Embryonic RNA expression patterns of the *c-kit* receptor and its cognate ligand suggest multiple functional roles in mouse development

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Mutations at the dominant *white spotting* (*W*) and *Steel* (*Sl*) loci in mouse exert deleterious effects on three migratory cell lineages (primordial germ cells, melanocytes and hematopoietic stem cells) resulting in loss of pigmentation, reduced fertility and anemia. The *W* locus encodes the *c-kit* protein tyrosine kinase (TK) receptor. More recently, the *Sl* locus has been shown to encode a ligand for *c-kit*, which is variously known as mast cell growth factor (MGF), stem cell growth factor and *c-kit* ligand. Here we report an *in situ* hybridization analysis comparing the expression profiles of MGF and *c-kit* transcripts during mouse embryogenesis. The data are consistent with the *c-kit* receptor–ligand complex providing a homing mechanism during stem cell migration in early development and in stem cell proliferation, differentiation, or survival in late development. In the nervous system, an unexpected and complex pattern of expression is uncovered that suggests involvement of the *W* and *Sl* gene products in the organization of the neural tube and brain.

Key words: development/*c-kit*/mouse/*Steel*/*white spotting*

Introduction

The *Steel* (*Sl*) (Altus *et al.*, 1971; Fried *et al.*, 1973; Kitamura and Go, 1978; Mayer, 1970) and dominant *white spotting* (*W*) (Russell and Bernstein, 1968; Bennett, 1956; Geissler *et al.*, 1981; Harrison and Russell, 1972; McCulloch *et al.*, 1964) loci are among the most studied mutations in mouse (for review see Silvers, 1979). Many alleles of these two unlinked loci have been identified as spontaneous mutations or have been induced experimentally. Although the many mutations display phenotypes of varying severity, all *W* and *Sl* mutants are deficient in three embryonic migratory cell lineages; primordial germ cells (PGC), melanocytes and hematopoietic stem cells. In the homozygous condition, deficiencies at the *W* and *Sl* loci often result in lethality while in the heterozygous or compound heterozygous condition, varying degrees of diluted coat color, anemia and reduced fertility are observed (Russell, 1979). Transplantation experiments with neural crest, spleen and bone marrow have indicated that the product of the *W*

locus is cell autonomous. In contrast, the defects associated with the *Sl* mutation reside in the microenvironment of the affected cells (for review, see Silvers, 1979).

Over the past two years, the *W* gene product has been identified and characterized at the molecular level (Chabot *et al.*, 1988; Geissler *et al.*, 1988). The *W* phenotype results from mutations that alter the *c-kit* protooncogene, which encodes a protein tyrosine kinase (TK) receptor. The *c-kit* receptor is believed to function as a homodimer, a hypothesis that is supported by the observed dominant loss of function effects of *W* point mutations versus the recessive lethal effects observed in alleles that delete the locus.

More recently, characterization of a factor that stimulates the growth of mast cells in culture (MGF) and that binds the *c-kit* receptor has led to the identification of the *Sl* gene product (Copeland *et al.*, 1990; Huang *et al.*, 1990; Zsebo *et al.*, 1990; Williams *et al.*, 1990b). Analysis of cDNA clones suggests that the MGF molecule is synthesized as a membrane bound form although soluble recombinant protein is biologically active in *in vitro* assays (Anderson *et al.*, 1990). Presumably, a soluble form of the MGF protein can occur *in vivo* by proteolytic cleavage of the membrane form.

It has been suggested that *W* and *Sl* mutations may affect the homing of the affected stem cells as well as their proliferative or survival capacity (Mintz and Russell, 1957; Silvers, 1979). In vertebrates, TK receptors are believed to function primarily as mediators of signals that stimulate cell proliferation (for review, see Hanks *et al.*, 1988). Although recent studies with the *trk* family of TK receptors indicates that TK receptors can transmit neurotrophic signals (Kaplan *et al.*, 1991a,b; Hempstead *et al.*, 1991). In *Drosophila melanogaster*, TK receptors can act as transmitters of morphogenic signals. For example, expression of the *sevenless* gene, encoding a TK receptor, is required in the signal transduction pathway that triggers the development of photoreceptor number seven from its progenitor; no associated mitogenic response has been observed (Basler and Hafen, 1988).

To address further the functions of the *W/Sl* receptor–ligand complex in mouse development, including the nature of the signal (whether mitogenic, chemotactic or both) transmitted by the *W/Sl* receptor–ligand complex, we have analyzed the expression of these two genes by *in situ* hybridization. We reasoned that simultaneous analysis and direct comparison of receptor and membrane bound ligand expression would provide the most relevant information about the timing, location and nature of ligand–receptor interactions occurring *in vivo*. In this study, we have focused primarily on the development of pigment cells, the germ line and the nervous system although we document additional sites of *c-kit* and MGF gene expression. The patterns observed in skin and gonads suggest that MGF mediates a chemotactic signal for *c-kit* expressing migratory cells that is consistent with the known *W* and *Sl* phenotypes. Additionally, we have uncovered an unexpected profile of

expression for *c-kit* and MGF in the nervous system, which suggests a role for these genes in neural development.

Results

The probes used in this study were derived from murine cDNAs (for details, see Materials and methods). The linearized *c-kit* probe spans ~3.0 kb of sequences while the MGF probe is a 1 kb fragment. These probes recognize the expected transcripts in Northern blot analysis (not shown). ³⁵S-labeled antisense probes were hybridized to paraffin sections of embryos from each day of post-implantation

development. At our level of detection, no significant MGF expression was observed in embryonic structures prior to day nine (12 somite) embryos.

Figure 1 shows sections of day 11.5 (40 somites) and day 13.5 (50 somites) embryos hybridized with MGF (panels A and D) and *c-kit* (panels C and F) probes. This example illustrates that *c-kit* and MGF expression is not limited to tissues displaying phenotypic abnormalities in *W* and *Sl* mice. For example, at 11.5 (Figure 1A), MGF transcripts are present at high levels in the forming septa of the heart (of neural crest origin), in the maxillary process, and in the floor plate and motor columns of the neural tube (see also Figure 6). Less apparent here is expression in skin, liver

Fig. 1. Expression of MGF and *c-kit* in day 11.5 and 13.5 embryos. (A) MGF and (C) *c-kit* dark field views of adjacent day 11.5 embryo frontal sections. (B) light field of panel A. Note that the head in panel C is torn and displaced upward such that, by artifact, the sections appears longer. (D) MGF and (F) *c-kit* dark field views and (E) light field view of a day 13.0 frontal embryo section. (t) telocoel; (h) heart; (l) liver; (n) neural tube; (br) brain.

(a hematopoietic organ) and genital ridges which will be discussed more fully below. *c-kit* at this stage is expressed diffusely in the brain and neural tube while transcripts are most evident in liver. At day 13.5, MGF transcripts are most prominent in skin (Figure 1D) while lower levels are found in gonads, kidney, brain and neural tube. *c-kit* expression is highest at this stage in presumptive melanocytes (see below) and in various structures of the brain (Figure 1F) (see also Orr-Utreger *et al.*, 1990).

***c-kit* and MGF expression along PGC migratory pathways**

Primordial germ cells (PGC) originate in the allantois and migrate through the dorsal mesentery and hindgut to the

forming genital ridges between embryonic days 9.5 and 11.5 (Donovan *et al.*, 1986; Bennett, 1956; Mintz and Russell, 1957). Thus, colonization of the genital ridge begins on about embryonic day 10 and is completed by day 12.5, when overt differentiation of the ridges begins. Figure 2 shows a developing genital ridge of a day 10 (28 somite) embryo. In panel A, MGF expression is seen at its highest level within the genital ridge. In contrast, *c-kit* expression is present mainly along the mesentery in the vicinity of the dorsal aorta (Figure 2C). The enlargements corresponding to Figures 2A and C are shown in the lower panels (Figure 2D–F and D'–F'; see the regions labeled D, E and F respectively in panel B). Comparison of MGF grain density (D–F) with that of *c-kit* (D'–F') in adjacent sections illustrates the

Fig. 2. Expression of MGF and *c-kit* in embryonic day 10 genital ridges. (A) MGF and (C) *c-kit* dark field views of adjacent sections. (B) light field view of panel A. (D), (E) and (F) MGF and (D'), (E') and (F') *c-kit*; light field magnification of regions indicated in brackets in panel B. Dark grains indicate hybridization. (a) dorsal aorta; (D) genital ridge; (E) dorsal mesentery; (F) dorsal mesentery adjacent to dorsal aorta.

inverse gradient of expression for these two genes in cells along the dorsal mesentery from the dorsal aorta to the genital ridge. MGF expression is highest at the genital ridge (panel D) while the greatest number of *c-kit* grains are present in the vicinity of the dorsal aorta (panel F') with a decreasing gradient of grains seen toward the genital ridge (panels D' and E').

Once PGC colonization of the genital ridge is complete, the gradient of MGF mRNA expression along the migratory path disappears and transcripts are confined to the developing gonad (Figure 3, panels A–F). These data suggest that cells expressing MGF outline the pathway and guide the *c-kit*

expressing cells toward the genital ridge (Orr-Utreger *et al.*, 1990). It is likely the MGF functions here in its membrane bound form since the soluble form should only require transcript expression from the site of destination (i.e. genital ridges) rather than along the migratory path. Additionally, the observed shutdown of MGF expression along the migratory pathway upon completion of genital ridge colonization (Figure 3B and F) may reflect a mechanism of down regulation triggered by direct interaction of the membrane bound MGF with the *c-kit* receptor on the bypassing PGCs. Sexual differentiation of genital ridges into either testis or ovary at midgestation, does not result in

Fig. 3. MGF expression in genital ridges and gonads. (A) light field and (E) dark field views of a frontal section through genital ridges (arrows) of a day 9.5 embryo. (B) light field and (F) dark field view of a frontal section through genital ridges of a day 11.5 embryo. (C) light field and (G) dark field view of a sagittal section through testis of a day 13 embryo. (D) light field and (H) dark field view of a frontal section through a day 14.5 ovary. (da) dorsal aorta; (ki) kidney; (te) testis; (ov) ovary.

shutdown of MGF expression (Figure 3C, D, G and H), although levels may vary. Transitory expression of MGF was also observed in kidney tubules with transcripts present at embryonic day 13 and decreasing to levels below detection by day 15.5 (Figure 3G and H).

Analysis of *c-kit* and MGF expression in adult ovaries (Figure 4) revealed a complementary pattern of expression. MGF transcripts are present outside the oocytes and at highest levels in the surrounding granulosa cells. *c-kit* receptor mRNA expression is confined to the oocyte.

The expression patterns observed for *c-kit* and MGF throughout the course of gonadal differentiation suggest that this receptor–ligand complex may serve multiple or evolving functions during the progression of germ cell and gonad development. During premigratory and migratory stages of germ cell development, high concentrations of MGF transcript are present in the uncolonized genital ridges, with a lower but discernible quantity of transcripts along the migratory pathway. This suggests that MGF may provide a homing, chemotactic gradient for *c-kit* expressing migratory PGCs. Once the PGCs enter the genital ridge, germ cells begin a period of proliferation and undergo differentiation. During this period, *c-kit* and MGF are still expressed and it is possible that the *c-kit*–MGF complex may play a role in these processes. Finally, in the mature ovary, neither chemotaxis nor proliferation is an important feature. The oocyte is in a state of homeostasis and *c-kit*–MGF signal transduction may be involved in the maintenance of the oocyte.

***c-kit* and MGF expression in developing skin**

Melanocytes derive from neural crest cells that take a migratory route laterally from the apex of the neural tube

toward the dorsal region of the somites (Searle, 1968). In the mouse, melanoblast migration begins around day 8.5 of gestation and the precursors are dispersed throughout the trunk skin ectoderm and adhering mesoderm by day 11.5. Limb bud colonization is slightly retarded and occurs between days 11.5 and 12.5 (Rawles, 1947; Silvers, 1979). As observed in developing gonads, the pattern of MGF and *c-kit* expression seen in developing skin is also consistent with a function in chemotaxis of pigment cell migration. MGF expression was first observed in the somitic compartment that is the precursor of skin (dermamyotome) at a time preceding the influx of pigment cells (not shown). Figure 5 illustrates the relationship of MGF and *c-kit* mRNA expression in the mesenchyme underlying developing skin of a limb bud at day 13.0 when melanoblast colonization is recently completed (panels A–G) and at day 15.5 when melanocytes are in the process of proliferation and differentiation. The forelimb of a day 13 embryo shows high levels of MGF transcripts in the mesenchymal cells underlying the dermis (Figure 5A, D and E). *c-kit* expression is more punctate and reflects the presence of transcripts in melanoblasts that are in the process of colonizing the region (Figure 5C, F and G). The hindlimb of a day 15.5 embryo is seen in panels H–J. Although melanoblast colonization of skin is essentially complete at this stage, the complementary patterns of MGF and *c-kit* expression remain present. The relative number of melanocytes has increased dramatically due to proliferation in site (Figure 5J, L and M). It is possible that expression of *c-kit* in these regions may also reflect the presence of tissue mast cells. In late development, expression of *c-kit* and its ligand decreases markedly (not shown) suggesting that the principal role in skin may be in the migratory and early proliferative stages.

Fig. 4. MGF and *c-kit* expression in adjacent sections of adult ovary. (A) light field and (B) dark field view with MGF probe. (C) light field and (D) dark field view with *c-kit* probe. (gr) granulosa cells; (oo) oocyte.

Fig. 5. Comparative expression of MGF and *c-kit* in skin during mid-gestation. Day 13.0 embryo forelimb: (A) MGF dark field, (B) hematoxylin/eosin, light field, and (C) *c-kit* dark field views of adjacent sections. (D) light field and (E) dark field magnifications of (A). (F) Light field and (G) dark field magnifications of (C). Day 15.5 embryo hindlimb: (H) MGF dark field, (I) *c-kit* light field and (J) *c-kit* dark field. (K) MGF dark field, (L) light field and (M) *c-kit* dark field magnifications. (N) *c-kit* light field magnification of (M). Arrows point to pigment cells.

Fig. 6. MGF and *c-kit* expression in adjacent sections of embryonic neural tube. (A) MGF light field and (B) MGF dark field view of a day 9.5 embryo neural tube. (C) MGF dark field, (D) *c-kit* light field and (E) *c-kit* dark field view of a day 12 embryo neural tube. (F) MGF dark field, (G) MGF light field and (H) *c-kit* dark field of a day 14.5 neural tube. (fp) floor plate, (M) motor neurons, (arrows) floor plate and motor neurons; (d) dorsal root ganglion.

***c-kit* and MGF expression in neural tube and brain development**

The phenotypes described for *Sl* and *W* mutants have so far been confined to three migratory stem cell populations that affect pigmentation, fertility and hemopoiesis (Silvers, 1979). We have detected transcripts for the *c-kit* receptor and for MGF in various tissues not known to be affected in *W* and *Sl* mice. The strong and complementary patterns of expression seen for these genes in various locations of the developing brain and neural tube are most striking. *c-kit* (Orr-Utreger *et al.*, 1990) and MGF transcripts are visible from the earliest stages of neural differentiation with a pattern of expression that gains complexity with ongoing development. Figure 6 shows transverse sections of the lumbar neural tube at three stages of embryonic development [days 9.5 (A and B), 12.0 (C–E) and 14.5 (F–H)]. At day 9.5, *c-kit* expression in the neural tube is at our limits of detection (not shown). MGF transcripts are more abundant and localized to the discrete group of cells at the ventral boundary known as the floor plate (Figure 6B). By day 12.0, MGF expression appears in two additional sites to that of the floor plate (Figure 6C, middle arrow); along the ventrolateral boundary of the neural tube in the region where the motor

neuron columns form (Figure 6C, lateral arrows). An adjacent section shows that in the neural tube, *c-kit* expression is confined to the post-mitotic cells in the marginal zone at the dorsal aspect of the neural tube (Figure 6E). Additionally, the outer boundary of the neural tube is circumscribed by cells that express *c-kit* transcripts. Under high magnification, these cells appear to be migratory neural crest cells that travel between the dorsal root ganglia (DRG) and the neural tube (not shown).

At embryonic day 14.5, both MGF and *c-kit* transcripts are more abundant in the neural tube. MGF expression is seen in the neurons of the forming motor columns and in cells of the ventral marginal zone which appear to migrate toward the motor columns (Figure 6F). *c-kit* transcripts remain most abundant in the dorso-lateral marginal zone although a dorsal to ventral gradient of expression is evident throughout the neural tube (see also Orr-Utreger *et al.*, 1990). *c-kit* transcripts are also present in DRG at this stage and throughout the remainder of development (Figure 6H). The neural tube is precisely organized with a dorsal to ventral (D–V) gradient alignment of neurons (Jacobsen, 1978; Altman and Bayer, 1984). The contrasting D–V expression profiles observed here suggest that MGF and its receptor

Fig. 7. MGF and *c-kit* expression in adjacent sections of embryonic brain. (A) MGF light field and (B) MGF dark field of a frontal section through a day 9.5 embryo myelencephalon. (C) MGF dark field, (D) *c-kit* light field and (E) *c-kit* dark field frontal views along the mesencephalon of a day 11.5 embryo. (F) MGF dark field, (G) MGF bright field, and (H) *c-kit* dark field frontal view through the fourth ventricle of a day 13.5 embryo. (I) MGF dark field, (J) *c-kit* light field and (K) *c-kit* dark field sagittal view through a 15.5 day forebrain. (L) MGF dark field, (M) MGF light field and (N) *c-kit* dark field of a transverse section through a day 15.5 embryo head. Arrows point to the floor plate; (my) myelocoel; (me) mesocoel; (th) dorsal thalamus; (te) telocoel; (ob) olfactory bulbs.

may have a function in the organization of neurons along the D–V axis.

Analysis of the developing brain (Figure 7) provided similar results to those described for the neural tube although the observed pattern of expression is of greater complexity. At embryonic day 9.5, *c-kit* mRNA expression in the myelencephalon is low (not shown); however, MGF transcripts are prominent at the floor plate as illustrated in a transverse section through the hindbrain (Figure 7A and B) and throughout the length of the neural tube (not shown). MGF expression increases by day 11.5 along the floor plate from the caudal portion of the mesencephalon (mid-brain) along the length of the neural tube. Thus, a longitudinal section through the head region exposes the mid-brain and its ventricle (Figure 7C). In comparison, *c-kit* is expressed in two nuclei of cells adjacent to the floor plate in the mesencephalon (Figure 7E). In Figure 7, panels F–H, a longitudinal section shows expression of MGF and *c-kit* along the fourth ventricle at the mid-brain/hindbrain junction (metencephalon forms the upper boundary and myelencephalon forms the lower). In this section, MGF is predominantly expressed along the floor plate of the myelencephalon (hindbrain), although transcripts are also present in two groups of cells close to the lateral boundaries of the floor of the myelencephalon (see below). *c-kit* expression in this region of the brain is predominant along the entire marginal layer of the fourth ventricle with the exception of the cells adjacent to the floor plate (Figure 7H). It is interesting to note that the two motor nuclei of lateral cells expressing MGF in Figure 7, panel F correspond to regions that do not express *c-kit* in panel H.

By embryonic day 15.5, *c-kit* and MGF expression in the brain is quite complex. However, similar themes to those observed in the neural tube are present. Figure 7, panels I–K, shows a sagittal section through the head at the level of the caudal telencephalon (forebrain) and dorsal thalamus. *c-kit* expression is again highest in the layer of cells that are preparing to undergo differentiation after having emerged from the underlying mitotic (ependymal) cell layer. In contrast, MGF transcripts are present at high levels within the dorsal thalamus just caudal to the layer of *c-kit* expressing cells. An additional site of high expression for both the receptor and the ligand are the cells surrounding and within the olfactory epithelium and bulb (Figure 7L–N) with *c-kit* expression confined to outlying cells within the intermediate zone while MGF is present at high levels in punctate cells of the intermediate zone and in the ventricular epithelium.

The final stage of embryogenesis at which we compared MGF and *c-kit* mRNA expression was embryonic day 17.5. The abundance of MGF transcripts is reduced in skin and liver (not shown) but remains high in brain, spinal cord and in cranial and spinal ganglia (Figure 8). *c-kit* transcripts are also seen in the above noted structures albeit at lower levels (Figure 8). In particular, trigeminal and dorsal root ganglia display a punctate profile of *c-kit* expression, indicating expression in a limited number of neurons within the ganglia (Figure 8A). In dorsal root ganglia, MGF transcripts appear to be distributed in a larger proportion of cells (Figure 8C). At high magnification, *c-kit* grains can be localized only over neurons while MGF grains are evenly distributed and do not allow precise localization (not shown).

In our analysis of the developing nervous system, *c-kit*

Fig. 8. MGF and *c-kit* expression in day 17.5 embryo spinal ganglia. (A) MGF dark field, (B) MGF light field and (C) *c-kit* dark field of cervical spinal ganglion.

receptor transcripts were localized primarily to regions that correspond to cells having recently undergone mitosis and *en route* to differentiation along the various neuronal or glial pathways. In contrast, MGF expression was found in discrete clusters of cells, primarily along the floor plate, in the thalamus and in the olfactory epithelium.

Discussion

The known targets of *Sl* and *W* are three migratory stem cell lineages that give rise to primordial germ cells, pigment cells and hemopoietic cells. The absence of these cells at their final destination in *W* and *Sl* mice can result from inability of the precursors to survive or to undergo proper guidance, from incapacity to proliferate (McCoshen and McCallion, 1975) or from a combination of these defects (Mintz and Russell, 1957; Mayer and Green, 1968; Morrison-Graham *et al.*, 1990). Our *in situ* hybridization experiments are consistent with the notion that in early development, cell homing is a primary function of the *c-kit*–MGF receptor–ligand complex. Both in germ cell and pigment cell migration, MGF transcripts are abundantly expressed at the site of destination for the migrating cells

prior to their arrival. A gradient of MGF expressing cells is also observed along the migratory pathway of primordial germ cells providing additional evidence for a chemotactic function. However, both *c-kit* and MGF remain transcriptionally active after cell migration is completed, suggesting that additional functions such as those involved in proliferation or maintenance may be attributed to this receptor–ligand complex. Consistent with this view, certain IL-3 dependent mast cell lines have been shown to require MGF for proliferation (Boswell *et al.*, 1990; Williams *et al.*, 1990a). It is possible that *in vivo*, similar synergism of unrelated polypeptide growth factors may contribute to cell growth or survival.

Like colony stimulating growth factor (Rettenmier, 1989), the *Sl* product can exist in a membrane bound and in a soluble form. In the past, it has been unclear whether both forms of *Sl* are biologically functional *in vivo*. The analysis of MGF expression in the early developing gonad is most consistent with a function for the membrane bound form. Two main criteria support, but do not prove, this view. First, a gradient of cells expressing MGF transcript is observed along the PGC migratory pathway rather than a focalized point of expression at the target (genital ridge). Second, a shutdown of MGF expression occurs along the migratory pathway once the PGCs have migrated through. These data suggest the possibility of a feedback down-regulatory mechanism transmitted through the membrane bound MGF and initiated by interaction with the *c-kit* receptor-expressing PGCs.

Further indirect support for this interpretation has recently been provided by Donovan and colleagues (Dolci, S., Williams, D.E., Ernst, M.K., Resnick, J.L., Brannan, C.I., Lock, L.F., Lyman, S.D., Boswell, H.S. and Donovan, P.J., submitted for publication) who have shown that the membrane form, but not the soluble form, of MGF enhances the survival of mouse PGCs in primary cultures.

Expression of *c-kit* is maintained in female germ cells after birth (Orr-Utreger *et al.*, 1990) and into adulthood. The presence of high levels of MGF transcripts in the supporting granulosa cells suggests a role for this receptor–ligand pair in maintenance of the oocyte. Consistent with this possibility, female mice homozygous for the *Steel contrasted* (*Sl^{con}*) allele exhibit diminishing fertility with age. Ovaries of young *Sl^{con}/Sl^{con}* females contain about half the normal number of maturing follicles and by three months of age only the corpora lutea, devoid of oocytes, are seen in ovary sections (Beechey and Searle, 1983). It will be of considerable interest to measure MGF and *c-kit* expression levels in maturing ovaries of *Sl^{con}/+* and *Sl^{con}/Sl^{con}* mice. Reduced or aberrant expression of MGF transcripts in the granulosa cells would provide direct support for a role of the *c-kit*–MGF complex in maintenance of mouse oocytes.

MGF and *c-kit* expression is not limited to the sites predicted from the known phenotypes. For example, MGF transcripts are also present in the neural crest derived septa of the heart, in facial chondrogenic nuclei and in mid-gestation mesonephric tubules.

Examination of the developing neural tube has also revealed an unexpected expression pattern for MGF that suggests a role for this gene in the organization of neurons along the dorsoventral axis. Early presence of MGF transcripts in the cells of the floor plate is followed by appearance of transcripts in the ventrolateral regions associated with motor neuron differentiation (Jessell *et al.*,

1989). The arrangement of the neural tube in its dorsoventral axis is bilaterally symmetrical and differentiation of distinct neuronal classes occurs in a patterned fashion along its axis. Thus motor neurons differentiate in a series of columns laterally adjacent to the floor plate (Altman and Bayer, 1984) while commissural neurons differentiate in the dorsal marginal zone (Placzek *et al.*, 1990) and neural crest cells form on the dorsal apex of the neural tube (Le Douarin, 1982).

Recent floor plate grafting experiments in chick embryos by T.Jessell, J.Dodd and their co-workers provide evidence that the floor plate functions as the organizer for the adjacent motor neuron tracts (Yamada *et al.*, 1991). These data lend direct support to the notion that inductive events are important in the orderly arrangement and differentiation of distinct classes of neurons along the dorso-ventral axis. The gradient of *c-kit* expression observed in differentiating neurons in the dorsal neural tube and along its outer boundary, coupled with MGF expression in the floor plate and in two lateral regions, suggests that MGF may function as a chemoattractant molecule for neurons or for axons.

The floor plate has also been demonstrated to exert chemotrophic action on at least one set of specific neural tube axons in tissue explant experiments (Tessier-Lavigne *et al.*, 1988). This chemotrophic guidance is selective for commissural neuron axons that form in the dorsal spinal cord, extend their axons laterally to the motor columns, and then deviate ventromedially to the floor plate (Placzek *et al.*, 1990). The presence of *c-kit* expressing cells at the dorsal (roof plate) region of the neural tube coupled with the expression pattern displayed by MGF transcripts at the intermediate and ventral target of commissural axons, make the products of the *W* and *Sl* loci potential candidates to play a role in commissural axon guidance. Use of antibodies that detect only commissural neurons in the neural tube (Dodd and Jessell, 1988) together with *c-kit* directed antibodies and purified MGF should allow analysis of this possibility. The localized expression seen for MGF in the neural tube is in contrast to the gradients observed in the developing germ cell pathway. The absence of an MGF transcript gradient suggests that a protein gradient may exist and that the possible function of MGF (i.e. axonal guidance and neuron alignment) may be mediated by soluble factor.

Expression of *c-kit* in the brain is highest in the regions just outlying the mitotic layers of the third and fourth ventricles. Cells in this region are neural and glial precursors that are thought to acquire specific fates in response to local cell interactions (see Purves and Lichtman, 1984; Turner and Cepko, 1987). After the establishment of cellular identity, axonal extension and synaptic formation ensues. These processes require trophic interactions to aid in axonal guidance. The focalized expression seen of MGF may provide one of many sources for trophic alignment of neurons and glia during brain development.

High levels of MGF transcripts are also found in the embryonic thalamus. The thalamus serves as a relay station in the brain, interconnecting sensory and motor input to the cerebral cortex as well as hearing, balance, vision and taste. Thus, afferent inputs from the hypothalamus, the trigeminal sensory nuclei, olfactory, optic and spinal tracts, among others, are mediated by the thalamus (Kelly, 1985 and references therein; Hebel and Stromberg, 1986). A variety of trophic signals must interplay to result in the successful

guidance and formation of the intricate networks present within the thalamic nuclei. Presence of high levels of MGF transcripts during embryonic formation of this region is consistent with the possibility that this ligand may function in the guidance of neurons or their axons from any of the afferent origins to the thalamic nuclei.

To date, no neurological phenotype has been described for any *W* and *Sl* mutation. Embryonic lethality observed with some *W* and *Sl* alleles has been attributed to the deleterious consequences of severe anemia. It remains possible, however, that previously unrecognized neural defects may be the primary underlying cause of some lethal alleles. Alternatively, redundancy of function may exist with other genes that compensate for *W* and *Sl* mutations, thus minimizing neurological phenotypes. A potential extreme example of this latter possibility has been reported by Joyner *et al.* (1991). These investigators have mutated one of the *engrailed* genes (*En-2*) in ES cells and generated mice that are homozygous for the null allele at this locus. Unexpectedly, these mice are viable, suggesting that the highly related and similarly expressed *En-1* gene may function as a compensatory substitute for the damaged *En-2* locus. The precise nature of MGF and *c-kit* involvement in neural development will be better understood following detailed analysis of neural tube and brain development in mice carrying the various *W* and *Sl* alleles.

While this manuscript was in preparation, Matsui *et al.* (1990) reported a study of stem cell growth factor (SCF) expression during limited stages of embryonic development. SCF is identical with MGF (Copeland *et al.*, 1990; Williams *et al.*, 1990a; Zsebo *et al.*, 1990) and, in general, the SCF study is in agreement with our data although these investigators did not observe MGF (SCF) transcripts in spinal ganglia where we observe considerable levels of MGF after day 14.5 and in the adult. Comprehensive analysis throughout embryonic development, directly comparing gene expression for the *c-kit* receptor and its ligand described in these studies has provided a number of interesting new insights into the function of this receptor-ligand pair.

Summary

The availability of molecular clones for the *W*-encoded *c-kit* receptor and its ligand encoded by *Sl* has allowed elucidation of the molecular basis for the pleiotropic defects caused by mutations at these loci. In the present study, we have shown that the expression profile of both genes during embryogenesis is consistent with their presumed role in migratory and proliferative stages of germ and pigment stem cells. The data also suggest a role in the maintenance of mature oocytes and in the establishment of neural architecture in formation of the neural tube and brain. Further *in situ* analysis of *W* and *Sl* mutants, together with experiments on the behavior of embryonic neuronal and germ cells in explant and tissue culture, should provide additional insights into the function of these two interacting gene products.

Materials and methods

Mice and embryos

Mouse embryos were derived from C57BL/6Ncr × C3H/HeN MTV⁻ F2 litters. Prior to paraffin embedding, embryos were staged under the dissecting microscope by counting somites through midgestation, and when somites were no longer visible in older embryos, external markers such as state of limb development were used (Theiler, 1972). The morning of vaginal plug was considered as day 0.5 (E0.5).

In situ hybridization

In situ hybridization protocols were as described (Martin-Zanca *et al.*, 1990). Briefly, dissected embryos were fixed overnight in 4% paraformaldehyde, dehydrated with alcohols and xylenes, and embedded in paraffin. Embryos were sectioned at 5 μ m thickness and mounted on gelatin-coated slides. Slides were deparaffinized, digested with proteinase K (Boehringer Mannheim), acetylated with triethanolamine, dehydrated, hybridized overnight with 5×10^5 c.p.m. of 35 S-labeled riboprobe at 50°C, washed in hybridization buffer treated with ribonucleases at 65°C, rinsed in $2 \times$ SSC, dehydrated, and dipped in Kodak emulsion NTB-2. After drying, the slides were stored for six days at 4°C and then developed in Kodak D-19 and fixed as recommended by the manufacturer. All dark field and bright field photomicroscopy was done with a Zeiss Axiophot microscope.

All the data presented here represents examples of multiple *in situ* experiments on serial sections from independent embryos. All experiments provided similar results. Further adjacent sections have been hybridized to *trk* and *trkB* probes which hybridize transcripts of two genes encoding TK receptors that display distinct patterns of expression from those described here (not shown) (Martin-Zanca *et al.*, 1990; Klein *et al.*, 1990).

Probes

The MGF cDNA probe pBS-SK-MGF-4 has been described in detail elsewhere (Copeland *et al.*, 1990). This Bluescript vector contains a 1 kb insert at the *SalI* site of Bluescript SK (Stratagene) and was linearized with *Bam*HI to generate a T3 polymerase 35 S-labeled cDNA antisense probe encompassing the entire insert.

The *c-kit* probe is a 3.0 kb murine cDNA fragment isolated from MC-6 mast cells and cloned into Bluescript (Stratagene). Generation of this clone is described in detail elsewhere (Williams *et al.*, 1990). Linearization with *Bam*HI results in a 2.5 kb 35 S-labeled cRNA antisense probe. 35 S-labeled RNA probes were prepared by standard procedures (Krieg and Melton, 1987; Martin-Zanca *et al.*, 1990) using UTP (Amersham) as the labeled nucleotide.

Histology

Embryo sections were stained by the Dopa oxidase method for the presence of melanin containing cells (Sheehan and Hrapshak, 1980) and the pattern seen was identical to that of *c-kit* expression (not shown).

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