Development 122, 2163-2171 (1996)
Printed in Great Britain © The Company of Biologists Limited 1996
DEV4649

Expression of the *met* receptor tyrosine kinase in muscle progenitor cells in somites and limbs is absent in *Splotch* mice

Xiu-Ming Yang[1,3], Kyle Vogan[2], Philippe Gros[2] and Morag Park*[,1,2,3,4]

[1]Molecular Oncology Group, Royal Victoria Hospital, 687 Pine Avenue West, Montreal, Quebec, Canada H3A 1A1
Departments of [2]Biochemistry, [3]Medicine and [4]Oncology, McGill University, Montreal, Quebec, Canada

*Author for correspondence

SUMMARY

Hepatocyte growth factor/scatter factor (HGF/SF) stimulates proliferation, dissociation, migration and morphogenesis of cells in culture. To investigate a possible role for HGF/SF and its receptor, the Met tyrosine kinase, in embryonic development, we have analyzed their expression in mouse embryos from day 7.5 of gestation by whole-mount in situ hybridization. *Met* expression is first detected in the ventral portion of somites at day 9.25 of gestation (22 somite embryo) at the level of fore limb buds. As somites mature, *met* expression is detected in caudal somites, and is confined to the lateral and medial tips of the dermomyotome and dermomyotome/myotome respectively. In contrast, *HGF/SF* is expressed exclusively in the mesodermal core of the limb bud. As the dermomyotome elongates ventrolaterally, the *met*-expressing cells at the lateral tip appear to detach from the somite, invade the limb bud and localize at the dorsal and ventral limb sides in close proximity to *HGF/SF*-expressing cells. At later stages, both *met*- and *HGF/SF*-expressing cells appear to migrate distally and localize to the digit forming area of the developing hand plate. *Met* expression in the lateral dermomyotome and limb bud coincides with expression of *Pax-3*, a marker for migrating muscle precursor cells in the somite and limb. *Splotch-2H* and *Splotch-delayed* mice, which harbor mutations in *Pax-3*, show major disruptions in early limb muscle development. Significantly, no *met*-expressing cells were observed in the limbs of homozygous *Splotch-2H* and *Splotch-delayed* animals, whereas *HGF/SF* expression was not affected. The restricted expression of *met* to a subpopulation of *Pax-3*-expressing cells in the lateral tip of the dermomyotome, demonstrates that *met* represents a unique molecular marker for this migratory cell population. From these observations, together with the biological activities of HGF/SF, we propose that in homozygous *Splotch* embryos the failure of muscle precursors to migrate into and populate the limb bud results from a loss of *met* expression in the cells at the ventrolateral edge of the somitic dermomyotome.

Key words: Met receptor tyrosine kinase, hepatocyte growth factor/scatter factor, somites, *Splotch*, Pax-3, cell migration, dermomyotome, myoblasts, mouse

INTRODUCTION

Hepatocyte growth factor (HGF), also known as scatter factor (SF), is a multifunctional cytokine secreted by embryonal fibroblasts with activities on epithelial and endothelial cells. HGF/SF promotes proliferation, dissociation and motility of epithelial cells and endothelial cells in culture (Stoker et al., 1987; Grant et al., 1993), and stimulates the invasion of carcinoma cells (Weidner et al., 1990) and the branching morphogenesis of tubular epithelial cells (Montesano et al., 1991). HGF/SF is essential for embryonic development (Schmidt et al., 1995; Uehara et al., 1995), is a potent angiogenic factor (Grant et al., 1993) and is involved in organ regeneration (Matsumoto and Nakamura, 1992). The high affinity receptor for HGF/SF has been identified as the product of the *met* proto-oncogene, which encodes a receptor tyrosine kinase (RTK) (Bottaro et al., 1991; Naldini et al., 1991). Activation of the Met RTK is sufficient to mediate the pleiotropic biological activities of HGF/SF in culture (Weidner et al., 1993; Komada and Kitamura, 1993; Zhu et al., 1994), suggesting that the Met RTK mediates the physiological responses to HGF/SF in vivo.

The *met* and *HGF/SF* genes are expressed in the majority of adult and embryonic tissues (Chan et al., 1988; Iyer et al., 1990; Tashiro et al., 1990; Sonnenberg et al., 1993), with *met* showing highest levels of expression in epithelia that line tubules and ducts (Di Renzo et al., 1991; Yang and Park, 1995). During embryogenesis, *HGF/SF* is expressed in mesenchymal cells in close proximity to *met*-expressing epithelia (Sonnenberg et al., 1993), supporting a role for Met and HGF/SF in mesenchymal/epithelial cell interactions important for organogenesis during embryonic development. However, while mice lacking a functional HGF/SF protein die in utero with developmental defects in liver and placenta, they fail to show defects in other epithelial organs (Schmidt et al., 1995; Uehara et al., 1995). Moreover, the observation that the ectopic expression of *HGF/SF* in chick embryos at gastrulation induces the formation of neuronal tissues and markers (Stern et al., 1990; Streit et al., 1995), plus the ability of HGF/SF to stimulate dis-

sociation, motility and invasion of epithelia, have also suggested a role for HGF/SF and its receptor, Met, during early murine development, at stages prior to organogenesis.

To investigate the possible function of the Met RTK and HGF/SF during early development, we have examined the temporal and spatial expression pattern of *met* and *HGF/SF* in mouse embryos from day 7.5 of gestation by whole-mount in situ hybridization. We demonstrate that *HGF/SF* is expressed in the mesodermal core of the developing limb buds, whereas the *met* RTK is expressed in somites in two distinct domains; one localized to the lateral tip of the dermomyotome marking a population of cells that will migrate into the limb bud, and a second localized to the medial tip of the dermomyotome/myotome. Moreover, we show that the cell population that expresses *met* in the lateral tip of the dermomyotome and in the limb is missing in the *Pax-3* mutants *Splotch-2H* (*Sp^{2H}*) and *Splotch-delayed* (*Sp^d*) which lack limb bud musculature, whereas *HGF/SF* expression is apparently normal in these mutant animals. These results identify *met* as a unique marker for limb muscle progenitor cells, and suggests a possible interaction between *Pax-3* and *met* during the development of this specific cell population at the ventrolateral edge of the somitic dermomyotome.

MATERIALS AND METHODS

Mouse embryos and staging

Embryos for in situ hybridizations were dissected from the F_1 female mice of a B6 and C3H cross. *Sp^{2H}*/+ mice on a mixed C3H/HeH and 101/H background (MRC Radiobiology Unit, Harwell, England) and *Sp^d*/+ on a C57BL/6J background (The Jackson Laboratory, Bar Harbor, ME) were maintained in our animal facility by brother-sister mating. Embryos were generated using timed matings, with the morning of the vaginal plug designated E0.5. To allow a more precise system of staging, embryos were viewed under a dissecting microscope and classified according to their somite numbers (Rugh, 1990). Embryos with 20-25 somites are assigned to 9.25 days post coitium (d.p.c.); those with 26-30 somites, 9.5 d.p.c.; 30-36 somites, 9.75 d.p.c.; 37-45 somites, 10 d.p.c.; 45-50 somites, 10.5 d.p.c.

Genotyping of *Splotch* embryos

To determine the genotypes of the embryos used in this study, the yolk sac and amniotic membrane was removed from each embryo and was used to prepare genomic DNA, according to a modified protocol using proteinase K digestion, serial phenol and chloroform extractions, and ethanol precipitation. Oligonucleotide primers P_3B (5′ CCTCGGTAAGCTTCGCCCTCTG 3′) and P_3E (5′ CAGCGCAGGAGCAGAACCACCTTC 3′), flanking the 32 bp genomic region deleted from the *Pax-3* gene in *Sp^{2H}* mice (Epstein et al., 1991) were used to amplify a 127 bp fragment specific to the wild-type *Pax-3* allele, and a 95 bp fragment from the *Sp^{2H}* mutant *Pax-3* allele. For each sample, 10 ng of genomic DNA was subjected to 30 cycles of amplification consisting of denaturing (94°C, 40 seconds), annealing (65°C, 40 seconds), and extension (72°C, 40 seconds), followed by an additional extension at 72°C for 10 minutes. To type the offspring of crosses between *Sp^d* mice, primers P_3K (5′ TTGGCCAGGGCCGAGTCAAC 3′) and P_3L (5′ CTGTCTCCTGGTACCTGCAC 3′) were used to amplify a 183 bp *Pax-3* genomic DNA fragment that includes the guanine at position 421 which is mutated to cytosine in the *Sp^d* mutant (Vogan et al., 1993). The amplified products were radiolabeled by including [α-^{32}P]dATP in the PCR reaction mix, and were then analyzed by single strand conformational polymorphism analysis, essentially as described by Orita et al. (1989).

Plasmids and riboprobe synthesis

As probes for hybridization studies, an 0.5 kb *Eco*RI fragment (p*met* SC1) (Yang and Park, 1993) derived from the 5′-portion of the murine *met* cDNA (pcD) (Iyer et al., 1990), or a 1.5 kb *Eco*RI fragment (p*met* SC2) located at the 3′ portion of p*met* SC1 were subcloned into the pBluescript KSII$^+$ vector (Stratagene). The mouse *HGF/SF* cDNA was kindly provided by Dr Walter Birchmeier. Non-radioactive antisense and sense riboprobes were synthesized by in vitro transcription using digoxigenin-UTP following the manufacturers instructions (Boehringer Mannheim). The *met* antisense probes used in these experiments were as follows: *met*1, corresponding to bp 479-720 in the murine cDNA; *met*2, bp 268-720; *met*3, bp 1876-2298 (Chan et al., 1988). *HGF/SF* antisense probes were as described by Sonnenberg et al. (1993). The *myogenin*, *MyoD* antisense probes (Sassoon et al., 1989), and *Pax-3* probes (Goulding et al., 1991), have been described previously.

Whole-mount in situ hybridization

Whole-mount in situ hybridization was performed according to a revised protocol kindly provided by Dr Janet Rossant (Conlon and Rossant, 1992). Embryos were dissected free in DEPC (diethyl pyrocarbonate) treated phosphate-buffered saline (PBS) and fixed in 4% paraformaldehyde in PBS for 2 hours at 4°C with rocking. After washing three times, embryos were incubated for 1 hour with cold PBS containing 0.1% Tween 20 (PBT) at 4°C, then stored in 100% methanol at −20°C for less than a month. For hybridization, embryos were incubated at 65°C overnight in hybridization buffer with 0.4 μg/ml digoxigenin-labeled RNA probes.

To determine the hybridization at the cellular level, stained embryos were re-fixed with 4% paraformaldehyde in PBS for 20 minutes at room temperature, dehydrated with a TBST (137 mM NaCl, 25 mM Tris-HCl pH 7.6, 3 mm KCl, 0.1% Tween 20)/methanol series (30%, 50%, 75%, 95% and 100% twice, 5 minutes each), and incubated with 100% methanol twice for 1 hour each, xylene twice for 1 hour each, 1:1 xylene/paraffin twice for 30 minutes each at 60°C and paraffin three times for 1 hour each at 60°C. Embryos were then embedded in wax and sectioned at 7 μm. Sections were dewaxed for 2× 15 minutes in xylene and rehydrated in an ethanol series (100% ×2; 95%, 70%, 50% and water, 5 minutes each), followed by counterstaining with 0.002% eosin solution, and then mounted with Permount.

RNA isolation and RT-PCR

RNA was isolated from frozen sections prepared from mouse embryos of 11 d.p.c, and first strand cDNA was synthesized from 2 μg of total RNA as described previously (Vogan et al., 1993). *Pax-3*-, *met*-, *HGF/SF*-, and *GAPDH*- (*glyceraldehyde-3-phosphate dehyrogenase*) specific cDNA fragments were amplified by PCR from 50 ng of template using the following sets of primers under the reaction conditions outlined below: *Pax-3* (sense: 5′ ^{538}GTTGCGTCTCTAAGATCCTG^{558} 3′; antisense: 5′ ^{719}GCGTCCTTGAGCAATTTGTC^{699} 3′; 25 cycles, annealing: 62°C), *met* (sense: 5′ ^{272}AGATGAACGTGAACATGAAG^{291} 3′; antisense:5′ ^{566}CTAATGAGTTGATCATCATAG^{546} 3′; 30 cycles, annealing: 52°C), *HGF/SF* (sense: 5′ ^{484}CCATGAATTTGACCTCTATG^{503} 3′; antisense: 5′ ^{760}ACTGAGGAATGTCACAGACT^{741} 3′; 25 cycles, annealing: 55°C), and *GAPDH* (sense: 5′ ^{376}CCATGGAGAAGGCTGGGG^{393} 3′; antisense: 5′ ^{570}CAAAGTTGTCATGGATGACC^{551} 3′; 20 cycles, annealing: 62°C). Amplification products were radiolabeled by including 2.5 μCi of [α-^{32}P]dATP in each reaction, and products were resolved on 12% polyacrylamide gels and exposed to X-ray film for autoradiography.

RESULTS

Localization of the *met* RTK transcripts to developing somites

To investigate the involvement of the Met RTK and its ligand

HGF/SF in early embryonic development, we have studied the expression of these genes in mouse embryos from day 7.5 of gestation by whole-mount in situ hybridization. A comparison of embryos hybridized with digoxigenin-labelled antisense (Fig. 1A-C) and sense *met* cRNA probes (Fig. 1D) revealed that *met* transcripts are not detected at 7 or 8 days post coitum (d.p.c.) and are first identified in the ventral portion of somites at 9.25-9.5 d.p.c. (20-30 somite embryos; Fig. 1A). Somites first appear at day 8 of gestation and rapidly partition into the sclerotome, which will give rise to skeletal structures and into the dermomyotome, which will subsequently give rise to myotome and dermatome (reviewed by Emerson, 1993). Somites mature in a rostrocaudal direction where newly formed undifferentiated somites and fully differentiated somites can be found in the same embryo in caudal and cranial regions respectively.

Met expression was localized to two discrete domains within somites. By 9.25-9.5 d.p.c., *met* was first expressed in the ventral portion of rostral somites at the level where the fore limb buds are formed (Fig. 1A). *Met* expression progressed to caudal somites and by 9.75 d.p.c. *met* was expressed in the ventral region of thoracic somites and in somites at the level of the hind limb buds (Fig. 1B) and in the ventral region of the most caudal somites by 10 d.p.c. (Fig. 1C). A second domain of *met* expression was observed in the dorsal portion of the somites. A low level of expression was first identified in the dorsal aspect of somites rostal to the fore limb bud at 9.5 d.p.c. (Fig. 1A). *Met* expression increased and progressed to more caudal somites such that *met* was expressed in both the ventral and dorsal tip of the same somite (Fig. 1C). From a comparison of 10 embryos at 9.75 d.p.c. (Fig. 1B), *met* expression within a given somite begins in the dorsal portion several hours later than in the ventral portion of the same somite. In addition, at 9.75 d.p.c., *met* is expressed in the dorsal tip of somites rostral to the fore limb bud; somites that do not appear to express *met* in their ventral domain (Fig. 1B,C).

Location of *met*-expressing cells in the developing limb buds

At 9.75 d.p.c. (Fig. 1B) and 10 d.p.c. (Fig. 1C,E,I), *met* transcripts were no longer detected in the ventral portion of somites at the level of the fore (Fig. 1I) and hind limb buds (Fig. 1 C,E). Instead, at 9.75 d.p.c., *met*-expressing cells were detected as a diffuse signal localized to the mesodermal core at the base of the developing fore limb bud (Fig. 1B,I) and hind limb bud at 10 d.p.c. (Fig. 1C,E). Expression increased and progressed distally by 11.0 d.p.c. where it was concentrated in the distal half of the limb bud over the dorsal (data not shown) and ventral limb sides (Fig. 1J). Expression decreased by 11.5 d.p.c. and localized to the digit forming area in the developing hand plate (Fig. 1K,L). A similar localization of *met*-expressing cells was observed in the hind limbs lagging 0.5 days behind the fore limbs (Fig. 1E).

Expression of *HGF/SF* in the developing limb buds

To establish whether Met/HGF/SF interactions occur in the developing limb bud, we localized *HGF/SF* expression by whole-mount in situ hybridization. A diffuse signal for *HGF/SF* was detected at 9.75 d.p.c. (Fig. 1F). However, by early 10 d.p.c. (Fig. 1G,M), when compared with embryos hybridized with a sense riboprobe (Fig. 1H), *HGF/SF*-specific transcripts were concentrated to the mesodermal core of the limb bud (Fig. 1G) and were localized over the dorsal (Fig. 1M) and ventral (data not shown) limb sides. By 11-11.75 d.p.c., *HGF/SF*-expressing cells progressed distally and become concentrated ventrally to a subapical domain in the distal half of the fore limb (Fig. 1N) then subsequently to the digit forming area of the developing hand plate (Fig. 1P). The progression of HGF/SF-expressing cells to the distal half of the limb buds correlated spatially and temporally with *met*-expressing cells in the limb bud at this stage (Fig 1J-L). The amplification of *HGF/SF*-specific cDNA fragments from head, trunk, tail and limb sections of embryos by polymerase chain reaction further demonstrated that only basal levels of *HGF/SF* were present in head, trunk and tail sections, whereas high levels of *HGF/SF* were observed in the limbs (Fig. 5, left panel).

Met expression overlaps with *Pax-3*, a marker for migratory muscle precursor cells

Somites give rise to several myogenic cell populations, one derived from migratory muscle precursor cells at the lateral tip of somitic dermomyotome at the level of the limb buds, a second originates from the lateral tip of the somitic dermomyotome of the trunk from which abdominal muscles derive, and a third derives from the medial half of the dermomyotome and gives rise to axial, back and intercostal muscle (Christ et al., 1983; Ordahl and Le Douarin, 1992). Expression of *met* in the lateral tip of somites adjacent to the limb buds (Fig. 1A-C) and expression of *HGF/SF* (Fig. 1J-L) and *met* (Fig. 1N-P) in adjacent regions of the limb buds, suggest a potential role for *met* and *HGF/SF* in the migration of muscle precursor cells from the somite. In order to establish if *met* expression correlates with the location of muscle precursor cells in the developing embryo, *met* expression was compared with that of *Pax-3* and of myogenic regulatory factors *myogenin* and *MyoD*.

Pax-3, a member of the Pax family of transcriptional regulators, is expressed in the somitic dermomyotome and marks a population of cells that migrate from the lateral dermomyotome into the limbs (Bober et al., 1994; Goulding et al., 1994; Williams and Ordahl, 1994). To identify the cell types in somites that express *met* transcripts following whole-mount in situ hybridization, embryos at various stages (9.25-10.5 d.p.c.) were postfixed in paraformaldehyde, sectioned and compared with sections from embryos hybridized with *Pax-3*. At 9.75 d.p.c., *Pax-3* is expressed in cells throughout the dermomyotome (Fig. 2A; Bober et al., 1994; Goulding et al., 1994) whereas *met* expression was concentrated in cells at the lateral tip of the dermomyotome (Fig. 2C), that overlap with a population of *Pax-3*-expressing cells (Fig. 2A).

At 9.75 d.p.c., *met*- and *Pax-3*-expressing cells localized to the lateral tip of the dermomyotome, appeared to detach from the somite domain, accumulate in clusters at the body-limb junction (Fig. 2D and B respectively), and then further invade the limb (Fig. 2E). The expression of *met* in the limb buds parallels that of *Pax-3*-expressing cells (Fig. 2B) and by 10.5 d.p.c., both the *met*- (Fig. 3B) and *Pax-3*- (Fig. 3C) expressing cell populations have segregated into dorsal and ventral limb regions. At this stage, *met*-expressing cells (Fig. 3B) overlap with the dorsal and ventral expression domains of *MyoD* (Fig. 3D and data not shown) and *myogenin* (Fig. 3E and data not shown), which later give rise to limb muscles (Sassoon et al., 1989; Ott et al., 1991). However, *met*-expressing cells (Fig.

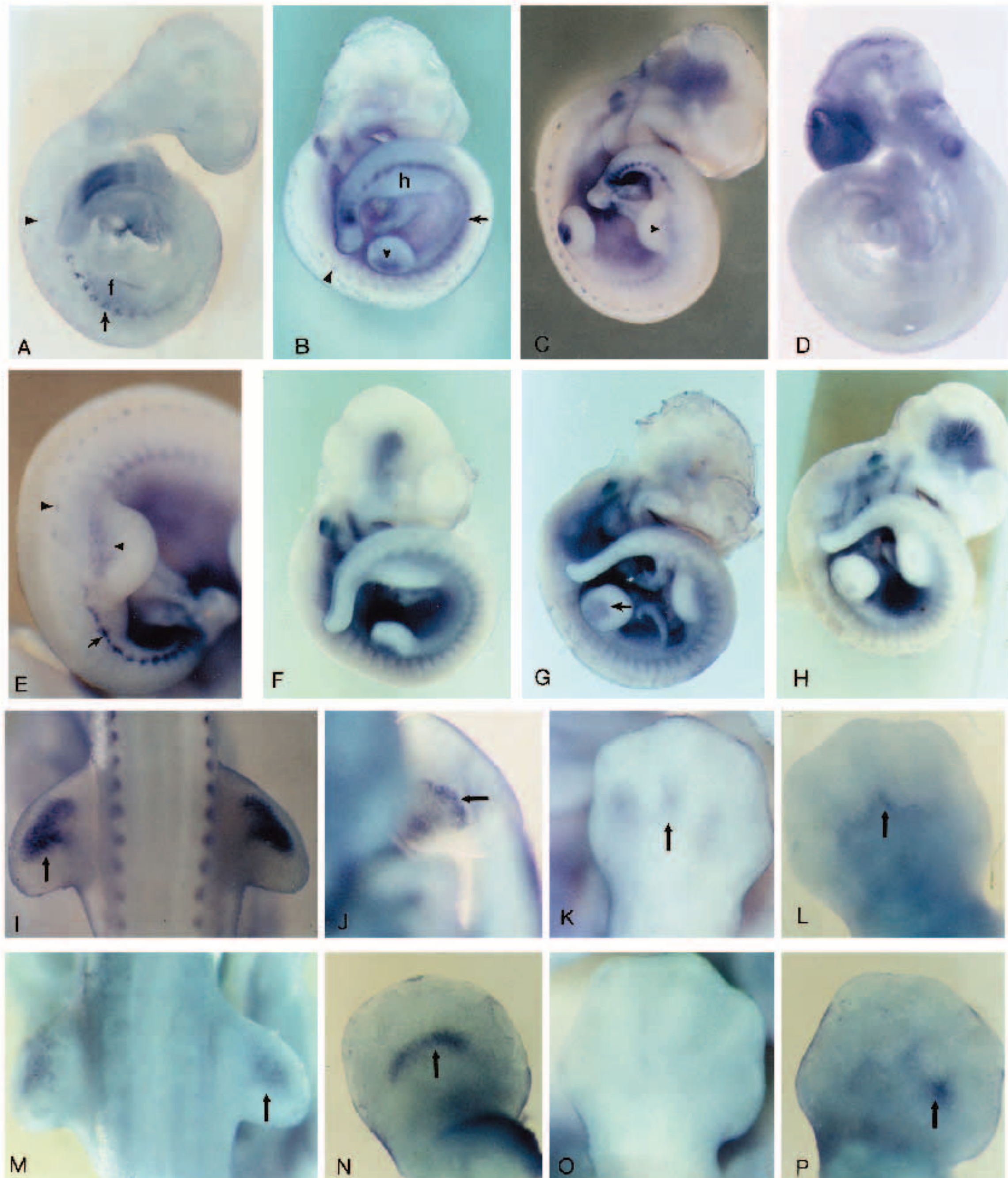

Fig. 1. Expression of *met* and *HGF/SF* during somite and limb development. Lateral views of whole-mount in situ hybridization with digoxigenin labelled antisense *met* (A-C,E,I-L), *HGF/SF* (F,G,M-P), sense *met* (D) or *HGF/SF* (H) cRNA probes. Specific hybrids are visualised as a blue precipitate. In embryos hybridized with sense probes (D,H), backgroud staining is detected, mostly in the head, whereas (A) at 9.5 d.p.c. (30 somites), specific *met* transcripts are detected in the ventral portion (arrow) of somites at the level of the fore limb buds (f), in the dorsal tip of a few rostral somites (arrowhead), as well as in the tail bud. (B) At 9.75 d.p.c. (36 somites), *met* is no longer expressed in the ventral tip of somites at the fore limb, but is expressed in the fore limb bud (small arrowhead), the ventral region of thoracic (arrow), hind limb (h) and tail somites, and in the dorsal tip of somites (large arrowhead). (C) At 10 d.p.c. (45 somites), *met* transcripts are no longer observed in the ventral tip of somites at the level of the hind limb, but are concentrated in the hind limb bud (arrowhead). E is an enlargement of C showing *met* expression in the dorsal (large arrowhead) and ventral (arrow) tips of somites and *met* expression in the hind limb bud (small arrowhead). When compared with control sense *HGF/SF* cRNA probe (H), at 9.75 d.p.c., a diffuse signal for *HGF/SF* is observed throughout the whole embryo (F) which becomes concentrated in the fore limbs at 10 d.p.c. (45 somites) (G) (arrow). At 10 d.p.c., arrows indicate *met* (I) and *HGF/SF* (M) expression domains in the fore limb bud. At 11 d.p.c. (53 somites), expression of *met* (J) (arrow) and *HGF/SF* (N) (arrow) progresses distally and colocalizes on the ventral limb side. By 11.5 d.p.c. (58 somites), *met* is expressed in the dorsal (K, arrow) and ventral (L, arrow) sides of the fore hand plate at the digit forming area, whereas *HGF/SF* expression is not detected on the dorsal side (O) and appears to colocalize with the *met* expression domain on the ventral side (P, arrow) of the developing hand plate.

3B) were predominantly concentrated distal to that of *MyoD* (Fig. 3D) or *myogenin* (Fig. 3E) and localized adjacent to *HGF/SF*-expressing mesenchymal cells (Fig. 3A). Furthermore, from whole-mount in situ hybridizations at 10. 5 d.p.c. (Fig. 1E) and from transverse sections at the level of the fore limb bud at 9.75-10.75 d.p.c., we have also localized *met* expression to the dorsal/medial tip of the dermomyotome/early myotome (Fig. 2E).

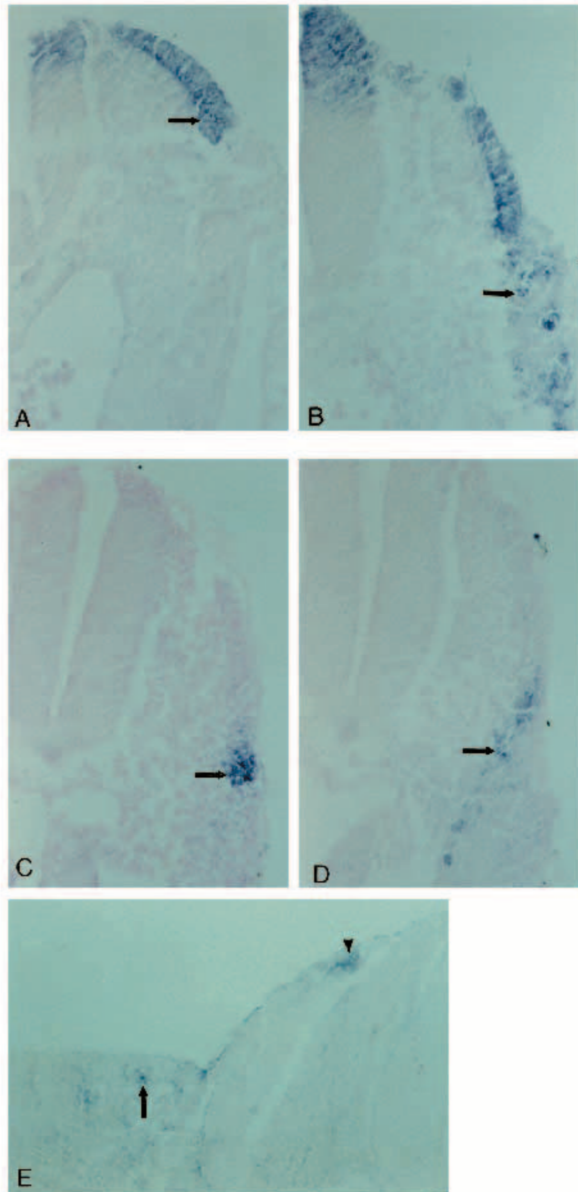

Fig. 2. Expression of *met* and *Pax-3* in the dermomyotome (transverse sections obtained from whole-mount in situ embryos). Sections at fore limb level at 9.75 d.p.c. (36 somites) hybridized with *Pax-3* (A,B) or *met* (C,D) antisense probes. *Pax-3* is expressed throughout the dermomyotome, but is concentrated at the lateral tip (A, arrow). *Met* is expressed in cells located at the ventrolateral tip of the dermomyotome (C, arrow), colocalizing with a subpoulation of *Pax-3*-expressing cells. All *met*-expressing cells (D, arrow) and a subpopulation of *Pax-3*-expressing cells (B, arrow) at the ventrolateral tip of the dermomyotome, appear to migrate towards the base of the fore limb bud. At 10 d.p.c. (40 somites), *met*-expressing cells are found in the fore limb buds (arrow) and *met* expression is also detected in the dorsal-medial tip (arrowhead) of the dermomyotome/early myotome (E).

Met-expressing cells are absent in the developing limbs of *Splotch-2H* embryos

The coexpression of *met* and *Pax-3* in the lateral dermomyotome and the apparent progression of *met*-expressing cells into the limb buds, suggests a potential role for *met* in the development of limb musculature. In order to investigate whether *met* is expressed in the myoblast precursor cells of the limb buds, *met* expression was analyzed in Sp^{2H} mutant mice. In Sp^{2H} mice, which carry a mutant allele of the *Pax-3* gene (Epstein et al., 1991), myoblast precursor cells expressing *Pax-3* fail to migrate into the limb (Bober et al., 1994; Goulding et al., 1994). Thus, expression of myogenic genes is not detected in the limbs (Bober et al., 1994) and as a consequence, Sp^{2H} mice are devoid of limb musculature (Franz, 1993; Franz et al., 1993).

To examine *met* and *HGF/SF* expression in Sp^{2H} embryos, a series of whole-mount in situ hybridizations were performed between 9 and 11.5 d.p.c. In homozygous Sp^{2H} embryos, distinct differences in the distribution of *met* transcripts were observed (Fig. 4G-I) when compared to the normal (Fig. 4A-C) and heterozygote (Fig. 4D-F) embryos, while those of *HGF/SF* remained normal (Fig. 4J). No positive cells hybridizing with *met* were observed in the ventral portion of somites at 9.75-11.5 d.p.c (Fig. 4G,H) (the development of Sp^{2H} embryos is delayed approximately half a day compared to C3H/B6 F1 embryos). Significantly, no *met*-expressing cells that leave the somites or populate the limbs between 9.5-11.5 d.p.c. were observed in homozygous Sp^{2H} embryos (Fig. 4H,I). Moreover, in homozygous Sp^{2H} animals, *met* expression in the dorsal portion of the dermomyotome is severly decreased and/or dispersed in thoracic somites (Fig. 4G,H), but is maintained in somites rostral and caudal to the fore and hind limbs respectively. A second *Splotch* allele, *Splotch-delayed*, which contains a point mutation in the paired domain of *Pax-3* (Vogan et al., 1993) with a similar defect in limb muscle (Franz, 1993), shows a pattern of *met* and *HGF/SF* expression similar to Sp^{2H} (data not shown). RT-PCR analyses using RNA prepared from wild-type and mutant limbs confirm the observations that *HGF/SF* expression is not affected in mutant embryos (Fig. 5 right panel), whereas *met* expression is severely decreased.

DISCUSSION

The *met* RTK is expressed in migratory cells of the lateral dermomyotome; prospective myogenic precursors of the limbs

In this paper, we present data showing that the migratory precursor cells of limb bud muscle express the *met* RTK, whereas *HGF/SF* is expressed in the mesodermal core of the developing limb buds. Whole-mount in situ hybridizations on mouse embryos indicated that *met* is first expressed in the ventrolateral tip of the dermomyotome in somites at the level of the fore limb buds at 9.25 d.p.c. and at the level of the hind limb bud by 9.75 d.p.c (Fig. 1A,B). As somites mature, the

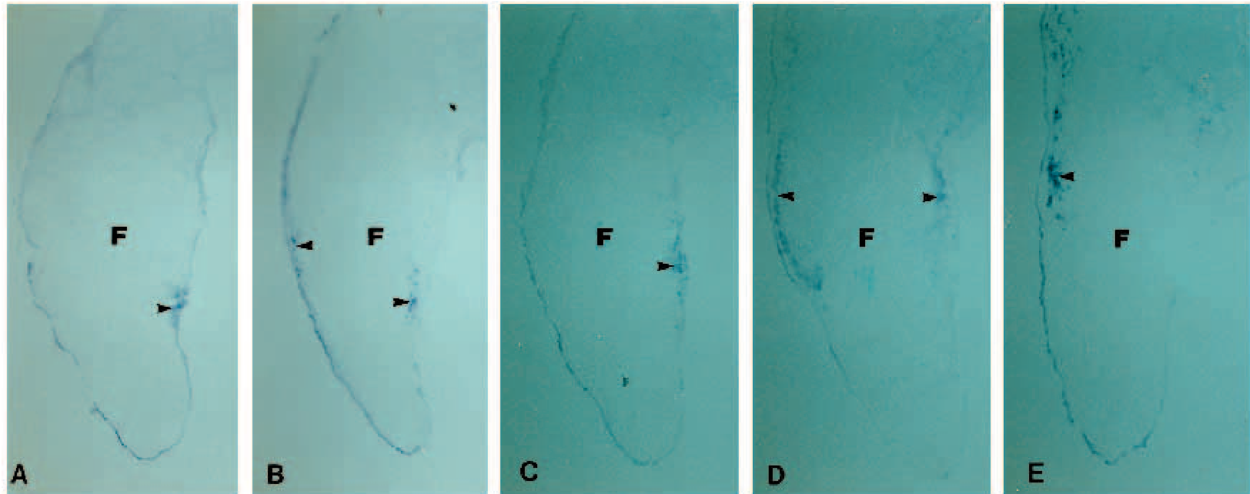

Fig. 3. Expression of *HGF/SF*, *met*, *Pax-3,* and myogenic factors *MyoD* and *myogenin,* in the fore limb of 10.5 d.p.c. embryos (46-50 somites). Transverse sections at fore limb level at 10.5 d.p.c. hybridised with *HGF/SF* (A), *met* (B), *Pax-3* (C), *MyoD* (D) and *myogenin* (E). *Met*-expressing cells (B) are localized in close proximity with but central to the *HGF/SF*-expressing cells (A). *Met*-expressing cells partially overlap with *Pax-3*- (C) and *MyoD*- (D) expressing cells, but are predomonantly concentrated distal to the *MyoD* (D) and *myogenin* (E) expression domains.

met-expressing cells in the lateral dermomyotome of 5-6 somites at the level of the fore and hind limb buds begin to invade adjacent limbs (Figs 1B,C,E, 2C,D). At later stages, *met*-expressing cells become organized into ventral and dorsal sides of the limb bud (Figs 1I-L, 3B) and appear to localize with muscle precursor cells that migrate into the mesodermal core of the fore and hind limb buds as identified from chick/quail grafting experiments (Christ et al., 1977, 1983; Ordahl and Le Douarin, 1992). As myogenic factors (*MyoD* and *myogenin*) begin to be expressed in the limb (Fig. 3D,E) and muscle differentiation occurs, the *met* signal disappears gradually from this region and is localized to a subapical domain on the dorsal and ventral limb side (Figs 1J-L, 3B) in close proximity to *HGF/SF*-expressing mesenchyme (Figs 1N,P, 3A).

This evidence suggests that the *met*-expressing cells from the lateral dermomyotome invade the limbs and correspond to muscle precursor cells. This is further supported by the observation that cells expressing *met*, in both the lateral tip of the dermomyotome (Fig. 2A,C) and in the limb bud (Fig. 3B), overlap with cells expressing *Pax-3*; a molecular marker for migratory precursor cells of limb muscle (Figs 2B,D, 3C) (Bober et al., 1994; Goulding et al., 1994; Williams and Ordahl, 1994). Moreover, evidence for *met* expression in myogenic precursor cells, rather than the induction of *met* expression in the limb mesenchyme, is provided by the observation that no *met*-expressing cells are localized in the limbs of *Splotch-2H* (Fig. 4G-I) or *Splotch-delayed* mutant mice (data not shown), which have mutations in *Pax-3* (Epstein et al., 1991; Vogan et al., 1993) and are devoid of limb musculature (Franz, 1993; Franz et al., 1993). Interestingly, *met* is also expressed in muscle derivatives which differentiate in cultures of pluripotent P19 teratocarcinoma cells (Yang and Park, 1993). However, *met* is not expressed in terminally differentiated muscles in the limb bud (Fig. 3B) or in skeletal muscles in the adult animal (Yang and Park, 1995). Thus, the expression of the *met* RTK appears restricted to proliferating and/or migrating muscle precursor cells.

Met-expressing cells align adjacent to *HGF/SF*-expressing cells in the limb bud

HGF/SF affects cultured epithelia by stimulating cell proliferation, breakdown of cell junctions, increased cell motility and cell scatter, which are coupled with the ability of HGF/SF to induce an epithelial-mesenchymal transition of cells in culture (Stoker et al., 1987; Weidner et al., 1990). The spatiotemporal distribution of *HGF/SF*-expressing cells in the developing limb (Fig. 1G,M-P), their organization into dorsal and ventral cell masses (Fig. 3A), and their subsequent localization to a subapical domain in the digit forming area to which *met*-expressing cells concentrate (Fig. 1N-P), is in agreement with recent reports demonstrating expression of HGF/SF in the murine (Bladt et al., 1995; Takebayashi et al., 1995) and chicken (Myokai et al., 1995) limb bud and supports a role for *HGF/SF* in the localization of migratory *met*-expressing cells to the limb bud. Although it is formally possible that *met* expression is induced in cells in the limb bud in the vicinity of the *HGF/SF*-expressing cells, the observation that the Sp^{2H} and Sp^{d} mutants express *HGF/SF* in the limb buds at apparently wild-type levels (Figs 4J, 5), whereas no *met*-expressing cells are observed in the limb buds by in situ hybridization (Fig. 4G-I), make this unlikely. However, we cannot exclude that the low level of *met* expression detected by RT-PCR in the limb buds of *Splotch-2H* mutant mice (Fig. 5) corresponds to de novo *met* expression in the limb bud. While this manuscript was in preparation, Bladt et al. (1995), reported an absence of limb musculature in $Met^{-/-}$ and $HGF/SF^{-/-}$ mice, demonstrating a direct role for the Met RTK and its ligand HGF/SF in the formation of limb bud musculature. These data are in agreement with our data and support a role for HGF/SF in stimulating the transition of epithelial, *met*-expressing lateral dermomyotome cells, into migratory, proliferating mesenchymal cells that invade the limbs and give rise to muscle.

In addition to the migration of *met*-expressing cells from the somites, both *met*- and *HGF/SF*-expressing cells within the limb bud appear to migrate to the base of the digit forming area of the hand plate (Fig. 1J-L,N-P). The signal for the migration

Fig. 4. *Met* expression is absent in the limb buds of the *Splotch-2H* mutant. In control (+/+) embryos at 11 d.p.c. (48 somites), *met* expression is detected in the mesodermal core of the developing fore and hind limb buds (A-C), in the ventral tip of thoracic and caudal somites (A,B), and in the dorsal tip of nearly all somites (A). In a heterozygous embryo (+/–) with spina bifida (D-F), the *met* expression pattern is similar to that of +/+ embryos. In mutant embryos (Sp^{2H}/Sp^{2H}) with exencephaly and spina bifida (G-I), *met* expression is no longer detected in the fore (G,H) and hind limb buds (G,I) or in the ventral tip of thoracic somites, and is severely decreased in the dorsal tip of thoracic somites (G,H). Expression of *HGF/SF* in the dorsal and ventral sides of the developing fore and hind limb buds is normal in (Sp^{2H}/Sp^{2H}) mutant embryos (J, arrowhead). f, fore limb.

of the *HGF/SF*-expressing cells is unknown. However, Takebayashi et al. (1995) have recently demonstrated expression of *HGF/SF* in the digit forming area of the hand plate and although they failed to detect *met* expression, they demonstrated that Met/HGF/SF interactions are involved in cell motility, proliferation and proteoglycan synthesis of chondrocytes in culture. Thus the *met*-expressing cells localized in the digit forming area of the developing hand plate (Fig. 1K,L) may mark precursor cells for chondrocytes.

Met expression defines two distinct dermomyotomal domains

During embryogenesis, cells located in the lateral portion of

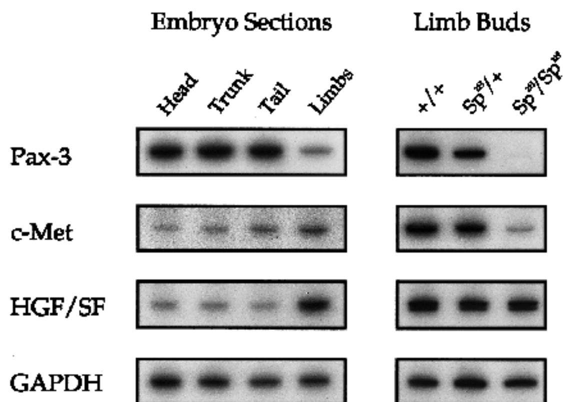

Fig. 5. Correlation of *met* and *HGF/SF* expression in normal and *Splotch-2H* embryos. First strand cDNA was synthesized from total RNA isolated from the head, trunk, tail and limb bud segments of 11 d.p.c. normal embryos (left panel), or from 11 d.p.c. limb buds of normal (+/+), heterozygous ($Sp^{2H}/+$) and mutant (Sp^{2H}/Sp^{2H}) embryos (left panel). Specific segments of *Pax-3*, *met* and *HGF/SF* cDNA were amplified by polymerase chain reaction using specific oligonucleotide primers (see Materials and Methods). A PCR amplification using oligonucleotide primers specific for *GAPDH* was used to control for the amount of cDNA in each reaction.

the somitic dermomyotome migrate and give rise to abdominal muscle and limb muscle, whereas cells in the dorsal medial quadrant of the somite give rise to myotome, which forms the axial, back and intercostal muscles and dermotome, which gives rise to cells of the dermis (Christ et al., 1983; Ordahl and LeDouarin, 1992; Williams and Ordahl, 1994). In addition to its expression in cells at the ventrolateral tip of the dermomyotome, *met* is also expressed in cells at the dorsal-medial tip of the dermomyotome/early myotome (Fig. 2E). These cells are the first cells in the medial portion of the somite to activate expression of muscle differentiation markers and express *MyoD* (Williams and Ordahl, 1994). Although the exact function of these cells is not known, the expression of *MyoD* is localized in the myotome at later stages (Sassoon et al., 1989; Williams and Ordahl, 1994). This has suggested that there are different cell populations within the myotome; one is first recognizable as a cell population at the anteriomedial edge of the somite (Christ et al., 1978; Kaehn et al., 1988) that expresses *myf-5* (Ott et al., 1991), a second may derive from a *Pax-3*-expressing cell population at the cranial edge of the medial half of the dermomyotome (Williams and Ordahl, 1994), and a third may derive from the dorsal-medial tip of the dermomyotome/myotome which expresses *met* and *MyoD* (Pownall and Emerson, 1991; Williams and Ordahl, 1994). These results, together with the observation that *met* is expressed in muscle of the intercostal region of embryos at 11-15 d.p.c. (Sonnenberg et al., 1993), suggest that *met*-expressing cells of the dorsal-medial tip of the dermomyotome/early myotome may be involved in the formation of intercostal and axial musculature. Although axial muscle does not appear to be affected in the *met* $RTK^{-/-}$ mice (Bladt et al., 1995), there may be functional redundancy in this cell population. For example in *Myo* $D^{-/-}$ or *Myf* $5^{-/-}$ mice there is no defect in axial musculature and this is only observed in *Myf 5/Myo* $D^{-/-}$ mice (Braun et al., 1992; Rudnicki et al., 1992, 1993).

Met is aberrantly expressed in *Splotch-2H* and *Splotch-delayed* embryos

From studies of Sp^{2H} and Sp^{d} mutant embryos, the absence of a functional *Pax-3* protein has profound effects on *met* expression. *met*-expressing cells are absent from limb buds and no *met*-expressing cells are detected in the ventral tip of somites, thus supporting a role for *Pax-3* in directly or indirectly regulating *met* expression in these domains, or in specifying the *met*-expressing cells in the lateral dermomyotome. In contrast, *met* expression is apparently retained at wild-type levels in the dorsal-medial tip of somites, rostral to the developing fore limb bud and caudal to the hind limb bud, but is severly decreased in the dorsal tip of somites within the trunk region (Fig. 4G,H). The dermomyotome of somites at the trunk level is disorganized in Sp^{2H} embryos (Franz, 1993; Franz et al., 1993; Bober et al., 1994), which may directly or indirectly affect *met* expression, or disperse *met*-expressing cells such that they are no longer detected by whole-mount in situ hybridization. Interestingly, although facial and body wall muscles develop almost normally in the Sp^{2H} mutant, a reduction in axial muscles in a rostral-caudal gradient has been observed (Franz, 1993; Franz et al., 1993) which is therefore concurrent with the decrease in *met*-expressing cells in the dorsal tip of the dermomyotome in the Sp^{2H} mutant (Fig. 4G,H).

Several lines of evidence suggest that Pax-3 may be involved in maintaining muscle precursor cells in an undifferentiated proliferative state (Galili et al., 1993; Epstein et al., 1995). This plus the observation, that in the $met^{-/-}$ mice (Bladt et al., 1995), *Pax-3* expression appears normal, yet no limb bud musculature is formed, supports a role for the Met RTK and HGF/SF in the dissociation and migration of limb muscle precursor cells from the somite. Therefore in *Splotch* embryos, the failure of muscle precursors to leave the somites and populate the limbs may result directly from the failure of mutant forms of Pax-3 to induce *Met* expression in the presumptive cells at the ventrolateral edge of the dermomyotome. The possibility that Pax-3 acts directly to activate *Met* expression in this cell population remains an interesting avenue for further study.

The authors would like to thank Dr A. Peterson and especially I. Tretjakoff for mating of B6 and C3H mice, Dr S-P Yee for the inspiration to pursue these studies and for his critical reading of the manuscript, Dr E. Daniels for useful discussions, Dr A. Karapolis for use of his microscope, and Drs M. Rudnicki, S.-P. Yee and W. Birchmeier for probes. We are indebted to Dr B.F. Hales and T.R.S. Ozolins for their technical expertise and experimental help. This research was supported by operating grants to M. P. from the National Cancer Institute of Canada with money from the Canadian Cancer Society and from the Medical Research Council of Canada, and to P. G. from the Howard Hughes Medical Foundation. X.-M. Y. is a recipient of a fellowship from the Research Institute of the Royal Victoria Hospital, K. V. is a recipient of a Howard Hughes studentship award, P. G. is a Centenial Scholar of the Medical Research Council of Canada, and M. P. is a Senior Scholar of the National Cancer Institute of Canada.

REFERENCES

Bladt, F., Riethmacher, D., Isenmann, S., Aguzzi, A. and Birchmeier, C. (1995). Essential role for the *c-met* receptor in the migration of myogenic precursor cells into the limb bud. *Nature* **376**, 768-771.

Bober, E., Frantz, T., Arnold, H.-H., Gruss, P. and Tremblay, P. (1994). Pax3 is required for the development of limb muscles: a possible role for the migration of dermomyotomal muscle progenitor cells. *Development* **120**, 603-612.

Bottaro, D. P., Rubin, J. S., Faletto, D. L., Chan, A. M.-L., Kmiecik, T. E., Vande Woude, G. F. and Aaronson, S. A. (1991). Identification of the HGF receptor as the c-met proto-oncogene product. *Science* **251**, 802-804.

Braun, T., Rudnicki, M. A., Arnold, H.-H. and Jaenisch, R. (1992). Targeted inactivation of the muscle regulatory gene *Myf-5* results in abnormal rib development and perinatal death. *Cell* **71**, 369-382.

Chan, A. M.-L., King, H. W. S. Deakin, E. A., Tempest, P. R., Hilkens, J., Kroezen, V., Edwards, D. R., Wills, A. J., Cooper, C. S. and Brooke, P. (1988). Characterization of the mouse *met* proto-oncogene. *Oncogene* **2**, 493-599.

Christ, B., Jacob, H. J. and Jacob, M. (1977). Experimental analysis of the origin of the wing musculature in avian embryos. *Anat. Embryol.* **150**, 171-186.

Christ, B., Jacob, H. J. and Jacob, M. (1978). On the formation of the myotomes in avian embryos. An experimental and scanning electron microscope study. *Experientia* **34**, 514-516.

Christ, B., Jacob, H. J. and Jacob, M. (1983). On the origin and development of the ventrolateral abdominal muscle in the avian embryo. *Anat. Embryol.* **166**, 87-101.

Conlon, R. A. and Rossant, T. (1992). Exogenous retinoic acid rapidly induces anterior ectopic expression of murine *Hox-2* gene *in vivo*. *Development* **116**, 357-368.

Di Renzo, M. F., Narshimhan, R. P., Olivero, M., Bretti, S., Giordano, S., Medico, E., Gaglia, P., Zara, P and Comoglio, P. M. (1991). Expression of the Met/HGF receptor in normal and neoplastic human tissues. *Oncogene* **6**, 1997-2003.

Emerson, C. P. Jr. (1993). Embryonic signals for skeletal myogenesis arriving at the beginning. *Curr. Opin. Cell Biol.* **5**, 1057-1064.

Epstein, D. J., Vekemans, M. and Gros, P. (1991). Splotch (Sp2H), a mutation affecting development of the mouse neural tube, shows a deletion within the paired homeodomain of *Pax3*. *Cell* **67**, 767-774.

Epstein, J. A., Lam, P., Jepeal, L., Maas, R. L. and Shapiro, D. N. (1995). Pax3 inhibits myogenic differentiation of cultured myoblast cells. *J. Biol. Chem.* **270**, 11719-11722.

Franz, T. (1993). The splotch (Sp^{1H}) and splotch-delayed (Sp^{d}) alleles: differential phenotypic effects on neural crest and limb musculature. *Anat. Embryol*. **187**, 371-377.

Franz, T., Kothary, R., Surani, M. A. H. and Grim, M. (1993). The splotch mutation interferes with muscle development in the limbs. *Anat. Embryol*. **187**, 153-160.

Galili, N., Davis, R., Fredericks, W. J., Mukhopadhyay, S., Rauscher III, F. J., Emanuel, B. S., Rovera, G. and Barr, F. G. (1993). Fusion of a fork head domain containing gene to PAX3 in the solid tumor alveolar rhabdomyosarcoma. *Nature Genet*. **5**, 230-235.

Goulding, M. D., Chalepakis, G., Deutsch, U., Erselius, J. R. and Gruss, P. (1991). *Pax3*, a novel murine DNA binding protein expressed during early neurogenesis. *EMBO J*. **10**, 1135-1147.

Goulding, M. D., Lumsden, A. and Paquette, A. J. (1994). Regulation of *Pax3* expression in the dermomyotome and its role in muscle development. *Development* **120**, 957-971.

Grant, D. S., Kleinman, H. K., Goldberg, I. D., Bhargava, M. M., Nickoloff, B. J., Kinsella, J. L., Polverini, P. and Rosen, E. M. (1993). Scatter factor induces blood vessel formation in vivo. *Proc. Natl. Acad. Sci. USA* **90**, 1937-41.

Iyer, A., Kmiecik, T. E., Park, M., Daar, I., Blair, D. G., Dunn, K. J., Sutrave, P., Ihle, J. N., Bodescot, M. and Vande Woude, G. F. (1990). Structure, tissue-specific expression, and transforming activity of the mouse *met* protooncogene. *Cell Growth Differ.* **1**, 87-95.

Kaehn, K., Jacob, H. J., Christ, B., Hinrichsen, K. and Poelman, R. E. (1988). The onset of myotome formation in the chick. *Anat. Embryol*. **177**, 191-201.

Komada, M. and Kitamura, N. (1993). The cell dissociation and motility triggered by scatter factor/hepatocyte growth factor are mediated through the cytoplasmic domain of the c-*met* receptor. *Oncogene* **8**, 2381-2390.

Matsumoto K. and Nakamura T. (1992) Hepatocyte growth factor: molecular structure, roles in liver regeneration, and other biological functions. *Critical Reviews in Oncogenesis.* 3(1-2), 27-54.

Montesano, R., Matsumodo, K., Nakamura, T. and Orci, L. (1991). Identification of a fibroblast-derived epithelial morphogen as hepatocyte growth factor. *Cell* **67**, 901-908.

Myokai, F., Washio, N., Asahara, Y., Yamaai, T., Tanda, Ishikawa, T., Aoki, S., Kurihara, H., Murayaama, Y., Saito, T., Matsumoto, K., Nakamura, T., Noji, S. and Nohno, T. (1995). Expression of the hepatocyte growth factor gene during chick limb development. *Dev. Dynamics* **202**, 80-90.

Naldini, L., Vigna, E., Narsimhan, R. P., Gaudino, G., Zarnegar, R., Michalopoulos, G. A. and Comoglio, P. M. (1991). Hepatocyte growth factor (HGF) stimulates the tyrosine kinase activity of the receptor encoded by the proto-oncogene c-*MET*. *Oncogene* **6**, 501-504.

Ordahl, C. P. and Le Douarin, N. M. (1992). Two myogenic lineages within the developing somite. *Development* **114**, 339-353.

Orita, M., Suzuki, Y., Sekiya, T., and Hayashi, K. (1989). Rapid and sensitive detection of point mutations and DNA polymorphisms using the polymerase chain reaction. *Genomics* **5**, 874-879.

Ott, M.-O., Bober, E., Lyons, G., Arnold, H. and Buckingham, M. (1991). Early expression of the myogenic regulatory gene, *myf-5*, in precursor cells of skeletal muscle in the mouse embryo. *Development* **111**, 1097-1107.

Pownall, M. E. and Emerson, C. P. Jr. (1992). Sequential activation of three myogenic regulatory genes during somite morphogenesis in quail embryos. *Dev. Biol.* **151**, 67-79.

Rudnicki, M. A., Braun, T., Hinuma, S. and Jaenisch, R. (1992). Inactivation of MyoD in mice leads to up-regulation of the myogenic HLH gene *Myf-5* and results in apparently normal muscle development. *Cell* **71**, 383-390.

Rudnicki, M. A., Schnegelsberg, P. N. J., Stead, R. H,. Braun, T., Arnold, H.-H., and Jaenisch, R. (1993). MyoD or myf-5 is required for the formation of skeletal muscle. *Cell* **75**, 1351-1359.

Rugh, R. (1990). *The Mouse. Its Reproduction and Development.* pp. 102-182. New York: Oxford University Press.

Sassoon, D. A., Lyons, G., Wright, W. E., Lin, V., Lassar, A., Weintraub, H. and Buckingham, M. (1989). Expression of two myogenic regulatory factors myogenin and Myo D1 during mouse embryogenesis. *Nature* **341**, 303-307.

Schmidt, C., Bladt, F., Goedecke, S., Brinkmann, V., Zschiesche, W., Sharpe, M., Gherardi, E and Bircheier, C. (1995). Scatter factor/hepatocyte growth factor is essential for liver development. *Nature* **373**, 699-702.

Sonnenberg, E., Meyer, D., Weidner, K. M. and Birchmeier, C. (1993). Scatter factor/hepatocyte growth factor and its receptor, the c-met tyrosine kinase, can mediate a signal exchange between mesenchyme and epithelia during mouse development. *J. Cell Biol.* **123**, 223-235.

Streit, A., Stern, C. D., Thery, C., Ireland, G. W., Aparicio, S., Sharpe, M. J. and Gherardi, E. (1995). A role for HGF/SF in neural induction and its expression in Hensen's node during gastrulation. *Development* **121**, 813-824.

Stern, C. D., Ireland, G. W., Herrick, S. E, Gherardi, E., Gray, J., Perryman, M. and Stoker, M. (1990). Epithelial scatter factor and development of the chick embryonic axis. *Development* **110**, 1271-1284.

Stoker, M., Gherardi, E. and Gray, J. (1987). Scatter factor is a fibroblast-derived modulator of epithelial cell mobility. *Nature* **327**, 239-242.

Takebayashi, T., Iwamoto, M., Jikko, A., Matsumura, T., Enomoto-Iwamoto, M., Myoukai, F., Koyama, E., Yamaai, T., Matsumoto, K., Nakamura, T., Kurisu, K. and Noji, S. (1995). Hepatocyte growth factor/scatter factor modulates cells motility, proliferation, and proteoglycan synthesis of chondrocytes. *J. Cell Biol.* **129**, 1411-1419.

Tashiro, K., Hagiya, M., Nishizawa, T., Seki, T., Shimonishi, M., Shimizu, S. and Nakamura, T. (1990). Deduced primary structure of rat hepatocyte growth factor and expression of the mRNA in rat tissues. *Proc. Natl. Acad. Sci. USA* **87**, 3200-3204.

Uehara, Y., Minowa, O., Mori, C., Shiota, K., Kuno, J., Noda, T. and Kitamura, N. (1995). Placental defect and embryonic lethality in mice lacking hepatocyte growth factor/scatter factor. *Nature* **373**, 702-705.

Vogan, K. J., Epstein, D. J., Trasler, D. G. and Gros, P. (1993). The splotch-delayed [Spd] mouse mutant carries a point mutation within the paired box of the *Pax-3* gene. *Genomics* **17**, 364-369.

Weidner, K. M., Behrens, J., Vandekerckhove, J. and Birchmeier, W. (1990). Scatter Factor: Molecular characteristics and effect on the invasiveness of epithelial cells. *J. Cell Biol*. **111**, 2097-2108.

Weidner, K. M., Sachs, M. and Birchmeier, W. (1993). The *met* Receptor Tyrosine Kinase Transduces Motility, Proliferation, and Morphogenic Signals of Scatter Factor/Hepatocyte Growth Factor in Epithelial Cells. *J. Cell Biol.* **121**, 145-154.

Williams, B. A. and Ordahl, C. P. (1994). *Pax3* expression in segmental mesoderm marks early stages in myogenic cell specification. *Development* **120**, 785-796.

Yang, X.-M. and Park. M. (1993). Expression of the met/hepatocyte growth factor/scatter factor receptor and its ligand during differentiation of murine P19 embryonal carcinoma cells. *Dev. Biol*. **157**, 308-320.

Yang, X.-M. and Park. M. (1995). Expression of the hepatocyte growth factor/scatter factor receptor tyrosine kinase is localized to epithelia in the adult mouse. *Lab Invest.* **73,** 483-491.

Yee, S.-P. and Rigby, P. W. J. (1993). The regulation of myogenin gene expression during the embryonic development of the mouse. *Genes Dev*. **7**, 1277-1289.

Zhu, H., Naujokas M. A. and Park, M. (1994). Receptor chimeras indicate that the *met* tyrosine kinase mediates the motility and morphogenic responses of hepatocyte growth/scatter factor. *Cell Growth Diff.* **5,** 359-366.

(Accepted 19 April 1996)