

## The expression of the homeobox gene *Msx1* reveals two populations of dermal progenitor cells originating from the somites

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### SUMMARY

Experimental manipulation in birds has shown that trunk dermis has a double origin: dorsally, it derives from the somite dermomyotome, while ventrally, it is formed by the somatopleure. Taking advantage of an *nlacZ* reporter gene integrated into the mouse *Msx1* locus (*Msx1<sup>nlacZ</sup>* allele), we detected segmental expression of the *Msx1* gene in cells of the dorsal mesenchyme of the trunk between embryonic days 11 and 14. Replacing somites from a chick host embryo by murine *Msx1<sup>nlacZ</sup>* somites allowed us to demonstrate that these *Msx1*- $\beta$ -galactosidase positive cells are of somitic origin. We propose that these cells are dermal progenitor cells that migrate from the somites and subsequently contribute to the dorsalmost dermis. By analysing *Msx1<sup>nlacZ</sup>* expression in a *Splotch* mutant, we observed that migration of these cells does not depend on *Pax3*, in contrast to other migratory populations such as limb muscle progenitor cells and neural crest cells.

*Msx1* expression was never detected in cells overlying the

dermomyotome, although these cells are also of somitic origin. Therefore, we propose that two somite-derived populations of dermis progenitor cells can be distinguished. Cells expressing the *Msx1* gene would migrate from the somite and contribute to the dermis of the dorsalmost trunk region. A second population of cells would disaggregate from the somite and contribute to the dermis overlying the dermomyotome. This population never expresses *Msx1*.

*Msx1* expression was investigated in the context of the onset of dermis formation monitored by the *Dermo1* gene expression. The gene is downregulated prior to the onset of dermis differentiation, suggesting a role for *Msx1* in the control of this process.

Key words: Dermis, Dermatome, Dermomyotome, Development, Mouse, Homeodomain protein, *Msx* gene, Heterospecific graft,  $\beta$ -galactosidase, Differentiation, In situ hybridisation

### INTRODUCTION

Although much attention has focused on the muscle and cartilage derivatives during somite differentiation, very little is known about the formation of another major derivative, the dermis of the back. Data from birds show that, in the head, dermis mostly differentiates from neural crest cells (Le Lièvre and Le Douarin, 1974, 1975; Couly et al., 1993). In the trunk, lateral and ventral dermis originate from the somatopleure (Murray, 1928; Mauger, 1972a), whereas the dorsolateral dermis derives from dermatomal cells originating from the somites (Mauger and Sengel, 1970; Mauger, 1972a; Christ et al., 1983). Signals from the neural tube are necessary for conversion of the epithelial dermatome into mesenchymal cells (Mauger, 1972b), possibly mediated by Neurotrophin-3 (Brill et al., 1995). Epithelio-mesenchymal interactions between dermis and the overlying ectodermal epithelium confer its morphogenetic properties on the skin, leading to the formation

of cutaneous appendages such as feathers and hairs (reviewed in Sengel, 1990; Hardy, 1992).

Recently, several markers for the early steps of dermis differentiation have been described in mammals and birds. The homeobox containing gene, *Mhox* (*Prx1*) is activated in the dermatome, under the control of the overlying ectoderm, and subsequently expressed in dermis (Cserjesi et al., 1992; Kuratani et al., 1994). However, no dermal defect has been reported for a null mutation in this gene (Martin et al., 1995). The *Dermo1* gene is expressed in the newly formed dermis, and subsequently restricted to the epidermal-dermal junction (Li et al., 1995). Therefore, it constitutes a convenient marker for the onset of dermis differentiation. Two other genes, *Wnt11* (Tanda et al., 1995) and *Sim1* (Fan et al., 1996) may also be markers of the early dermis. However, their late patterns of expression have not been precisely described.

The homeobox containing gene, *Msx1* (previously called *Hox7*), is regulated by epithelio-mesenchymal interactions

involved in the differentiation of many organs (reviewed in Davidson, 1995). Its expression has also been described in the lateral margin of the dermomyotome of some somites, and during migration of the limb muscle progenitor cells (Houzelstein et al., 1999), a lineage in which it may act as a repressor of differentiation (Song et al., 1992; Woloshin et al., 1995; Bendall et al., 1999).

Null mutations in *Msx1* have been generated by insertion of a *neo<sup>r</sup>* gene (Satokata and Maas, 1994), or by an in-frame insertion of the *nlacZ* reporter gene into its homeobox (*Msx1<sup>nlacZ</sup>* allele; Houzelstein et al., 1997). In both cases, homozygous mutants die at birth. Defects have been described in the facial region and include a complete cleft of the secondary palate, middle ear defect, failure of tooth differentiation and some defects in the skull. In *Msx1<sup>-/-</sup>* mutants, it has been proposed that the *Msx2* protein compensates for the lack of *Msx1* (Catron et al., 1996; Houzelstein et al., 1997), resulting in the unexpected absence of defects in structures such as limbs, which are major sites for *Msx1* expression during development.

Taking advantage of the functional  $\beta$ -galactosidase enzyme encoded by the *Msx1<sup>nlacZ</sup>* allele (Houzelstein et al., 1997), we show that *Msx1* is segmentally expressed in the dorsal mesenchyme of the embryo. Cells expressing the reporter gene are seen lying under the ectoderm, on either side of the neural tube, and cover 18-20 segments from the first cervical vertebra. In this region, they appear to be somite-derived, since grafting *Msx1<sup>nlacZ</sup>* mouse somites into chick embryos results in  $\beta$ -galactosidase positive (*Msx1*- $\beta$ -gal<sup>+</sup>) cells migrating dorsally from the grafted somites. We propose that these cells are dermis progenitor cells originating from the somite and that *Msx1* expression reveals a sub-population of dermis progenitor cells migrating from the somite. We show that this population of cells is present in the back of embryos from E11 (embryonic day 11). Contrary to other migrating cell lineages, such as limb muscle progenitor cells and neural crest cells, its migration is not dependent on *Pax3*. We also show that *Msx1* is downregulated prior to the onset of dermis differentiation, monitored by the expression of the *Dermo1* gene. We conclude that expression of the *Msx1* gene defines a subset of dermal progenitor cells and that it may play a role in regulating the onset of dermis differentiation.

## MATERIALS AND METHODS

### Mouse strains

Gene targeting of the *Msx1* locus with the *nlacZ* (*n*, nuclear localisation signal) reporter gene (*Msx1<sup>nlacZ</sup>*) was described previously (Houzelstein et al., 1997). Heterozygous *Msx1<sup>nlacZ</sup>* mice were backcrossed for 10 generations on the C57BL/6J background. They were also mated with *Splotch* heterozygous mice (C57BL/6J, Jackson Laboratory), resulting in double heterozygotes which are about 95% on the C57BL/6J background.

### $\beta$ -galactosidase staining and histochemistry

X-gal staining on whole embryos and cryostat sections was as previously described (Houzelstein et al., 1997). For codetection of  $\beta$ -galactosidase activity and cartilage formation,  $\beta$ -galactosidase was revealed as described but X-gal was replaced by Salmon-gal (Apollo Scientific Ltd), which forms a red precipitate. Embryos were subsequently processed to reveal cartilage formation with Alcian Blue, as described by Jegalian and De Robertis (1992).

Cryostat sections of chick/mouse chimeras were stained for  $\beta$ -galactosidase, washed twice in PBS before counterstaining with Hoechst (bisbenzimidazole Hoechst No. 33342, Sigma; saturated solution of Hoechst in ethanol diluted 1/1000 in PBS). They were subsequently washed twice in PBS and mounted in Mowiol (Calbiochem).

### In situ hybridisation

The *Msx1* probe corresponds to the 3' untranslated region of the mouse gene as previously described (Lyons et al., 1992). The *Dermo1* probe (a kind gift from Dr E. Olson) was as described by Li et al. (1995). The chick *MyoD* probe (a kind gift of Drs O. Saitoh and M. Periasamy) was described by Fontaine-Pérus et al., 1997. The *Pax3* (Goulding et al., 1994) probe is derived from a 519 bp *PstI/HindIII* fragment from the 3' coding end of the mouse cDNA (kindly provided by P. Gruss). In situ hybridisation on cryostat sections was performed according to Myat et al. (1996) with Boehringer AP substrate replacing NBT-BCIP.

### Mouse/chick heterospecific chimeras

Somite grafting was performed as previously described (Fontaine-Pérus et al., 1995) except that *Msx1<sup>nlacZ</sup>* mice were maintained on a C57BL/6J background. Somites 8-15 (forelimb level) were excised from 18- to 20-somite-stage mouse embryos. They were transplanted at the forelimb (somites 15-21) or the hindlimb (somites 25-32) level in chick hosts, without changing their orientation. The grafts were performed on 15- to 25-somite-stage chick embryos. Depending on the stage of the host embryos, either the segmental plates or the 4-6 caudalmost somites were replaced by mouse somites. Chick embryos were fixed between 20 and 72 hours following surgery.

Neural tube grafting was performed as previously described (Fontaine-Pérus et al., 1997). Donor neural tubes were excised from the caudalmost part of 15- to 18-somite-stage *Msx1<sup>nlacZ</sup>* mice. Hosts were fixed between 16 and 24 hours following surgery.

All pictures were scanned and assembled using Adobe Photoshop.

## RESULTS

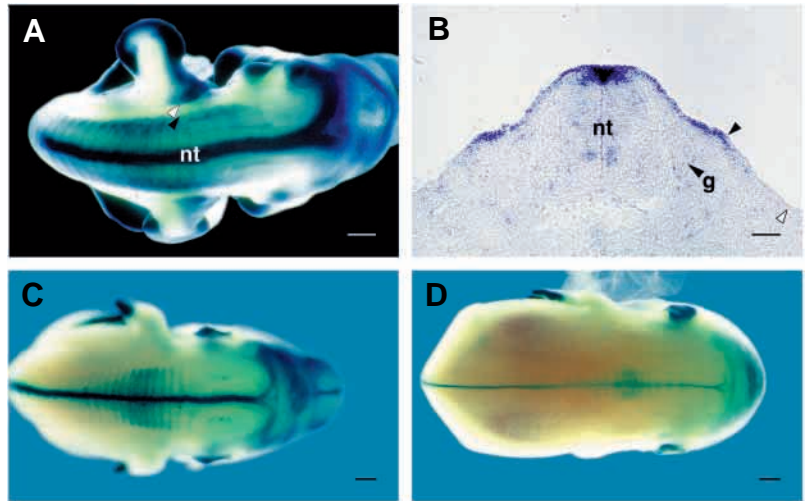
### *Msx1* is expressed in the dorsal region of the trunk of E11 to E14 embryos

Taking advantage of the high sensitivity of  $\beta$ -galactosidase detection on whole-mount embryos, we detected a new domain of *Msx1* expression in the back of the trunk of embryos from E10.75-E11 (Fig. 1). The signal, seen on either side of the strongly labelled neural tube, exhibits a segmental pattern and extends over 15-20 segments from the first cervical vertebra to the level of the hindlimbs (Fig. 1A). Expression was also detected as a weaker signal in the mesenchyme posterior to the hindlimbs (data not shown). Cells expressing the reporter gene are located in the mesenchyme under the dorsal ectoderm, overlying the neural tube down to the most medial part of the dermomyotome (black arrowhead in Fig. 1A,B; see also Figs 8A,C and 9). *Msx1* expression was not detected in the cells overlying the dermomyotome between the black and white arrowheads in Fig. 1A,B (see also Fig. 8A,C), in a region where, in chick, dermis forms from cells disaggregating from the dermatome (Christ et al., 1983; Brill et al., 1995).

This site of expression, although not previously described, is not an artefact of the insertion of the *nlacZ* gene into the *Msx1* locus, since the *Msx1* mRNA was detected in the same location by in situ hybridisation on normal embryos (Fig. 1B, compare with *Msx1<sup>nlacZ</sup>* expression in Fig. 8A).

Expression is most intense at about E12 (Fig. 2A) and diminishes subsequently. At E13,  $\beta$ -galactosidase was still

**Fig. 1.** Expression of the *Msx1* gene in the dorsal trunk of mouse embryos between E11 and E14. (A) An E11 embryo, heterozygous for the *Msx1<sup>lacZ</sup>* allele, showing *Msx1*- $\beta$ -gal<sup>+</sup> cells (stained blue with X-gal) in the dorsal mesenchyme. The black arrowhead indicates the lateral margin of  $\beta$ -gal<sup>+</sup> cells in the dorsal mesenchyme. The white arrowhead indicates the lateral margin of the somite-derived dermis precursor cells, as deduced from grafting in the chick (see text and Figs 4 and 5). nt, neural tube. (B) A transverse cryostat section from an E11 wild-type embryo, hybridised with an *Msx1* antisense probe. *Msx1* expression is detectable in the roof plate of the neural tube and in mesenchymal cells located under the axial ectoderm, in a similar pattern to that of *Msx1<sup>lacZ</sup>* (compare with expression of the reporter gene in Fig. 8A). White and black arrowheads mark the same limits as in A. nt, neural tube; g, spinal ganglion. (C) An *Msx1<sup>lacZ/+</sup>* E13 embryo, processed as in A. Expression of the reporter gene is clearly segmented and remains detectable at the thoracic level. (D) An *Msx1<sup>lacZ/+</sup>* E14 embryo, processed as in A. Only a few  $\beta$ -gal<sup>+</sup> cells are detected in the dorsal mesenchyme of the embryo. Bars, 500  $\mu$ m (A,C,D); 100  $\mu$ m (B).



detected in the dorsal mesenchyme and expression was most pronounced at the level of forelimbs (Fig. 1C). At these two developmental stages, *Msx1*- $\beta$ -gal<sup>+</sup> cells form stripes, with each stripe corresponding to one vertebral condensation (Fig. 2A). At E13, each neural arch separates two adjacent *Msx1*- $\beta$ -gal<sup>+</sup> stripes (Fig. 2B). The fact that a stripe is out of phase with a vertebra, and therefore in phase with the somite, is due to somite resegmentation (see Christ and Ordahl, 1995, for a review). The expression of *Msx1<sup>lacZ</sup>* does not shift towards more posterior parts of the body, as it would do if it were linked to the rostro-caudal gradient of differentiation (Fig. 1C). *Msx1<sup>lacZ</sup>* was still detectable at E14 at the level of the forelimbs (Fig. 1D). At E14, the few remaining *Msx1*- $\beta$ -gal<sup>+</sup> cells are located deep in the dorsal mesenchyme, and are restricted to a narrow region just above the central part of the neural tube (Fig. 3A enlarged in 3B). In chick, this region has been shown to contribute to the spinous process, the dorsalmost aspect of the vertebra (Takahashi et al., 1992; Monsoro-Burq et al., 1994, 1996).

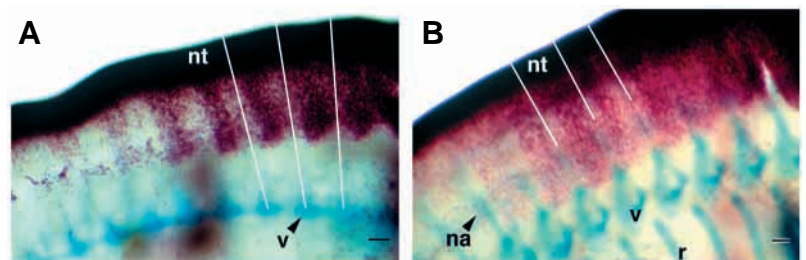
***Msx1*-expressing cells located in the dorsal region of the trunk originate from the somites**

Two main cell types are present in the dorsal mesenchyme of the embryo, at the stage when *Msx1* expression is detected

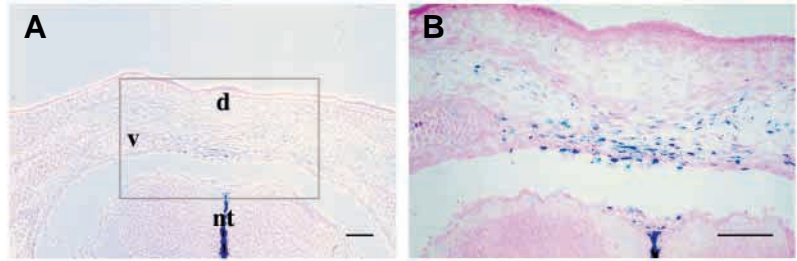
there: neural crest cells and dermal progenitor cells. Judging from their location and their segmental distribution, the *Msx1*- $\beta$ -gal<sup>+</sup> cells were likely to be dermal progenitor cells. However, in mouse the melanoblasts enter into dermis between E11 and E14 (Mayer, 1973), at a time when the *Msx1*- $\beta$ -gal<sup>+</sup> cells were detected in the back of the trunk, so it was necessary to investigate whether these *Msx1*- $\beta$ -gal<sup>+</sup> cells were of neural crest origin.

Mouse neural tubes were grafted at the segmental plate level, caudal to the most newly formed somite of a 15- to 18-somite chick embryo. A previous study demonstrated that active emigration of neural crest cells from the mouse neural tube could occur after its in ovo-engrafting. Under these conditions, cells from the implant contribute to the development of the peripheral nervous system and to the pigmentation through migration of neural crest cells associated with the mouse neuroepithelium (Fontaine-Pérus et al., 1997). A total of ten cases were examined between 16 and 24 hours following surgery. 16 hours post-surgery, neural crest cells left the grafted mouse neural tube. 20 hours post-surgery, the *Msx1<sup>lacZ</sup>* transgene expression was restricted to the dorsal area of the implanted neural tube (Fig. 4A,B). 24 hours post-surgery, the mouse neural crest cells compacted and constituted the early spinal ganglionic primordia. At this stage, transgene

**Fig. 2.** Stripes of *Msx1*- $\beta$ -gal<sup>+</sup> cells are in phase with the somitic segmentation. (A) Dorsal thoracic region of an E12 heterozygous embryo. *Msx1*- $\beta$ -gal<sup>+</sup> cells are detected with Salmon-gal (red precipitate). Cartilage of the vertebral body (v) is stained by Alcian Blue. Ribs have not yet formed. The white bars mark the limits of two adjacent stripes. Note that one vertebral body corresponds to each *Msx1*- $\beta$ -gal<sup>+</sup> stripe. nt, neural tube; v, vertebral body. (B) Dorsal thoracic region of an E13 heterozygous embryo, processed as in A. Ribs (r) and neural arches of the vertebra (na) have formed. One neural arch separates two adjacent *Msx1*- $\beta$ -gal<sup>+</sup> stripes (white bars). Each vertebra derives from the posterior half of one somite and the anterior half of the following one. Therefore, the centre of a vertebra is located between two adjacent somites. Since each vertebra comes inbetween two adjacent *Msx1*- $\beta$ -gal<sup>+</sup> stripes, each stripe is in register with one somite. nt, neural tube; na, neural arch; v, vertebral body; r, rib. Bars, 100  $\mu$ m.



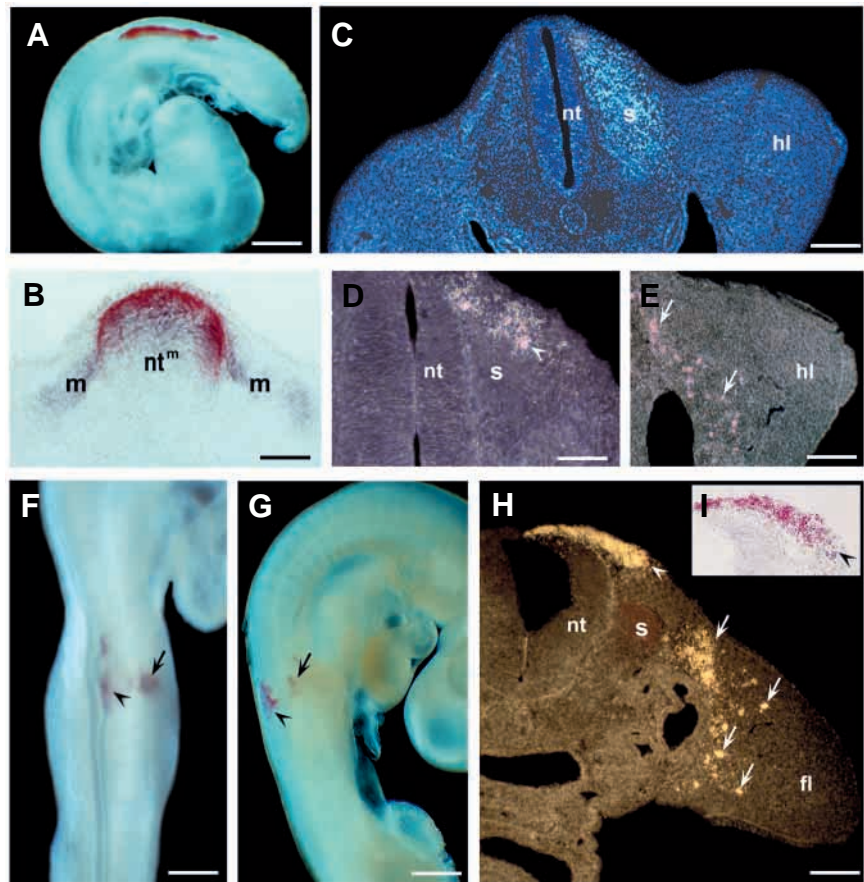
**Fig. 3.** Expression of the *Msx1<sup>nlacZ</sup>* gene in the dorsal trunk of embryos at E14. (A) A cryostat section from an *Msx1<sup>nlacZ/+</sup>* E14 embryo, assayed for  $\beta$ -galactosidase activity (phase contrast picture). A few *Msx1*- $\beta$ -gal<sup>+</sup> cells (blue) are detected in the mesenchyme that may subsequently form the spinous process of the vertebra. d, dermis; v, vertebra; nt, neural tube. (B) Enlargement of the square shown in A, photographed in bright field. Bars, 100  $\mu$ m.



expression was revealed in the same area as 4 hours before (data not shown). No transgene expression was observed outside the mouse neural tube, indicating that associated neural crest cells, which migrate away from the implant, do not express *Msx1<sup>nlacZ</sup>* under these conditions.

In the chick, dermis ventral to the lateralmost limit of the somite has been proposed to originate from the somatopleure, whereas dermis overlying the somite and neural tube has been shown to originate from somitic cells of the dermomyotome (Murray, 1928; Mauger, 1972a; Christ et al., 1983; Brill et al.,

**Fig. 4.** Expression of *Msx1<sup>nlacZ</sup>* in dermal progenitor cells of mouse/chick chimeras. (A,B) Chimeras grafted with mouse neural tubes. (C-I) Chimeras grafted with mouse somites. (A) An E3 mouse/chick chimeric embryo, whole-mount stained 20 hours post-surgery. The caudal part of the neural tube from an E9 *Msx1<sup>nlacZ</sup>* mouse embryo was implanted to replace the chick neural tube, at the level of the segmental plate in a 15-somite chick embryo. *Msx1*- $\beta$ -gal<sup>+</sup> cells are detected in the dorsal portion of the grafted neural tube (stained red with salmon-gal). Bar, 500  $\mu$ m. (B) Transverse section through the grafted region of the embryo shown in A. The section has been hybridised with a mouse *Pax3* probe. Dorsal cells in the mouse neural tube (nt<sup>m</sup>) express both the *Msx1*- $\beta$ -gal (red) and *Pax3* (faint purple). The *Pax3* probe also labels, by cross-hybridisation with the chick mRNA, the lateral lip of the dermomyotome. A chick-specific *MyoD* probe reveals the position of the host myotome (faint purple; m). No *Msx1*- $\beta$ -gal<sup>+</sup> cells could be detected migrating from the grafted neural tube, indicating that the *Msx1*- $\beta$ -gal<sup>+</sup> cells detected in the dorsal mesenchyme of the mouse embryos are not melanoblasts originating from the neural crest. Bar, 40  $\mu$ m. (C) Transverse section through the grafted region of an E3 mouse/chick chimeric embryo stained with bisbenzimidide, 24 hours after grafting. Nine mouse somites from the forelimb level replaced the segmental plate in a 25-somite chick embryo. The mouse somite (s) and cells originating from it appear as a brighter domain. hl, hindlimb, nt, chick neural tube. Bar, 50  $\mu$ m. (D) Serial section to the one shown in C. *Msx1*- $\beta$ -gal<sup>+</sup> cells (arrowhead) are located in the dorsal mesenchyme overlying the most medial part of the grafted mouse somite (s) and the chick neural tube (nt). Bar, 25  $\mu$ m. (E) Serial section to the one viewed in D. *Msx1*- $\beta$ -gal<sup>+</sup> cells (arrows) were also identified emigrating from the ventrolateral part of the grafted somite to the host hindlimb (hl). Bar, 40  $\mu$ m. (F) An E3 mouse chick chimera, whole-mount-stained 20 hours post-surgery. Somites 8-10 from an E9 *Msx1<sup>nlacZ</sup>* mouse were grafted to replace part of the segmental plate in a 15-somite chick embryo. The arrowhead shows the *Msx1*- $\beta$ -gal<sup>+</sup> red cells located in the vicinity of the host neural tube and the arrow shows the *Msx1*- $\beta$ -gal<sup>+</sup> red cells migrating to the forelimb. Bar, 250  $\mu$ m. (G) An E3 mouse/chick chimeric embryo, whole-mount-stained 24 hours post-surgery. Somites 8-10 from an E9 *Msx1<sup>nlacZ</sup>* mouse were implanted at the brachial level of an 18-somite chick embryo. As in F, *Msx1*- $\beta$ -gal<sup>+</sup> cells are confined dorsally in the vicinity of the neural tube (arrowhead) and, ventrally, penetrate into the forelimb (arrow). Bar, 350  $\mu$ m. (H) Section through the grafted region of the embryo shown in G at the forelimb level. *Msx1*- $\beta$ -gal<sup>+</sup> cells are detected in dark field as bright zones under the dorsal ectoderm, down to the most medial region of the grafted somite (arrowhead). *Msx1*- $\beta$ -gal<sup>+</sup> cells (arrows) are also present in the lateral margin of the dermomyotome and in the proximal region of the forelimb (fl). nt, chick neural tube; s, mouse somite. Bar, 40  $\mu$ m. (I) Magnification of part of the section shown in H. *Msx1*- $\beta$ -gal<sup>+</sup> cells located under the dorsal host ectoderm (arrowhead) are stained with Salmon-gal and appear red in bright field microscopy.



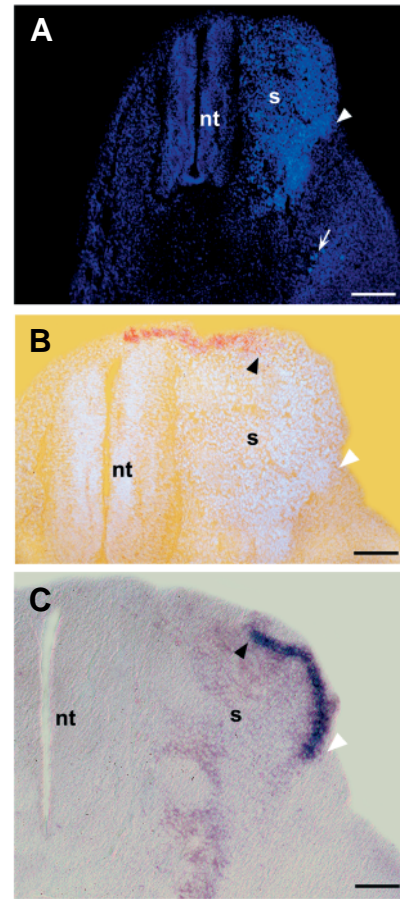
1995; Huang et al., 1997). Previous work (Fontaine-Pérus et al., 1995) showed that mouse somites transplanted into a chick host survived and rapidly developed into the three somite derivatives: dermatome, myotome and sclerotome. To follow the fate of somitic cells, somites dissected from an E9 *Msx1<sup>nlacZ</sup>* mouse embryo were grafted in place of forelimb or hindlimb level somites of an E2 chick embryo. Cells from the mouse somites were followed by bisbenzimidazole staining. They colonised the area located beneath the chick ectoderm from the junction with somatopleure up to the most dorsal part of the trunk (Fig. 4C; white arrowhead in Fig. 5A). The arrowhead indicates the limit between the dermis of somitic origin (mouse cells, brightly stained) and the dermis of somatopleural origin (host cells, fainter staining). This result is in agreement with the position of the border between dermis of somitic and of somatopleural origin observed in birds.

The segmental plates of 15- and 25- somite chick embryos or the last 4-6 somites of 18- to 20-somite chick embryos were replaced unilaterally with somites 8-15 from E9 *Msx1<sup>nlacZ</sup>* mouse embryos. From 20 hours post-surgery on, *Msx1*- $\beta$ -gal<sup>+</sup> cells originating from the mouse somites were detected in the dorsalmost mesenchyme beneath the host ectoderm, in the vicinity of the dorsal neural tube (arrowheads on the sections shown in Fig. 4D,H,I; see also Fig. 4F,G for whole-mount aspects). This result proves that the *Msx1*- $\beta$ -gal<sup>+</sup> cells detected in the back of the embryos are of somitic origin.

We never detected *Msx1*- $\beta$ -gal<sup>+</sup> cells among the cells directly overlying the grafted somite in the region lateral to the arrowhead in Fig. 4D,H or between the black and white arrowheads in Fig. 5B, although the cells from this region also originate from the grafted somite (see Fig. 5A). By this time, these cells already express *Dermo1*, a marker of dermis differentiation (revealed with a mouse-specific *Dermo1* probe, between the black and white arrowheads in Fig. 5C). On the contrary, *Msx1*- $\beta$ -gal<sup>+</sup> cells migrating from the graft are still *Dermo1* negative (compare Fig. 5B and C; the limit between *Msx1* and *Dermo1* expressing cells is shown by a black arrowhead).

*Msx1*- $\beta$ -gal<sup>+</sup> cells were simultaneously detected in the proximal region of the wing (Fig. 4E-H, arrows). We previously showed that these cells are muscle progenitor cells migrating from the grafted somite (Houzelstein et al., 1999). In mouse, in contrast to grafted chick, muscle progenitor cells migrate 24 hours before the first *Msx1*- $\beta$ -gal<sup>+</sup> cells are detected in the dorsal trunk. Signals extrinsic to the somite regulate its differentiation. This observation may reflect either the fact that the timing of these signals is slightly different between chick and mouse or that it is slightly altered in this heterospecific context.

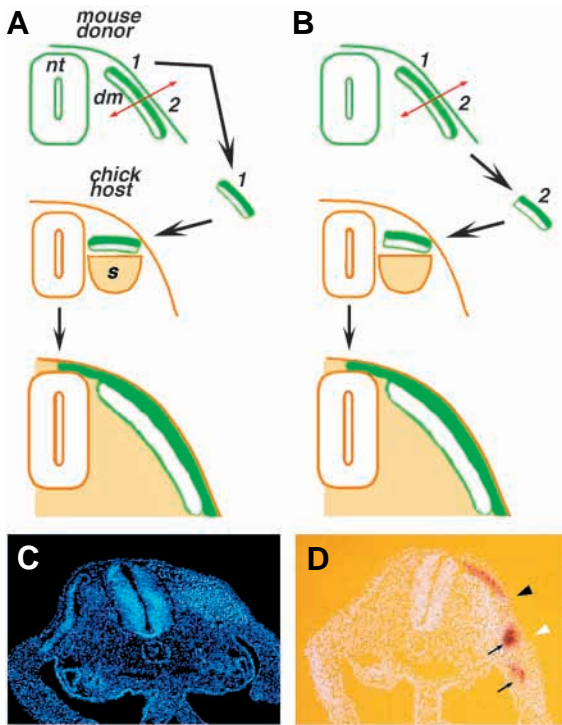
Since *Msx1*- $\beta$ -gal<sup>+</sup> cells populate the most dorsal regions of presumptive dermis, we addressed the question of whether the dermomyotome itself, from which they originate, is already regionalised. For this purpose, we replaced the dorsal half of an epithelial somite in a chick host by either the lateral or the medial half of the dermomyotome from a mouse somite (Fig. 6A,B). In either case, the grafted dermomyotome piece contributed progenitor cells to the dorsal dermis. Therefore, the medial (Fig. 6C) and the lateral (Fig. 6D) halves of the dermomyotome may be induced to provide these progenitor cells. Furthermore, cells from a grafted lateral dermomyotome piece do express *Msx1*- $\beta$ -gal as they migrate



**Fig. 5.** Codetection of *Msx1<sup>nlacZ</sup>* and *Dermo1* in mouse/chick chimeras. (A) A section from an E4 mouse/chick chimeric embryo, 48 hours after grafting. Eight mouse somites replaced the whole segmental plate in a 22-somite chick embryo (the graft extended until the 29<sup>th</sup> presumed somite, i.e. a part of the hindlimb level). The bisbenzimidazole treatment stains the mouse cells brighter. The white arrowhead indicates the lateral limit of the territory colonised by the mesenchymal cells originating from the grafted mouse somite (s). A few bright cells are emigrating to the hindlimb (arrow). nt, chick neural tube; s, mouse somite. Bar, 35  $\mu$ m. (B) Same section as in A, showing the *Msx1*- $\beta$ -gal<sup>+</sup> area in red. The black arrowhead indicates the lateral limit of the *Msx1<sup>nlacZ</sup>* expression territory. The white arrowhead marks the same territory limit as in A. nt, chick neural tube; s, mouse somite. Bar, 25  $\mu$ m. (C) Serial section to the one shown in B, hybridised with a mouse specific *Dermo1* probe. The black arrowhead defines the medial limit of the *Dermo1* expression domain. The white arrowhead marks the same territory limit as in A and B. Comparing C and B demonstrates that the areas where *Dermo1* and *Msx1<sup>nlacZ</sup>* are detected do not overlap. nt, chick neural tube; s, mouse somite. Bar, 20  $\mu$ m.

into the back (Fig. 6D). They also express the reporter gene in the lateral region of the dermomyotome and as they migrate to the wing bud (Fig. 6D; Houzelstein et al., 1999). These results suggest that, regarding dermis formation, the dermomyotomal fate can be modulated until late in development. It also suggests that signals extrinsic to the dermomyotome regulate this fate.

From the grafting experiments of *Msx1<sup>nlacZ</sup>* mouse somites into a chick host, we conclude that the cell population



**Fig. 6.** Capacity of the medial and lateral parts of the dermomyotome to contribute dorsal dermis progenitor cells. (A,B) Scheme of the grafting strategy. The dermomyotome from a mouse somite was cut into a medial (1) or a lateral (2) part. (A) The medial and (B) the lateral dermomyotome halves were implanted in place of the dorsal halves of epithelial somites in chick embryos. Whatever its origin (1 or 2), the dermomyotomal piece contributes cells to the whole dorsal dermis of the chick host embryo at the graft level. (C,D) Transverse sections of E3 mouse/chick chimeric embryos grafted at the 19-somite stage. (C) The medial or (D) the lateral parts of mouse dermomyotomes were implanted at the 16-19 somite level in chick embryos. (C) The mouse cells (bright cells; stained with Hoechst) spread out of the medial half dermomyotome to provide dermis progenitors to a domain extending from the upper part of the neural tube to the lateral plate. (D) Dorsal dermis progenitor cells can originate from the lateral part of the dermomyotome. They are induced to express the *Msx1<sup>lacZ</sup>* gene (stained red by Salmon-gal) only in the region medial to the grafted somite (black arrowhead), while dermis progenitor cells colonise the territory extending laterally down to the white arrowhead. Note also *Msx1<sup>lacZ</sup>* expression in the lateral region of the grafted dermomyotome and in cells migrating in the proximal region of the wing bud (arrows), which takes place into myogenic progenitor cells ( $\times 160$ ).

expressing *Msx1* in the dorsal mesenchyme of the trunk corresponds to dermis progenitor cells migrating from the somites and not to neural crest cells migrating from the neural tube. Our results also show that we can define two populations of somite-derived dermis progenitors, based on *Msx1* expression. The first expresses *Msx1* during its migration from the somite, while the second differentiates in situ and never expresses *Msx1*.

#### The *Msx1* pattern of expression is not altered in the trunk of *Splotch* embryos

*Pax3* is necessary for the migration of different cell

populations, including neural crest cells and limb muscle progenitor cells (Franz and Kothary, 1993; Bober et al., 1994; Goulding et al., 1994). In order to determine whether *Pax3* is also necessary for dermis progenitor cell migration from the somites, we generated mice carrying one *Msx1<sup>lacZ</sup>* allele on a *Splotch* (*Pax3<sup>-/-</sup>*) homozygous mutant background. In E11 *Splotch* homozygous embryos, *Msx1<sup>lacZ</sup>* reporter gene expression in the dorsal mesenchyme was similar to that in wild-type embryos (Fig. 7A, compare with Fig. 1A). In E13 *Splotch* embryos, *Msx1*- $\beta$ -gal<sup>+</sup> cells are located in the dorsal mesenchyme, as in normal embryos (compare Figs 7B and 8E). These results further confirm that these cells are not neural crest-derived melanoblasts, since neural crest migration is impaired in the *Splotch* mutant (Serbedzija and McMahon, 1997). They also show that, although *Pax3* expression is detected in the dermomyotome (which will subsequently form somite-derived dermis and muscle), the *Msx1*- $\beta$ -gal<sup>+</sup> dermal progenitor cells do not depend on *Pax3* expression for their migration, in contrast to other migratory populations.

#### *Msx1* and *Dermo1* expression patterns are mutually exclusive during trunk dermis development

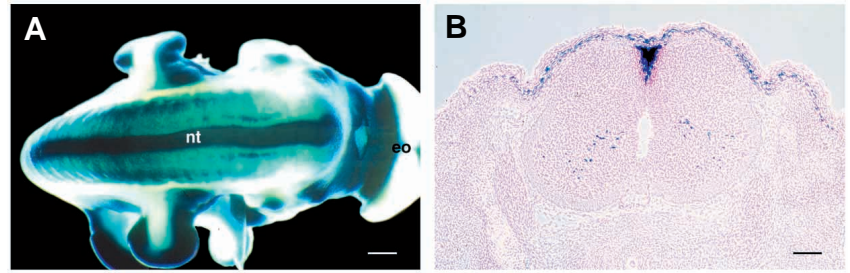
*Dermo1* is a bHLH (basic-helix-loop-helix) protein and its mRNA has been detected in differentiating dermis (Li et al., 1995). At E11, *Msx1<sup>lacZ</sup>* is expressed in the dorsal mesenchyme of the embryo, and its domain of expression complements that of *Dermo1* (Fig. 8A,B). In E12 embryos, *Msx1<sup>lacZ</sup>* and *Dermo1* expression domains remain mutually exclusive. In particular, in the lateral part of the *Msx1<sup>lacZ</sup>* expression domain, the reporter gene is expressed deeper in the mesenchyme at E12 than at E11 (black arrowhead in Fig. 8C), whereas *Dermo1* begins to be expressed in the mesenchymal layer immediately overlying the *Msx1* expressing layer (Fig. 8D). In E13 embryos, *Dermo1* is expressed over the whole dorsal region of the trunk (Fig. 8F) in a cell layer immediately overlying the deeper mesenchymal cell layer that expresses *Msx1<sup>lacZ</sup>* (Fig. 8E).

These results suggest that, in the mouse, dermis from the flank up to the dorsal margin of the somite differentiates by E11, about 2 days before dermis derived from cells migrating from the somite. This differentiation first occurs in the outer layers, before extending to the innermost layers. Our results also suggest that *Msx1<sup>lacZ</sup>* is downregulated prior to the onset of dermis differentiation.

## DISCUSSION

We have detected *Msx1* expression in mesenchymal cells underlying the ectoderm of the back of the trunk; these cells are mainly fated to form dermis. Grafting experiments have shown that these cells originate from the somites. To our knowledge, the *Msx1* gene is the only early marker of dorsal dermis progenitor cells described to date. It distinguishes two somite-derived dermis populations. The first one disaggregates from the dermomyotome and never expresses the *Msx1* gene, while the second one expresses *Msx1* during its dorsal migration from the somite. Our results also show that migration of the latter is independent of *Pax3*, contrary to other somite- and neural tube-derived cell populations.

**Fig. 7.** Expression of the *Msx1<sup>lacZ</sup>* in *Spotch* mutant embryos. (A) An E11 embryo heterozygous for the *Msx1<sup>lacZ</sup>* gene and homozygous for the *Spotch* (*Pax3<sup>-/-</sup>*) mutation. This embryo exhibits an exocephaly (eo) characteristic of *Spotch* mutants. *Msx1<sup>lacZ</sup>* expression is unaffected in this mutant (compare with the normal embryo in Fig. 1A). nt, neural tube. (B) Transverse section from an E13 embryo heterozygous for the *Msx1<sup>lacZ</sup>* gene and homozygous for the *Spotch* (*Pax3<sup>-/-</sup>*) mutation. Putative dermis and spinous process progenitor cells, which are  $\beta$ -gal<sup>+</sup>, appear unaffected in this mutant (compare with normal embryo in Fig. 8E). Bars, 500  $\mu$ m (A); 100  $\mu$ m (B).



***Msx1* is expressed in the dorsal mesenchyme of the mouse embryo**

In the dorsal mesenchyme of the mouse embryo trunk, *Msx1*-expressing cells were revealed as stripes, in register with the segmentation of the somites, at the cervical and thoracic levels. In E12 embryos, we show that a very large majority of the mesenchymal cells overlying the neural tube express *Msx1*. This segmented pattern is reminiscent of the fate map of the somitic contribution to the spinal pterygia in chick, which corresponds to the somite-derived dermis (Mauger and Sengel, 1970). Mouse/chick heterospecific grafts demonstrate that these *Msx1*-expressing cells originate from the somites and contribute to the dorsal dermis of the trunk.

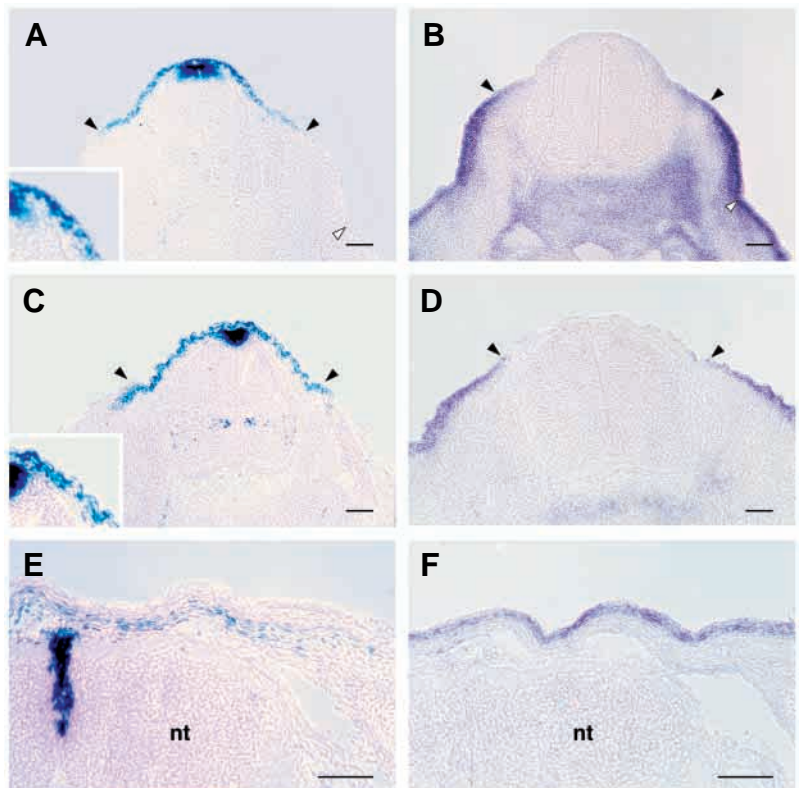
Quail/chick chimera studies have shown that some of the dorsal mesenchymal cells that overlie the neural tube contribute to the dorsalmost aspect of the vertebra, the spinous process (Takahashi et al., 1992). In E14 *Msx1<sup>lacZ</sup>* mouse embryos, the latest *Msx1*- $\beta$ -gal<sup>+</sup> cells detected in the dorsal mesenchyme of the trunk, given their location immediately above the central aspect of the neural tube, are likely to

correspond to this cell population. It has previously been suggested that progenitors of the spinous process derive from the dermatome (Monsoro-Burq et al., 1996). The fact that we observe *Msx1* expression both in somite-derived progenitors of the dermis and in the potential progenitors of the spinous process is in agreement with this hypothesis. Thus, dermatomal cells would migrate and occupy the space between the neural tube and ectoderm; outer layers would differentiate into dermis, while cells in the medially situated inner layers differentiate into cartilage. This resembles the situation for neural crest-derived skull bones and dermis (Couly et al., 1993). It is noteworthy that in both cases *Msx1* is initially expressed in the entire mesenchyme. Differentiation then begins in the outer layers (as monitored by *Dermol* expression), while *Msx1* becomes progressively restricted to the inner layers (this article and data not shown).

**Migration of the dermis progenitor cells of the back**

Using *Msx1* as a marker, we show that dermis progenitor cells are present in the dorsal mesenchyme from early E11. Since

**Fig. 8.** Comparison of *Msx1<sup>lacZ</sup>* and *Dermol* expression in axial dermis progenitor cells. All embryos in this figure are heterozygous for the *Msx1<sup>lacZ</sup>* gene. They have been cryostat-sectioned and subsequently processed for  $\beta$ -galactosidase activity (A,C,E) or hybridised with a *Dermol* antisense probe (B,D,F). (A,B) Parallel sections through the trunk region of an E11 embryo. (A) *Msx1<sup>lacZ</sup>* gene is expressed in the mesenchyme overlying the neural tube and extending, laterally, to the most medial region of the somite (black arrowhead). (B) *Dermol* is detected in the dermis overlying the dermomyotome (between the black arrowhead and the white arrowhead), and in the lateral dermis (lateral to the white arrowhead). The black and white arrowheads mark equivalent levels in both pictures, and show that the expression domains of these two genes are virtually exclusive. (C,D) Parallel sections through the trunk region of an E12 embryo. Expression of the *Msx1<sup>lacZ</sup>* (C) and *Dermol* (D) genes are still exclusive. In the lateral part, *Msx1<sup>lacZ</sup>* is expressed in a deeper layer of the mesenchyme than *Dermol* (arrowheads). (E,F) Parallel sections through the trunk region of an E13 embryo. *Dermol* is expressed over the whole dorsal region of the embryo (F) in a cell layer immediately overlying the deeper mesenchymal layer that now expresses *Msx1<sup>lacZ</sup>* (E). nt, neural tube. The insets in A and C are enlargements of the mesenchymal layer expressing *Msx1*. Bars, 100  $\mu$ m.



*Msx1*- $\beta$ -gal<sup>+</sup> cells form a continuum in the back of the trunk between the dorsal neural tube and the somite, it is difficult to define precisely when dermal cells cease to leave the somites. However, our results show that the migration is completed by E13.

Somite maturation follows an anterior to posterior gradient of differentiation. Unexpectedly, *Msx1* expression does not follow this gradient. Immediately after the onset of its expression, it covers at least 18 segments down to the level of the hindlimbs. Furthermore, when medial or lateral dermomyotome halves replace the dorsal epithelial somite in mouse/chick chimeras, cells from the grafted halves are induced to migrate and form the totality of the somite-derived dermis, independent of the medial-lateral origin of the grafted piece. These results suggest that the capacity of dermomyotomal cells to form dermis is modulated by extrinsic signals until late in development.

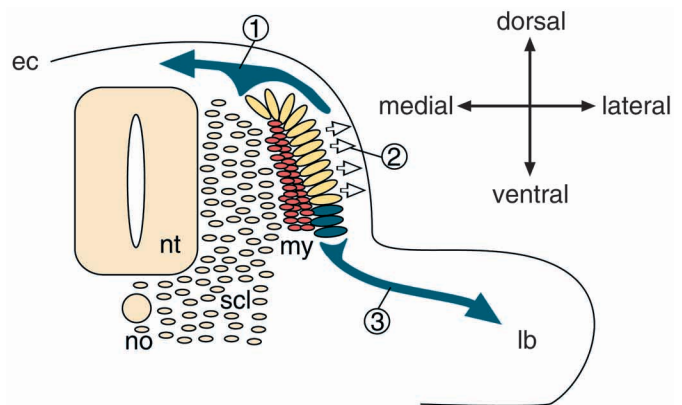
Limb muscle progenitor cells and dorsal dermis progenitor cells originate from the dermomyotome (reviewed in Christ and Ordahl, 1995). In mice, forelimb muscle progenitor cells migrate from the lateral edge of dermomyotomes at about E9 (Sze et al., 1995; Houzelstein et al., 1999). In *Splotch* mutants, lacking a functional *Pax3* gene, these cells do not migrate (Bober et al., 1994; Goulding et al., 1994; Daston et al., 1996). In this mutant, migration of trunk neural crest cells from the dorsal neural tube is also impaired. The defect is not intrinsic to the neural crest cells themselves, but appears to reflect inappropriate cell interactions either within the neural tube or between the neural tube and the somites (Serbedzija and McMahon, 1997). We show that, contrary to muscle progenitors and neural crest cells, dermis progenitor cells do not depend on *Pax3* for their migration from the somite.

### Two populations of dermis progenitor cells originate from the somite

An unexpected finding from our study was that *Msx1*<sup>lacZ</sup>-expressing cells were never detected in dermis progenitor cells overlying most of the dermomyotome, at any stage of development (see Fig. 9). However, in the chick, as well as in mouse/chick chimeras, these cells also originate from the somite and disaggregate from the dermatome (Mauger, 1972a; Christ et al., 1983; Brill et al., 1995 and our results).

We propose that two different dermis progenitor populations originating from the somites can be discriminated on the basis of *Msx1* and *Dermol* expression. The first, which expresses the *Msx1* gene, migrates and forms the dermis of the back; some cells in this population probably also contribute to the spinous process of the vertebra. In this population, *Dermol* is not detected before E13. The second forms laterally to the somite through disaggregation of the underlying dermatome and does not express the *Msx1* gene. In this population, *Dermol* is expressed from E11 (Fig. 9).

This is reminiscent of muscle cell differentiation. Limb muscle progenitor cells, derived from the lateral dermomyotome, express the *Msx1* gene during their migration from the somites into limb buds, while medially derived myotomal cells never express it (Houzelstein et al., 1999; Bendall et al., 1999). During both dermis and muscle differentiation, *Msx1* expression may be associated with a cell population that undergoes extensive migration and proliferation prior to differentiation.



**Fig. 9.** Scheme of *Msx1* expression pattern in the somitic derivatives. Progenitor cells of the most dorsal dermis migrate from the dermomyotome under the ectoderm and express *Msx1* (1; blue arrow). They express *Dermol* from E13. Progenitor cells of the dorso-lateral dermis delaminate from the dermomyotome and never express *Msx1* (2; white arrows). They express *Dermol* from E11. At the level of the forelimbs, muscle progenitor cells express *Msx1* in the lateral dermomyotome and during their migration into the limb bud (3; blue arrow). ec, ectoderm; lb, limb bud; my, myotome; no, notochord; nt, neural tube; scl, sclerotome.

Since *Msx1*-expressing cells are only detected in the most medial region of the trunk, it is possible that they originate from the most medial portion of the dermomyotome. Using a transgenic approach, Mackenzie et al. (1997) reported *Msx1* to be expressed in the dorsomedial lip of the dermomyotome. Cells of this lip express *Myf5* before differentiating into muscle. In *Myf5*<sup>-/-</sup> mutant, some of these cells migrate in the back of the embryo and express *Dermol* (Tajbakhsh et al., 1996). This result was suggesting that, at least in this mutant, cells of the dorsomedial lip of the dermomyotome remain multipotent and are able to differentiate into dermis. Defining the precise dermomyotomal origin of the somitically derived dermis in chimeric embryos remains an open issue. This is mainly due to the high plasticity of the dermomyotome and its capacity to adapt its fate to environmental cues (cf. grafts of medial versus lateral halves of dermomyotomes). However, preliminary results are in agreement with a possible medial origin of the dorsalmost dermis (Y. C., G. A.-B. and J. F.-P, unpublished results). A dedicated analysis of the contribution of different dermomyotomal domains to dorsal dermis is currently in progress.

### Insights into dermis differentiation

The *Dermol* gene has been shown to be expressed in dermis (Li et al., 1995). It is activated when the first morphological signs of periderm differentiation are detected in the embryo (M'Boneko and Merker, 1988), in accordance with concomitant differentiation of epidermis and dermis (Li et al., 1995). This gene is therefore a very convenient marker for the onset of dermis differentiation during development, although it is also expressed in some non-dermal tissues. In early E11 embryos, *Dermol* is activated in dorsal and ventral limb dermis derived from the somatopleural mesoderm, as well as in the facial dermis. In the dermis of the back, derived from cells that



have migrated from the somite, its expression is detected from E13 (Li et al., 1995; our results).

We show that, in the dorsal region of the trunk of E11 embryos, *Msx1* is expressed 2 days before any known marker, specifically in an external layer of cells that underlies the ectoderm and which is fated to differentiate into dermis. Since it has been previously shown that *Msx1* is expressed in the head mesenchyme, in the limb buds and lateral mesoderm (see Davidson, 1995, for a review) immediately before *Dermo1* is detected in cells derived from these tissues, *Msx1* may be expressed in most dermal progenitor cells in the embryo. The mutually exclusive expression patterns of *Dermo1* and *Msx1* suggest that dermis differentiates progressively from the periphery of the mesenchyme, and that *Msx1* is downregulated prior to the onset of dermis differentiation.

It has been proposed that *Msx1* may act as a repressor of differentiation in the muscle lineage (Song et al., 1992; Woloshin et al., 1995; Houzelstein et al., 1999). Its function would be to antagonise the myogenic activity of *Pax3* (Bendall et al., 1999). We propose here that, as for limb muscle differentiation, *Msx1* is downregulated immediately prior to the onset of differentiation of dermal cells. Therefore, it may act as a repressor of differentiation in dermis, which would permit progenitor cell migration and proliferation from a small stem cell population. However, considering the absence of overlapping expression of *Pax3* and *Msx1* in dorsal dermis progenitor cells as well as the absence of dermis defect in *Spotch* mutants, *Msx1* does not seem to interfere with *Pax3* activity in this lineage.

No alteration in dermis differentiation has been described in *Msx1*<sup>-/-</sup> mutants (Satokata and Maas, 1994; Houzelstein et al., 1997). This result may be due to functional redundancy with the *Msx2* gene that has a pattern of expression very similar to that of *Msx1*. Both genes are expressed in the dorsal trunk where their expression depends on very similar regulatory cues (Takahashi et al., 1992; Monsoro-Burq et al., 1994, 1996). Confirmation of the role of these genes in the migration/proliferation of a sub-population of somitically derived dermal progenitors will come from the analysis of the phenotype of mice in which both *Msx1* and *Msx2* are mutated.

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