the *Sc1* promoter region does not show any similarity to astrocyte-specific gene promoters such as the glial fibrillary acidic protein promoter.

Using fluorescence in situ hybridization (FISH) analysis we have localized *Sc1* to mouse chromosome 5 (Fig. 4). Measurement of 10 specific chromosome 5 hybridizations demonstrated that *Sc1* is located at a position that is 64% of the distance from the heterochromatic–euchromatic boundary to the telomere of chromosome 5. This region of chromosome 5 corresponds to band 5E4, although the adjacent bands 5E3 and 5E5 cannot be excluded as band 5E4 is very small. The region spanning 5E3 to 5E5 corresponds to markers found on three different human chromosomes, and thus it is difficult to accurately establish potential relationships to human loci (homology data retrieved from Mouse Genome Database 3.1, The Jackson Laboratory, Bar Harbor, ME; http://www.informatics.jax.org).

DISCUSSION

We have found that *Sc1* and *Sparc*, in addition to having a high degree of amino acid identity, also

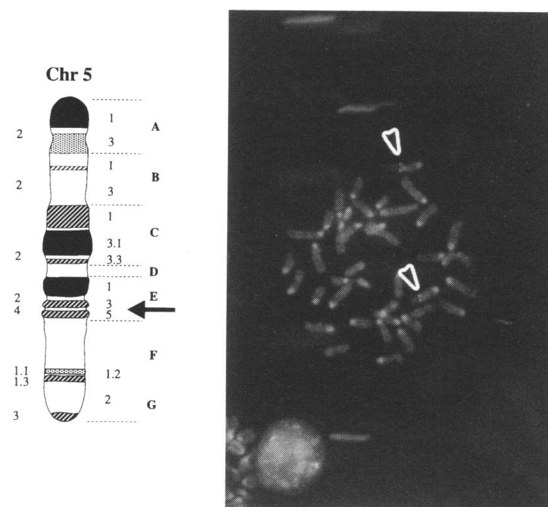

Figure 4 Assignment of *Sc1* to band 5E4 of mouse chromosome 5. FISH was used to localize *Sc1* to mouse chromosome 5 at band E4. The cartoon drawing of mouse chromosome 5 (Chr 5) shows the banding assignment, and the arrow indicates the position of *Sc1*. The photographic plate shows a metaphase spread of mouse chromosomes, and the arrowheads indicate the specific *Sc1* fluorescent signal present on chromosome 5.

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share a similar genomic organization. That these two genes share a high level of identity and precise exon/intron structure spanning the region of similarity suggests that they evolved from the same ancestral gene.

The presence in *C. elegans* of Sparc that is closely related to the vertebrate Sparc proteins suggests that the functions of Sparc have been well conserved (Schwarzbauer and Spencer 1993). Structural differences in the *C. elegans* Sparc compared with vertebrate Sparc are the absence of the protein sequence corresponding to exon 3, as well as that of exons 1 and 10, and the fusion of exons 6 and 7. However, these changes do not affect the strong homology of the *C. elegans* protein to that of the vertebrates. It will be interesting to determine whether Sc1 also occurs in *C. elegans*, as this would suggest an important early specification of function for Sc1, possibly relating to the protein sequence encoded by *Sc1* exons 3, 4, and 5.

SPARC has been assigned four domains (I–IV) that are distinguished by primary amino acid sequence, and all of the amino acid sequences in domains II–IV are present in Sc1 and Qr1 (Fig. 3B; Engel et al. 1987; Maurer et al. 1992, 1995). This modular nature of Sparc has a number of functional implications. Domain I (exons 3 and 4) is acidic and can bind Ca^{2+}; domain II (exons 5 and 6) contains a number of cysteine residues and is homologous to follistatin and Kazal type protease inhibitors (Patthy and Nikolics 1993); domain III (exons 7 and 8) appears to bind collagen IV (Mayer et al. 1991); and domain IV at the carboxyl terminus (exons 9 and 10) has been shown to bind Ca^{2+} via an EF hand motif (Engel et al. 1987; Maurer et al. 1992; Schwarzbauer and Spencer 1993; Maurer et al. 1995). Domains III and IV have been shown to form an extracellular Ca^{2+} binding module, termed EC, that is found in several other extracellular proteins (Maurer et al. 1995; Hohenester et al. 1996). The greatest variation between rat (Johnston et al. 1990), human (Girard and Springer 1995), and mouse Sc1 occurs in the region encoded by mouse exons 3, 4, and 5. Although the amino acid identity is diminished within this region, the region is highly acidic in all species, with an average pI of ~4 (this region is ~420 amino acids in length, and extends from amino acid position 20–417 of the mouse protein). This suggests that an acidic charge is required in this region rather than a specific amino acid sequence.

Perturbation of SPARC function with peptides has been used to correlate a number of biochemical properties to specific regions within the various domains of Sparc. These include an antiadhesive property associated with domains I and IV (Lane and Sage 1990), and the ability to modulate cell-cycle progression in certain cell lines associated with a region in domain II (Funk and Sage 1991, 1993). Recently, SC1 has been identified in the immune system as a component of the high endothelial venule (the protein has been called hevin to identify this fact). Functional studies with recombinant human SC1/hevin have also shown a striking antiadhesive property toward attachment of endothelial cells to different substrates (Girard and Springer 1996). Thus, SPARC analogous domains present in Sc1 may confer similar functional properties on Sc1 (and Qr1), while the Sc1 unique domains may contribute different activities.

METHODS

Isolation of *Scl* Genomic Clones

From a mouse (strain 129Sv/J) Lambda Fix II genomic library (Stratagene, La Jolla, CA) 1×10^6 plaques were transferred to Hybond nylon filters (Amersham, Arlington Heights, IL) and screened with a ^{32}P-labeled full-length mouse Sc1 cDNA (2.7 kb) under conditions described in Church and Gilbert 1984, and the 20 positive plaques obtained were taken to purity.

Three independent λ clones were isolated that collectively contained all of the *Sc1* exons (Fig. 1B). These three clones were subcloned as *Not*I fragments (using the *Not*I sites on the arms of λ FixII) or *Xba*I fragments (Fig. 1). These subclones were sequenced using a number of oligonucleotide primers designed from the mouse *Sc1* cDNA. Additional primers were designed as the sequencing project progressed. Oligonucleotides were synthesized on an Applied Biosystems, Inc. (Foster City, CA) oligonucleotide synthesizer and all sequencing was performed using an Applied Biosystems autosequencer and dye terminator technology. Both strands of the genomic clones corresponding to coding regions were sequenced.

Isolation of Pl Clones

As no λ clones were obtained that contained both exon 1 and exon 2 of *Sc1*, we obtained P1 clones to provide contiguous DNA that allowed us to map the large first intron. *Sc1* P1 clones containing both exon 1 and exon 2 were obtained from Genome Systems (St. Louis, MO). The P1 library was screened with PCR probes designed against exon 1, and those P1 phage that contained exon 1 were screened using PCR with specific primers for exon 2. The exon 1-specific primers were mSc1-P1-1 (forward), 5′-CAAAGCTCAGCAGCACGGAGG-3′, and mSc1-P1-2 (reverse), 5′-GTTTAACGCATGCAGTTGGGC-3′, generating a

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PCR product of 394 bp. The exon 2-specific primers were mSc1-P1-3 (forward), 5′-CCTTCAGCACTTAATTCAGAAG-3′, and mSc1-P1-4 (reverse), 5′-CTTCAGCCTTTAAGTCT-TGGG-3′, generating a PCR product of 349 bp. The DNA of interest from the P1 clones was mapped initially using Southern blot hybridization, and then subcloned into pBluescript (Stratagene) and subjected to analysis as described above for the λ subclones. Further mapping (and confirmation of Southern blot data) was done using long PCR (Elongase; Life Technologies, Gaithersburg, MD). Long PCR was performed according to the manufacturer's recommendations with primers derived from prior sequence analysis.

Mouse Chromosomal Localization

FISH was performed by Genome Systems using established methodologies (Stokke et al. 1995). DNA from the *Sc1* P1 clone described above was labeled with digoxigenin dUTP by nick translation, combined with sheared mouse DNA, and hybridized to normal metaphase chromosomes derived from male embryonic stem cells. Hybridization was done in 50% formamide, 10% dextran sulfate, and 2× SSC. Positive signal was detected using fluoresceinated antidigoxigenin antibodies followed by counterstaining with either propidium iodide or DAPI. Precise chromosomal assignment was obtained by cohybridization of *Sc1* with a chromosome 5-specific probe. A total of 80 metaphase cells were analyzed, with 73 exhibiting specific labeling.

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