Deletion and Interallelic Complementation Analysis of Steel Mutant Mice

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ABSTRACT

Mutations at the Steel (SI) locus produce pleiotropic effects on viability as well as hematopoiesis, pigmentation and fertility. Several homozygous viable SI alleles have previously been shown to contain either structural alterations in mast cell growth factor (Mgf) or regulatory mutations that affect expression of the Mgf gene. More severe SI alleles cause lethality to homozygous embryos and all lethal SI alleles examined to date contain deletions that remove the entire Mgf coding region. As the timing of the lethality varies from early to late in gestation, it is possible that some deletions may affect other closely linked genes in addition to Mgf. We have analyzed the extent of deleted sequences in seven homozygous lethal SI alleles. The results of this analysis suggest that late gestation lethality represents the SI null phenotype and that peri-implantation lethality results from the deletion of at least one essential gene that maps proximal to SI. We have also examined gene dosage effects of SI by comparing the phenotypes of mice homozygous and hemizygous for each of four viable SI alleles. Lastly, we show that certain combinations of the viable SI alleles exhibit interallelic complementation. Possible mechanisms by which such complementation could occur are discussed.

The pleiotropic effects on viability, hematopoiesis, pigmentation and fertility found in mice that carry Steel (SI) mutant alleles are due to mutations in mast cell growth factor (Mgf) (Copeland et al. 1990; Huang et al. 1990; Zsebo et al. 1990a). Mgf is the ligand for Kit (Flanagan and Leder 1990; Huang et al. 1990; Williams et al. 1990; Zsebo et al. 1990a), a receptor tyrosine kinase that is encoded by the Dominant White Spoting (W) locus (Chabot et al. 1988; Geissler et al. 1988). These two gene products comprise an intercellular signaling pathway that is required for the development of mast cells, erythroid cells, neural crest-derived melanocytes and germ cells. Mgf is biologically active as both a transmembrane, cell surface protein and as a soluble factor produced by proteolytic cleavage of the membrane bound form (Anderson et al. 1990; Flanagan et al. 1991; Torsoz et al. 1992). Gel filtration and sedimentation analysis has suggested that the soluble form of Mgf is a noncovalently associated homodimer (Arrakawa et al. 1991; Zsebo et al. 1990b); however, it is presently not known whether the membrane-bound form also functions as a dimer. Upon binding of Mgf to Kit, dimerization of the receptor is induced, leading to activation of the tyrosine kinase function with subsequent phosphorylation and binding to downstream signaling molecules (Blume-Jensen et al. 1991; Lev et al. 1991, 1992; Rottafel et al. 1991).

A large collection of SI and Wmutant alleles has been identified that are useful for elucidation of the in vivo functions of this signaling pathway. All SI and Wmutant alleles are semidominant in that they cause a mild phenotype in heterozygous mice (Silvers 1979a,b). However, the severity of the homozygous phenotype varies considerably between different alleles. In homozygous mice, the most severe SI alleles cause embryonic lethality while milder alleles allow viability but have characteristic effects on anemia, pigmentation and fertility. Previously, it was demonstrated that many embryonic lethal SI alleles are associated with complete deletions of Mgf coding sequences (Copeland et al. 1990; Huang et al. 1990; Zsebo et al. 1990a), suggesting that Mgf is essential for embryonic survival. However, the timing of embryonic death caused by various SI mutations varies from before implantation to late in gestation (Silvers 1979a; Cattanach et al. 1993). While severe anemia is thought to be the cause of death in late gestation SI embryos (Sarvela and Russell 1956; Silvers 1979a), lethality at earlier stages of embryogenesis is likely to result from deletion of other closely linked essential genes. In contrast to the lethal SI alleles, alleles such as Steel-Duckie (St\(^{d}\)), Steel-17H (St\(^{17H}\)), Steel-panda (St\(^{p}\)) and Steel-contrasted (St\(^{c}\)) are hypomorphic in that they contain residual Mgf activity that allows viability. The St\(^{d}\) allele contains an intragenic deletion that affects the transmembrane and cytoplasmic domains of Mgf such that only soluble protein is encoded (Branan et al. 1991; Flanagan et al. 1991) while the protein encoded by St\(^{17H}\) mice lacks the normal cytoplasmic domain due to a splicing defect (Branan et al. 1992). Last, St\(^{p}\) and St\(^{c}\) mice were shown to have regulatory mutations that both positively and negatively affect Mgf mRNA
levels but do not affect the Mgf coding sequences (Huang et al. 1993; Bedell et al. 1995). Each of these viable alleles causes characteristic defects in pigmentation, hematopoiesis and fertility in homozygous mice.

Here we describe a physical and genetic analysis of Sl mutants. These studies provide evidence for a second gene that lies proximal of Sl that affects embryonic viability and show that certain alleles of Sl can exhibit interallelic complementation. The implications of these studies for Sl gene function are discussed.

MATERIALS AND METHODS

Mice: The lethal and viable Sl alleles studied here are listed in Tables 1 and 3, respectively. Sl<sup>P</sup>, Sl<sup>10H</sup>, Sl<sup>12H</sup>, Sl<sup>31H</sup>, Sl<sup>25H</sup>, Sl<sup>24H</sup> and Sl<sup>23H</sup> mice were obtained from the MRC Radiobiology Unit (Chilton, Didcot, UK) while Sl<sup>2</sup> and Sl<sup>3</sup> mice and Sl<sup>1+<sup> DNA were obtained from The Jackson Laboratory (Bar Harbor, ME). Some Sl mutant stocks have been maintained at the NCI-FCRDC by backcrossing to C57BL/6J (Sl<sup>9<sup>) or C57/H<sup>/HeN (other Sl alleles) mice. Sl was maintained by brother-sister matings of Sl<sup>1+<sup> and Sl<sup>++<sup> segregants and was originally on a 129/Sv background. Homozygous embryos were obtained by intercrossing Sl heterozygotes. Mice heterozygous for different Sl alleles were mated to obtain F<sub>1</sub> progeny that were heteroallelic, i.e., Sl<sup>1+</sup> × Sl<sup>1+</sup> → Sl<sup>1+<sup> / Sl<sup>1+. The genotype of putative heteroallelic mice was determined by either Southern blot analysis, using probes that recognize allele-specific genomic alterations, or by progeny testing.

Southern blot analysis and pulsed field gel electrophoresis (PFGE): All DNAs analyzed were from mouse tissues except Sl/SI DNA that had been prepared from a fetal liver stromal cell line (Zsebo et al. 1990a) and was kindly provided by D. A. Williams. The isolation and analysis of genomic DNA by conventional Southern blot analysis was performed as previously described (Jenkins et al. 1982) except that either Zeta-bond (Gino, Inc., Meriden, CT) or Hybond N+ (Amersham, Arlington Heights, IL) nylon membranes were used and <sup>32</sup>P-labeled DNA probes were prepared using a Prime-It II kit (Stratagene, La Jefia, CA). The probes used are described below and were hybridized and washed under high stringency. The blots were first analyzed by autoradiography and then hybridization intensity was quantitated using a Phosphor Image System (Molecular Dynamics, Sunnyvale, CA). High molecular weight spleen DNA was prepared, digested and subjected to PFGE using methods described by Kingsley et al. (1992) and the blots were analyzed by autoradiography.

Probes that contain the 5′ flanking region and the full-length Mgf cDNA are described in the companion paper (Bedell et al. 1996). A YAC library of mouse DNA (ICRF reference library, Lefrach et al. 1990) was screened with an upstream genomic probe (EcoRI fragment B, Figure 4 of Bedell et al. 1996). Probes from the ends of the YAC inserts were prepared either by bubble PCR (Riley et al. 1990) or from cloned fragments isolated following hybridization of size-fractionated YAC fragments to vector sequences. Two probes from the proximal portion of chromosome 10 were used to control for DNA loading on Southern blots, Myb (Justice et al. 1990) and D10Fcr2 (Bedell et al. 1995). The Sl<sup>9<sup> deletion breakpoint fragment was cloned by size-fractionating EcoRI fragments of DNA from a Sl<sup>9<sup> homozygous embryo, ligating the fragments into λZAP vector followed by conventional screening, isolation and subcloning. Sequencing was performed using a dideoxy method (United States Biochemical, Cleveland, OH).

Interspecific backcross (IB) mapping: The chromosomal position of DNA probes was determined using a [(C57BL/6J × Mus musculus) × C57BL/6J] backcross mapping panel as described previously (Copeland and Jenkins 1991). The segregation pattern of probes polymorphic for the two species was compared with the segregation of 2000 other probes that have been typed in the same panel.

RESULTS

Deletion analysis of homozygous lethal Sl alleles: The extent of deleted sequences was determined in seven Sl alleles that cause lethality to homozygous embryos during various stages of development (see Table 1). All seven alleles were previously shown to be associated with deletions that remove the Mgf coding region (Copeland et al. 1990), a region that corresponds to ~40 kb of genomic DNA. To determine whether these deletions extend into sequences immediately flanking the Mgf coding region, Southern blot analyses of DNA from mice heterozygous for each of the lethal Sl alleles were performed with probes derived from the full-length Mgf transcription unit and ~14 kb of 5′ flanking region. These clones span ~64 kb of genomic DNA and are described in the companion paper (Bedell et al. 1996). In all heterozygous DNAs except for Sl<sup>de/++,</sup> the intensity of hybridization of each probe was approximately half that seen in wild-type DNA (data not shown). Hybridization of control probes, Myb (Justice et al. 1990) and D10Fcr2 (Bedell et al. 1995), to the same blots demonstrated that equivalent amounts of DNA had been loaded in each lane. These results indicate that, with the exception of Sl<sup>at</sup>, the sequences deleted in all Sl alleles examined includes the 5′ and 3′ flanking regions of Mgf and are therefore a minimum of 64 kb in size.

A probe from the 3′ untranslated region (UTR) of Mgf (see Bedell et al. 1996) detected numerous RFLPs in Sl<sup>de</sup> DNA, suggesting the presence of a deletion breakpoint. For example, a 5.5-kb EcoRI fragment was observed in Sl<sup>de/Sl<sup>de</sup> DNA while two EcoRI fragments of 5 and 2.7 kb are present in wild-type DNA upon hybridization with 3′ Mgf (Figure 1A, left). A subgenomic library of EcoRI-digested Sl<sup>de</sup>/Sl<sup>de</sup> DNA was prepared and the 5.5-kb mutant breakpoint fragment isolated. The approximate position of the Sl<sup>de</sup> breakpoint was determined to be in the distal region of the 3′ UTR of Mgf by restriction site mapping and hybridization analysis. Sequencing of the distal portion of the Sl<sup>de</sup> fragment revealed that the deletion breakpoint is at position 5287 of the 5.4-kb Mgf mRNA (Bedell et al. 1996). As the 3′ Mgf probe contains only ~100 bp that overlaps the sequence of Sl<sup>de</sup> genomic fragments, the hybridization intensity of this probe to mutant DNA is less intense than in wild-type DNA (Figure 1A, left). Hybridization of the gbb/H probe, derived from the Sl<sup>de</sup> breakpoint fragment, to Sl<sup>de/++</sup> DNAs confirmed that the authentic breakpoint fragment had been cloned (Figure 1A, right). Analysis of pulsed field gel...
### TABLE 1

**Lethal Steel alleles**

<table>
<thead>
<tr>
<th>Gene symbol(^a)</th>
<th>Gene name</th>
<th>Size of deletion (kb)</th>
<th>Phenotype(^b)</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>$Sl^{d}$</td>
<td>Steel-Grizzle Belly</td>
<td>120</td>
<td>Light-colored belly, dilution of coat, head spots</td>
<td>Anemia; embryos die after E15</td>
</tr>
<tr>
<td>$Sl^{f}$</td>
<td>Steel-J</td>
<td>650</td>
<td>Dilution of color on the belly; generally, a white tip on tail</td>
<td>Prenatal lethal; embryos die after E15</td>
</tr>
<tr>
<td>$Sl^{10H}$</td>
<td>Steel-10H</td>
<td>680</td>
<td>Lighter coat and feet than wild-type mice; occasional spotting</td>
<td>Anemia; embryos die after E15</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$Sl$</td>
<td>Steel</td>
<td>$&gt;810$</td>
<td>Slight dilution of coat color; occasional spotting</td>
<td>Anemia, prenatal lethal; embryos die after E15</td>
</tr>
<tr>
<td>$Sl^{5H}$</td>
<td>Steel-5H</td>
<td>$&gt;810$</td>
<td>Lighter coat and feet than wild-type mice; occasional spotting</td>
<td>Prenatal lethal (timing not reported)</td>
</tr>
<tr>
<td>$Sl^{12H}$</td>
<td>Steel-12H</td>
<td>$&gt;810$</td>
<td>Light coat; runting; reduced viability</td>
<td>Prenatal lethal: embryos die before implantation</td>
</tr>
<tr>
<td>$Sl^{18H}$</td>
<td>Steel-18H</td>
<td>$&gt;810$</td>
<td>Greyish coat; pale ears and tail; head spotting; occasional nose and belly spotting; reduced viability; anemia</td>
<td>Prenatal lethal: embryos die shortly after implantation</td>
</tr>
</tbody>
</table>

\(^a\) $Sl^{f}$ and $Sl$ all arose spontaneously whereas $Sl^{5H}$, $Sl^{10H}$, $Sl^{12H}$ and $Sl^{18H}$ were each induced by X-irradiation. Mutant stocks were maintained on a C3H/HeN background except $Sl^{f}$ that was maintained by brother-sister mating and was originally on a 129/Sv background. $Sl/Sl$ DNA was obtained from a stromal cell line derived from homozygous $Sl$ embryos (Zsebo et al. 1990a).

\(^b\) Unless otherwise noted, the phenotypes are as described in the original published reports for each allele. Differences in the phenotypes of both heterozygous and homozygous mice may be due to strain background effects. $Sl^{5H}/Sl^{5H}$ and $Sl^{10H}/Sl^{10H}$ were originally reported to be neonatal lethals. However, no liveborn homozygous mice were observed (M. A. Bedell, unpublished data) while live homozygous embryos have been observed at E12.5 and E15.

electrophoresis (PFGE) blots of YAC, wild-type, and $Sl^{d}+/+$ mouse DNAs placed the proximal end of the $Sl^{d}$ deletion $\sim$60 kb 5' of the $Mgf$ coding region and the size of the $Sl^{d}$ deletion was estimated to be $\sim$120 kb (Figure 2). The gbB/H probe was deleted in all other homozygous lethal $Sl$ alleles (Table 2).

Additional probes were obtained from YACs that were isolated from the ICRF Reference Library (Lehrach et al. 1990) after screening with EcoB (Figure 2), a genomic fragment from the 5' flanking region of $Mgf$ (see Bedell et al. 1996). Two YAC clones were obtained from a C3H library; clone ICRFy902G03127 (YLE3127), which is 400 kb and clone ICRFy902A0394 (YLE394), which is 610 kb. End-probes from each YAC were prepared and three of these (394R, 394L and 3127L) hybridized to unique sequences on Southern blots of mouse DNA while the fourth (3127R) detected highly repetitive sequences. The two YACs were aligned with respect to each other and the $Mgf$ coding region by partial and complete restriction digest analysis of YAC DNA, hybridization of $Mgf$ cDNA, genomic clones or YAC end probes to YAC DNA, and hybridization of YAC end probes to PFGE blots of mouse genomic DNA. These results revealed that 394L is $\sim$100 kb upstream of the $Mgf$ coding region, that 3127L is an additional 120 kb upstream from 394L and that 394R maps $\sim$590 kb 3' to $Mgf$ (see Figure 2). The total genomic region spanned by 3127L and 394L is estimated to be 810 kb. The chromosomal positions of 394R, 394L and 3127L were determined by interspecific backcross mapping (Copeland and Jenkins 1991). This analysis confirmed that 394L and 3127L are very closely linked to $Mgf$; they
FIGURE 1.—Representative Southern blots of DNA from Sl mutant mice. (A) DNAs from Sl/Sl embryos (lanes 1 and 3) and +/+ mice (lanes 2 and 4) were digested with EcoRI (lanes 1 and 2) or HindIII (lanes 3 and 4) and the blot was sequentially probed with a portion of the 3' UTR region of Mgfc cDNA (3' Mgfc) (see Bedell et al. 1996) and with gbB/H, a probe derived from the Sldeletion breakpoint fragment (see text). Note that the two probes detect the same band in Sl/Sl DNA but different bands in +/+ DNA. Hybridization with a control probe, Myb (not shown), revealed that each lane contained equal amounts of DNA. (B) DNAs from +/+ (lanes 1, 3, 5, 7, 9 and 11), Sl/+ (lane 2), Sl+/+ (lane 4), Sl+/+ (lane 6), Sl+/+ (lane 8), Sl/+ (lane 10) and Sl/+ (lane 12) mice were digested with EcoRI and the blot was sequentially probed with 3127L (the most proximal probe, top), 394R (the most distal probe, middle) and Myb (a control probe, bottom). Quantitation of the hybridization intensity with each of these probes, as well as other probes and other blots, are summarized in Table 2. In A and B, XHindIII size markers are shown to the left of the panels. (C) CHEF blot of spleen DNAs from +/+ (129/Sv and C57BL/6J, lanes 1 and 5, respectively), Sl/Sl (lane 2), Sl/+ (lane 3) and Sl/+ (lane 4) were digested with EcoRI and the blot probed with 3127L, the most proximal probe. The estimated sizes of the different fragments are indicated to the right of the panel. In panels A and C, arrowheads point to the mutant fragments.

did not recombine with each other or with the Mgfc cDNA probe in 174–183 N2 animals typed in common. In contrast, 394R mapped 0.5 +/- 0.5 cM distal to Mgfc cDNA after typing of 188 N2 animals. Because 394R was determined to be 3' to the Mgfc cDNA (see above), the genetic mapping results allowed the direction of Mgfc transcription to be oriented with respect to the centromere (Figure 2). Further analyses of the various Sl deletions were done using the YAC end probes, 3127L, 394L and 394R (Figure 1, B and C, Table 2). As expected, all three probes were present at normal intensity in Sldeletion DNA (Table 2). Sequences hybridizing to 3127L and 394R were also present at normal intensity in DNA from Sl/+ and Sl+/+ mice (Table 2, Figure 1B) indicating that the proximal and distal limits of these deletions are within the region encompassed by the two YACs. However, hybridization of 394L to both Sl/+ and Sl+/+ DNA
Steel Deletions and Complementation

**FIGURE 2.** The extent of deleted sequences in lethal Sl alleles. Schematics of chromosome 10 of wild-type C3H(+) and seven homozygous lethal Sl alleles are shown with the centromere (○) and telomere oriented left to right, respectively. Deleted regions are shown as gaps connected by offset thin lines. Hash marks represent portions not drawn to scale, the solid lines indicate intact DNA sequences and the dashed lines indicate sequences that were not analyzed so deletions are of unknown size. The probes shown are as follows: 3127L, left end probe of YAC YLE3127; 394L and 394R, left and right end probes, respectively, of YAC YLE394; EcoB, EcoRI fragment B of 5' flanking region of Mgf (see BDELI. et al. 1996); Mgf transcriptional unit of Mgf, oriented 5' to 3' with respect to the centromere (see text). Restriction enzyme sites: BssHII (B), EcoRI (E), SfiI (Sf). Other SfiI sites are not shown. See Table 1 for the estimated sizes of the deletions.

was half as intense as in wild-type (Table 2), suggesting that the proximal breakpoint of each deletion is located between 3127L and 394L, a distance of ~120 kb. To identify the exact location of the Sl and Sl\textsuperscript{10H} breakpoints, a chromosomal walk was initiated from these two probes. Overlapping phage clones of wild-type DNA were isolated and the direction of the walk determined by hybridization to DNA from YLE3127 and YLE394. Unique sequences derived from the walking clones were used as probes on conventional Southern blots of Sl\textsuperscript{10H} and DNA to identify probes that are located near the two breakpoints. The results of these analyses indicate that the Sl\textsuperscript{10H} deletion breakpoint lies ~38 kb proximal to the Sl\textsuperscript{10H} breakpoint (Figure 2). Although the distal extent of these two deletions has not been precisely determined, the sizes of mutant fragments on PFGE blots were used to estimate the total amount of deleted DNA. In DNA from wild-type mice, 3127L hybridizes to an EcoRI fragment of 680 kb while this probe detects mutant EcoRI fragments of 610 and 580 kb, respectively, in Sl\textsuperscript{10H} and DNA (Figure 1C). Wild-type DNA also contains a second EcoRI fragment of 580 kb, that contains the Mgf coding region (see Figure 2). However, an EcoRI site near the coding region is removed by the deletions in Sl\textsuperscript{10H} and DNA, so that only one EcoRI fragment of 580 kb, that contains the Mgf coding region (see Figure 2). However, an EcoRI site near the coding region is removed by the deletions in Sl\textsuperscript{10H} and DNA, so that only one EcoRI fragment of 580 kb, that contains the Mgf coding region (see Figure 2).

**TABLE 2**

<table>
<thead>
<tr>
<th>Mutant</th>
<th>3127L</th>
<th>394L</th>
<th>gbB/H</th>
<th>394R</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sl\textsuperscript{10H}/+</td>
<td>1.0</td>
<td>0.92</td>
<td>RFLPs</td>
<td>1.1</td>
</tr>
<tr>
<td>Sl\textsuperscript{10H}/+</td>
<td>0.84</td>
<td>0.51</td>
<td>0.48</td>
<td>0.90</td>
</tr>
<tr>
<td>Sl\textsuperscript{10H}/+</td>
<td>1.1</td>
<td>0.55</td>
<td>0.53</td>
<td>1.4</td>
</tr>
<tr>
<td>Sl\textsuperscript{10H}/+</td>
<td>0.56</td>
<td>0.36</td>
<td>0.44</td>
<td>0.99</td>
</tr>
<tr>
<td>Sl\textsuperscript{10H}/+</td>
<td>0.51</td>
<td>0.51</td>
<td>0.48</td>
<td>0.52</td>
</tr>
<tr>
<td>Sl\textsuperscript{10H}/+</td>
<td>0.64</td>
<td>0.56</td>
<td>0.49</td>
<td>0.56</td>
</tr>
<tr>
<td>Sl\textsuperscript{10H}/+</td>
<td>0.60</td>
<td>0.55</td>
<td>0.49</td>
<td>0.68</td>
</tr>
</tbody>
</table>

Southern blots of Sl/+ and Sl\textsuperscript{10H}/+ DNAs were sequentially hybridized to each probe; 3127L is 220 kb 5' to Mgf, 394L is 100 kb 5' to Mgf (see text and Figure 1), gbB/H is derived from Sl\textsuperscript{10H} deletion breakpoint and is 60 kb 5' to Mgf (see text), and 394R is 590 kb 5' to Mgf (see text and Figure 1). The intensity of hybridization of each experimental probe was normalized to that of control probes, Myb and D10Fcr2 (see text). For each heterozygous DNA, the data are expressed as the ratio of hybridization intensity relative to +/+ DNA and are the average of two separate samples. RFLPs, restriction fragment length polymorphisms.
viable with their containing two mutant lifespans. While both observed for homozygous mice shown in Figure 3. Mice with the genotype viability. By creating mice that are heteroallelic for amination than 

The pigmentation of approximately one-quarter of the first few weeks of birth. These hemizygous mice were completely white, anemic. The period of survival observed with neonatal SIIH/SIIH mice was invariably white. In SIIH/SI"m, much more pigment was observed than even the grey of SI"m/SI"m mice, with nearly normal pigmentation in most of the coat with the exception of a prominent white head spot (see Figure 3C). Thus, both SI"m and SI"m partially complement the color deficiencies produced by SI"m. In comparison, the pigmentation defects caused by SI"m, SI"m, and SI"m were not complemented by SI"m as SI"m/SI"m' and SI"m/SI"m were completely white (see Table 3) and SI"m/SI"m have about the same pigmentation as SI"m/SI"m mice (Figure 3A). In addition, SI"m/SI"m mice had slightly less coat pigment than SI"m/SI"m mice (Figure 3D). Comparison of the entire series of SI"m heteroallelic mice results in the following classification on the basis of pigmentation (from least to most pigment): SI"m/SI"m = SI"m/SI"m < SI"m/SI"m = SI"m/SI"m < SI"m/SI"m < SI"m/SI"m < SI"m/SI"m < wild type.

DISCUSSION

Deletion analysis of various lethal SI alleles has provided information regarding sequences that contribute to the lethal phenotype. The smallest deletion we have identified in the homozygous lethal class of SI mutants is ~120 kb in the SI" allele, while the next smallest deletions are 650 and 680 kb, respectively, in SI" and SI"H. The relatively small size of the SI" deletion suggests that it may not affect any genes other than Mgf that are essential for viability. Neonatal lethality has previously been reported to occur in both SI" and SI"H/SI"H mice (SCHAIBLE 1961, 1963; BEECHEY and SARLE 1985). However, no live-born homozygous offspring were observed when SI"/+ or SI"H/+ + mice were intercrossed (M. A. Bedell, unpublished results), suggesting that strain background may affect the viability of homozygous mice. Because our analysis indicates that the deletions in SI"H, SI" and SI"H are considerably smaller than that of the original SI allele (Figure 2) and analysis of SI/SI and SI/SI mice demonstrated that lethality occurred during embryogenesis after E15 (SARVELLA and RUSSELL 1956; P. J. DONOVAN, personal communication), all four alleles would be expected to cause homozygous lethality at the same stage of development when on a uniform genetic background. Late gestation lethality therefore appears to be the true null phenotype of SI mutations. However, detailed comparison of the development of SI"/SI" and SI/SI embryos will be required to determine if the larger deletion affects additional genes essential for embryonic survival. In comparison to deletions that remove all Mgf coding se-
Allele\textsuperscript{a} | Mutation | Consequence of mutation | Phenotype of heteroallelic Sl mice
<table>
<thead>
<tr>
<th></th>
<th></th>
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</thead>
<tbody>
<tr>
<td>+</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Sl\textsuperscript{eb}</td>
<td>120 kb deletion\textsuperscript{b}</td>
<td>Absence of Mgf</td>
</tr>
<tr>
<td>Sl\textsuperscript{d}</td>
<td>Intragenic deletion\textsuperscript{c}</td>
<td>Lack of membrane bound Mgf</td>
</tr>
<tr>
<td>Sl\textsuperscript{124H}</td>
<td>Point mutation in splice acceptor\textsuperscript{d}</td>
<td>Absence of normal Mgf cytoplasmic domain</td>
</tr>
<tr>
<td>Sl\textsuperscript{pos}</td>
<td>Distant rearrangement\textsuperscript{e}</td>
<td>Altered Mgf mRNA abundance</td>
</tr>
<tr>
<td>Sl\textsuperscript{dom}</td>
<td>Distant rearrangement\textsuperscript{f}</td>
<td>Altered Mgf mRNA abundance</td>
</tr>
</tbody>
</table>

\textsuperscript{a} All mutants were maintained on a C3H/HeN background except Sl\textsuperscript{d} that was maintained on C57BL/6J.

\textsuperscript{b} Present study.

\textsuperscript{c} Intragenic deletion of 4 kb that causes the absence of transmembrane and cytoplasmic domains (BRANNAN \textit{et al.} 1991; FLANAGAN \textit{et al.} 1991).

\textsuperscript{d} Point mutation in 3' splice site such that normal cytoplasmic domain is removed by splicing, leaving an out-of-frame cytoplasmic domain (BRANNAN \textit{et al.} 1992).

\textsuperscript{e} Chromosomal rearrangements in Sl\textsuperscript{pos} and Sl\textsuperscript{dom} that break 115 and 195 kb, respectively, upstream of Mgf coding sequences and have tissue-specific effects on Mgf mRNA abundance (BEDELL \textit{et al.} 1995).
Gene dosage appears to be a critical feature of the function of both wild-type and mutant Sl proteins for pigmentation and viability. All known Sl alleles exhibit haploinsufficiency in that heterozygous mice display mild pigmentation defects (see Tables 1 and 3) (Silvers 1979a; Cattanach et al. 1993). Because this occurs in mice heterozygous for Sl deletions, this semidominant effect must result from reduced gene dosage.
rather than from gain-of-function mutations in the protein. The profound effects of only a single copy of \( S^{117H} \) on viability are somewhat surprising. It is not presently known how the absence of the normal cytoplasmic domain in \( S^{117H} \) disrupts Mgf function but it may cause either decreased protein stability, lack of proper presentation in the membrane, absence of proteolytic cleavage or faulty signaling (BRANNAN et al. 1992). Although the lethality observed in \( S^{117H}/S^{66} \) could result from a gain-of-function mutation in the \( S^{117H} \)-encoded protein, this is unlikely because these hemizygous mice live longer than \( S^{66}/S^{66} \) mice. It is more likely that the amount of mutant protein expressed by only a single copy of the \( S^{117H} \) allele causes severe impairment of Mgf function. A threshold of functional Mgf activity may therefore be required for viability, as has been proposed for the activity of this growth factor during ovarian follicle development (HUANG et al. 1993; BEDELL et al. 1995). In sharp contrast to the lethality of \( S^{117H}/S^{66} \) mice, mice that are hemizygous for \( S^{66} \), \( S^{66} \) or \( S^{66} \) express sufficient functional Mgf for viability. One caveat to direct comparisons between these alleles is that the \( S^{66}/S^{66} \) mice were produced on a mixed (B6C3F1) background while all others were on a pure inbred background (C3H/HeN). Background effects could therefore be contributing to the observed differences in viability produced by these hypomorphic alleles.

Complementation of the pigmentation phenotype was observed with certain combinations of viable \( S^{l} \) alleles, i.e., more pigmentation was observed in \( S^{l^{2}}/S^{66} \) or \( S^{l^{2}}/S^{66} \) and \( S^{l^{2}}/S^{66} \) or \( S^{l^{2}}/S^{66} \) than in the respective homozygous mice. This complementation is even more striking when compared with the phenotypes of mice hemizygous for each of these alleles (see above). The nature of the mutations present in each of the complementing alleles is known. \( S^{l^{2}} \)-encoded Mgf lacks the normal cytoplasmic domain as the result of a splice mutation (BRANNAN et al. 1992), and both \( S^{66} \) and \( S^{66} \) contain intact coding sequences but have different rearrangements that affect the expression of the gene (BEDELL et al. 1995). Several possible mechanisms for this complementation have been considered. Because the \( S^{66} \) and \( S^{66} \) rearrangements are more than 100 kb from Mgf (BEDELL et al. 1995), they affect another gene, in addition to Mgf, that is required for pigmentation. If this were the case, the pigmentation of \( S^{l^{2}}/S^{66} \) or \( S^{l^{2}}/S^{66} \) and \( S^{l^{2}}/S^{66} \) or \( S^{l^{2}}/S^{66} \) mice would result from the mild effects of one mutant copy each of Mgf and the other gene. However, the \( S^{66} \) and \( S^{66} \) phenotypes are not complemented by \( S^{66} \) and \( S^{66} \) (BEDELL et al. 1995; present study), providing strong evidence that Mgf is the only gene required for pigmentation that is affected by the rearrangements. Therefore, the \( S^{l^{2}}/S^{66} \) and \( S^{l^{2}}/S^{66} \) and \( S^{l^{2}}/S^{66} \) phenotypes represent true interallelic complementation. Two mechanisms by which interallelic complementation may be produced are transvection, whereby regulatory elements of one allele affect expres-

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