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Differential regulation of the chick dorsal thoracic dermal progenitors from the medial dermomyotome

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SUMMARY

The chick dorsal feather-forming dermis originates from the dorsomedial somite and its formation depends primarily on Wnt1 from the dorsal neural tube. We investigate further the origin and specification of dermal progenitors from the medial dermomyotome. This comprises two distinct domains: the dorsomedial lip and a more central region (or intervening zone) that derives from it. We confirm that Wnt1 induces Wnt11 expression in the dorsomedial lip as previously shown, and show using DiI injections that some of these cells, which continue to express *Wnt11* migrate under the ectoderm, towards the midline, to form most of the dorsal dermis. Transplantation of left somites to the right side to reverse the mediolateral axis confirms this finding and moreover suggests the presence of an attractive or permissive environment produced by the midline tissues or/and a repellent or inadequate environment by the lateral tissues. By contrast, the dorsolateral dermal cells just delaminate from the surface of the intervening space, which expresses En1. Excision of the axial organs or the ectoderm, and grafting of Wnt1-secreting cells, shows that, although the two populations of dermal progenitors both requires Wnt1 for their survival, the signalling required for their specification differs. Indeed *Wnt11* expression relies on dorsal neural tube-derived *Wnt1*, while *En1* expression depends on the presence of the ectoderm. The dorsal feather-forming dermal progenitors thus appear to be differentially regulated by dorsal signals from the neural tube and the ectoderm, and derive directly and indirectly from the dorsomedial lip. As these two dermomyotomal populations are well known to also give rise to epaxial muscles, an isolated domain of the dermomyotome that contains only dermal precursors does not exist and none of the dermomyotomal domains can be considered uniquely as a dermatome.

Key words: Chick, Dermomyotome, Dermatome, Dermis, Feather, En1, Medial somite, Neural tube, Notochord, Skin, Somite, Wnt1, Wnt11

INTRODUCTION

The dorsal dermal cells appear progressively as part of a loose subectodermal mesenchyme that forms between days 3 (E3) and 5 (E5) of incubation in the chick. The origin of this dermis has been traced by chick/quail chimeras. In the dorsal region of the trunk, it derives from the somite dermomyotome (Mauger, 1972a), more precisely from its medial compartment (Olivera-Martinez et al., 2000). The *Dermo 1* gene is expressed in the newly dermis, which forms first at E4/E5 in the dorsomedial region in the chick (Scaal et al., 2001), and at an equivalent developmental stage (E11/E12) in the dorsolateral region in the mouse (Li et al., 1995). By grafts of mouse somites in chick hosts, it has been shown that the mouse subectodermal mesenchyme, which originates from the somites, is composed of two distinct medial and lateral populations that express, respectively, *Msx1* and *Dermo1* (Houzelstein et al., 2000). The same authors suggest that the most superficial dorsomedial mesenchyme downregulates *Msx1* prior turning on *Dermo1*. Besides the fact that the spatial appearance of dorsal subectodermal mesenchyme that expresses *Dermo1* is different in these two species, a basic question remains: where are the dermal progenitors located within the dermomyotome, which is known to be at the origin of other cell lineages, and how are they specified? In other words, the mechanisms of induction of dermal lineage(s) from the somite have not been explored.

Somites appear as epithelial structures that bud off from the presomitic mesoderm (Maroto and Pourquié, 2001). Soon after segmentation they become patterned along the dorsoventral and mediolateral axes. The ventral part forms a mesenchyme called the sclerotome, which gives rise to the vertebrae and at least part of the ribs (for reviews, see Christ and Ordahl, 1995; Ordahl et al., 2000). The dorsal epithelial part, called the dermomyotome, is known to produce the precursors of the striated muscles, and the scapular blade, as well as the dorsal

dermis (Aoyama and Asamoto, 1988; Christ and Ordahl, 1995; Huang and Christ, 2000; Huang et al., 2000a; Huang et al., 2000b; Mauger, 1972a). More precisely, the epaxial (dorsal) muscles (Denetclaw et al., 1997; Denetclaw and Ordahl, 2000; Kalcheim et al., 1999; Ordahl et al., 2001; Ordahl and le Douarin, 1992), as well as the feather-forming dorsal dermis (Olivera-Martinez et al., 2000), originate from the medial dermomyotome. Moreover, its dorsomedial lip (DML) has recently been shown (Ordahl et al., 2001) to drive growth and morphogenesis of both the primary myotome and the dermomyotome.

Extrinsic cues provided by surrounding tissues are responsible for the patterning of the somites (Aoyama and Asamoto, 1988; Borycki and Emerson, 2000; Dockter, 2000; Monsoro-Burq and le Douarin, 2000; Ordahl and le Douarin, 1992). The dorsal ectoderm and the dorsal neural tube have a dorsalizing effect on the somites, while the ventral notochord and floorplate exert a ventralizing effect (Brand-Saberi et al., 1993; Cossu et al., 1996a; Cossu et al., 1996b; Dietrich et al., 1997; Hirsinger et al., 1998). The lateral somite is specified by lateral plate-derived BMP4, that activates *Sim1* (Pourquié et al., 1996). This influence is antagonized by *Noggin* expression in the medial dermomyotome, which is activated by Wnt1 from the dorsal neural tube (Hirsinger et al., 1997; Marcelle et al., 1997). It is generally accepted that the epaxial myogenic lineage arises from the combined influences of notochord- and floorplate-derived Shh and dorsal neural tube Wnts (Borycki and Emerson, 2000; Munsterberg et al., 1995). In the absence of the neural tube and notochord, the medial somitic cells die, resulting in the absence of the vertebrae, dorsal muscles and ribs (Teillet et al., 1998), and also of the dorsal feather field (Mauger, 1972b; Olivera-Martinez et al., 2001).

Recently, we showed that the survival and specification of dorsal dermal progenitors relied on a signal from the dorsal neural tube (Olivera-Martinez et al., 2001). Wnt1 cells grafted in place of the axial organs (neural tube plus notochord) specifically restore the formation of a competent dorsal dermis, while no axial cartilage and almost no epaxial muscle form. This restored dermis possesses all the abilities that are characteristic (Dhouailly, 1977) of a dorsal dermis. It is able to induce the formation of a dorsal feather field in its overlying epidermis – the feather buds arising in longitudinal rows in a spatiotemporal sequence in accordance with the anteroposterior level of the Wnt1 cell graft (Olivera-Martinez et al., 2001).

We report that the medial compartment of the dermomyotome, which is the origin of the dermis (Olivera-Martinez et al., 2000), expresses *Wnt11* and *En1* in a complementary pattern, prior to their expression in the subectodermal mesenchyme. This allowed us to examine further the mechanism of induction and the involvement of these genes in the dermal lineage, and particularly the link between the dermomyotomal and mesenchymal populations. *Wnt11* has previously been shown to be expressed under the control of Wnt1 in the DML, and is suggested to be involved not only in myotomal but also in dorsal dermis development (Marcelle et al., 1997; Tanda et al., 1995). Active cell division in the DML leads to the formation of the dermomyotomal intervening space (Denetclaw et al., 1997; Denetclaw and Ordahl, 2000; Ordahl et al., 2001), which expresses *En1* and is suggested to produce myocytes in mice (Tajbakhsh and Buckingham, 2000). During hindbrain development, En1 expression was shown to be controlled by Wnt1 (Danielian and McMahon, 1996; Wurst et al., 1994). We analysed the possibility that *Wnt11* and *En1* are expressed under the control of Wnt1 in the dermomyotome, and are involved in the specification of dermal as well as myogenic progenitors, by using different experimental conditions that alter, inhibit or restore the formation of the dermis. Our results indicate that the chick feather-forming dorsal dermis derives directly and indirectly from the DML. The most medial dermal progenitors originate from the DML, turn on *Wnt11* under the influence of Wnt1 from the dorsal neural tube, and continue to express *Wnt11* while migrating under the dorsomedial ectoderm. A second, smaller non migrating population of dermal progenitors is located in the intermediate domain and derives indirectly from the DML, probably turning off *Wnt11* expression before turning on *En1* under the control of the ectoderm.

MATERIALS AND METHODS

In situ hybridization of whole mounts and sections

Chick *Sim1* probe was a gift of Dr Pourquié and has been described previously (Pourquié et al., 1996). *MyoD* probe was prepared as in Saitoh et al. (Saitoh et al., 1993). Chick *Wnt11* partial sequence (Tanda et al., 1995) was amplified by RT-PCR and cloned in pGEM-TEasy vector (Promega). The Chick *En1* cDNA plasmid was kindly provided by Dr C. Logan. Alkaline phosphatase-labelled whole-mount in situ hybridization were carried out as previously described (Wilkinson and Nieto, 1993). Hybridized embryos were embedded in 7.5% gelatin and 15% sucrose, frozen at –65°C and cryostat sectioned (30 μm).

Microsurgery

Fertilized chick eggs (JA957 strain, SFPA, St Marcellin, France) were incubated at 38°C until the embryos reached HH12-15 (17 to 24 somites). Microsurgery was performed in ovo as previously described (Teillet et al., 1998). To visualize the embryos, India ink (Pelikan) in Tyrode's solution (1/10) was injected between the embryonic tissues and the vitelline layer.

DiI injections

Dorsomedial lips of somites IV and V in HH14-15 embryos were microinjected with 0.5% (weight/volume) DiI (Molecular probes D-282) in dimethylformamide. After an additional 24 hours incubation period, embryos were embedded, sectioned and analysed under an Olympus AX 70 fluorescent microscope.

Transplantation of somites from the left to the right side

To reverse the mediolateral axis of differentiated somites, five adjacent somites (VIII to XII) from the left side of a donor embryo were transplanted in place of the right matched somites of a host embryo of the same stage (HH15-16). After an additional 36 hours incubation period, embryos were fixed for whole-mount in situ hybridization.

Axial organs excision (neural tube and notochord) and Wnt1 cell graft

The neural tube and the notochord were removed from somite X to chordoneural hinge. Cell aggregates were grafted from somite V to the unsegmented paraxial mesoderm or presomitic mesoderm (PSM), along a length equivalent to five presumptive somites, in order to maintain two excised, ungrafted regions anteriorly and posteriorly to the grafts as controls. A stable Wnt1-producing fibroblast Rat-B1a cell-line and the control cells were a gift of Dr R. Nusse. The day

before each operation, the cells were trypsinized and plated on uncoated bacterial Petri dishes to form the aggregates.

Neural tube excision

In order to study medial somitic lineages determination without the death observed in absence of the whole axial structures (Olivera-Martinez et al., 2001; Teillet et al., 1998), the neural tube alone was removed from somite X to the chordoneural hinge, leaving in place the notochord at all axial levels. To help separating the neural tube from the notochord, a drop of dispase (0.1 mg/ml) was added, then rinsed with Tyrode's solution and inactivated by foetal calf serum or albumin.

Ectoderm removal

The ectoderm was removed with tungsten needles after enzymatic treatment with dispase (0.1 mg/ml). The anteroposterior extension of the removal was from somite V to an equivalent length in the PSM. Along the mediolateral axis, the ectoderm removal was unilateral or bilateral. For unilateral excisions, a slit in the ectoderm was made between the somitic mesoderm and the neural tube, and another one in the ectoderm overlying the lateral plate mesoderm, at a distance from the segmental plate equivalent to the width of a somite. For bilateral excisions the ectoderm was removed from the right lateral plate to the left lateral plate as previously.

RESULTS

Wnt11, *En1* and *Sim1* are expressed in distinct domains in the dermomyotome and later in the subectodermal mesenchyme

In order to study the origin of the chick dorsal dermis precursors from the dermomyotome, we analysed the expression of three different dermomyotomal markers: *Wnt11*, *En1* and *Sim1*. As a myogenic marker we used *MyoD*, which is expressed only in postmitotic myocytes, in contrast to expression of *Myf*5, the onset of which is still controversial (Borycki et al., 1997; Hacker and Guthrie, 1998; Hirsinger et al., 1998; Pownall and Emerson, 1992) and which was recently shown to be transiently expressed in non-muscle progenitors (Kiefer and Hauschka, 2001).

Wnt11 transcripts first appear in the dorsomedial lip (DML) of the rostral somites at HH14, then expand caudally, but not beyond somite IV (±1), as already described (Marcelle et al., 1997; Tanda et al., 1995). We show that at HH18-19 at the forelimb level, *Wnt11* expression is detected not only in the DML, but also in isolated cells located between the DML and the dorsal neural tube (Fig. 1A-C). The last isolated *Wnt11*-expressing cells appear on the medial edge of somite XVI (±1). At the same stage, *En1* expression is detected in a subset of dermomyotomal cells, in a central position (Fig. 1D-F), from the VIIIth (±1) to the head somites. En1 domain can be allocated to the medial compartment since it is distinct from a third domain expressing *Sim1* (Fig. 1G-I). This *Sim1* somitic expression in chick characterizes the lateral territory (Pourquié et al., 1996) (Fig. 1G-I). Committed myoblasts expressing *MyoD* are detected under the DML (Fig. 1J-L), as previously shown (Hirsinger et al., 2001; Pownall and Emerson, 1992; Saitoh et al., 1993).

One day later (HH23), *Wnt11* transcripts are present in the subectodermal cells, that form the dorsomedial mesenchyme (Fig. 2A-C). *En1* transcripts are detected at the same stage in the mesenchyme in the lateral part of the trunk, its expression domain presenting a border near the trunk/limb junction (Fig. 2D-F). At this stage, *Sim1* is expressed in a few mesenchymal cells at the epaxial/hypaxial border (Fig. 2G-I). *MyoD* expression is localized underneath this loose mesenchyme (Fig. 2J-L) in epaxial myotome fibres known to derive from the DML (Christ et al., 1977; Cinnamon et al., 2001; Jacob et al., 1978; Ordahl et al., 2001; Solursh et al., 1987).

The dorsomedial subectodermal mesenchyme is formed by *Wnt11* positive cells, which migrate from the dorsomedial lip

In order to test whether the *Wnt11*-expressing cells of the subectodermal space at HH22-23 have effectively detached

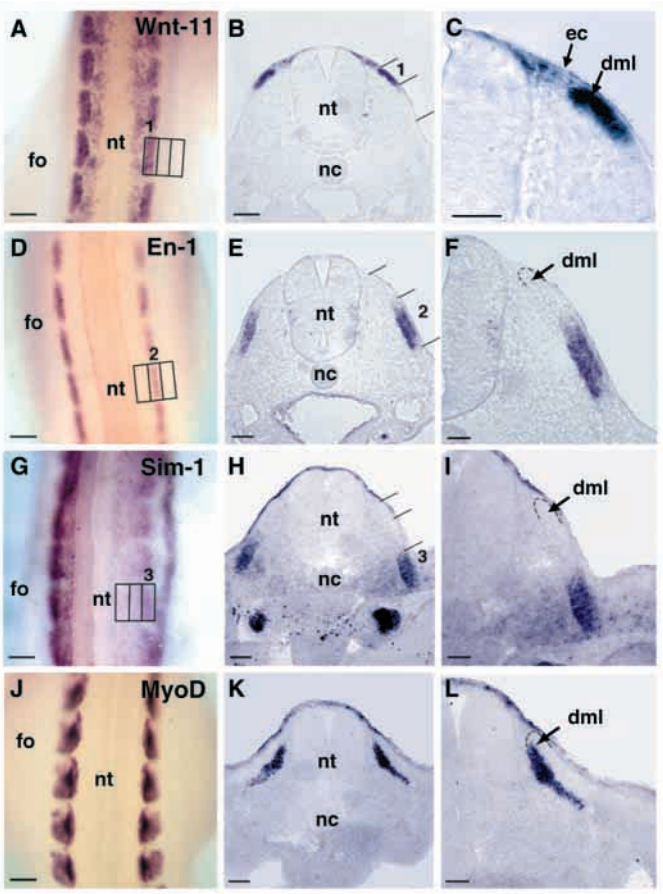

Fig. 1. *Wnt11* and *En1* are expressed in complementary domains in the chick medial dermomyotome. Dorsal views at HH18 of the forelimb (fo) level, and corresponding transversal sections, hybridized with *Wnt11* (A-C), *En1* (D-F), *Sim1* (G-I) and *MyoD* (J-L) probes. Rectangles outline one somite that involves three regions along the mediolateral axis. (A-C) *Wnt11* transcripts are detected in the dorsomedial lip (dml) (region 1) in the dermomyotomal cells closest to the dorsal neural tube (nt). Isolated *Wnt11*-expressing cells are also detected dorsomedially to the lip, under the ectoderm (ec). (D-F) *En1* is expressed in a central compartment of the dermomyotome (region 2), in a domain distinct from that of *Wnt11*. (G-I) The lateral third of the dermomyotome expresses *Sim1* (region 3) and is distinct from the *En1* domain. (J-L) *MyoD* positive cells are detected under the dermomyotomal epithelial sheet. nc: notochord. Scale bars: 100 μm in A,B,D,E,G,H,J,K; 50 μm in C,F,I,L.

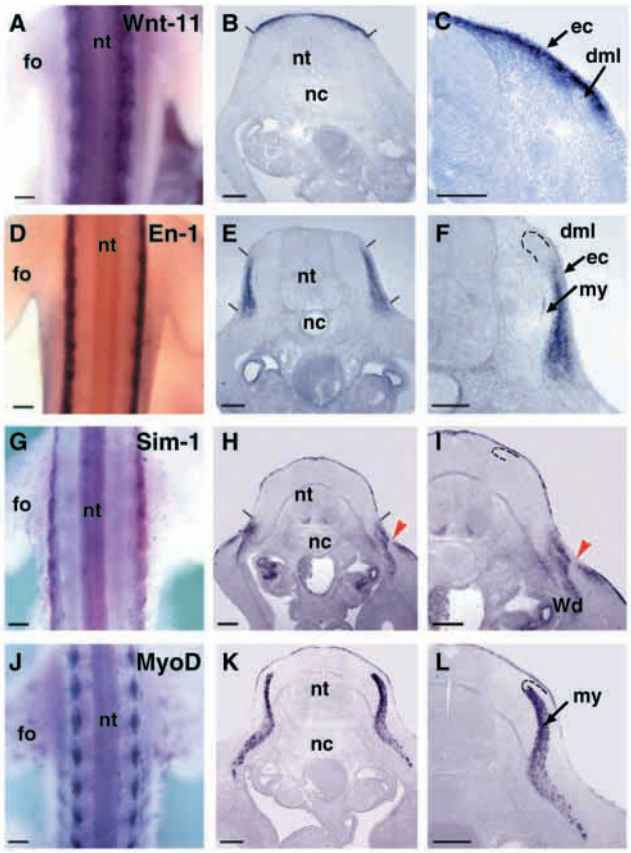

Fig. 2. *Wnt11* and *En1* are expressed in distinct populations of the chick dorsal subectodermal mesenchyme in the epaxial domain. Dorsal views at HH23 of the forelimb (fo) level, and corresponding sections, hybridized with *Wnt11* (A-C), *En1* (D-F), *Sim1* (G-I) and *MyoD* (J-L) probes. (A-C) *Wnt11* transcripts are detected in the dorsomedial subectodermal mesenchyme. (D-F) *En1* expression is detected in the dorsolateral mesenchyme. (G-I) *Sim1* is detected in a few mesenchymal cells on each side of a notch (arrowhead) in the ectoderm, at the limit between the epaxial and the hypaxial domain, as well as in the Wolffian duct (Wd). (J-L) *MyoD*-positive cells are localized below the *En1*-and the *Sim1*-expressing cells, in the myotome (my). ec, ectoderm; dml, dorsomedial lip; nc, notochord; nt, neural tube. Scale bars: 100 μm in A,B,D,E,G,H,J,K; 50 μm for C,F,I,L.

from the DML and migrated towards the midline, we carried out DiI injections in the DML at stage HH17 (Fig. 3A,B). After 24 hours (n=3) two groups of labelled cells were found (Fig. 3C). The first one, in the epaxial myotome as expected (Denetclaw and Ordahl, 2000), constituted by progenitors that translocate from the DML under the dermomyotome. Interestingly, a second group of labelled cells migrated dorsally towards the midline to form the subectodermal mesenchyme. This second group corresponds to cells that continue to express *Wnt11*. The injection of DiI in the central dermomyotome (data not shown) showed cells that detach from the intervening space and do not migrate further, as previously noted (Denetclaw et al., 1997; Denetclaw and Ordahl, 2000).

In order to challenge the capacity of DML cells to migrate towards the midline, we carried out somite transplantation from left to right to reverse the mediolateral axis (Fig. 3D). Six hours after the transplantation, the DML still expresses *Wnt11*, now in a lateral position, in the transplanted somites (data not shown). Ten hours later, *Wnt11* mesenchymal cells are also detected over the central part of the somite, below the ectoderm (data not shown). After 24 hours, they colonize the subectodermal space from the transposed DML towards the midline, by progressing over the entire dermomyotome (Fig. 3E,G).

Wnt11 and *En1* fail to be activated in absence of the neural tube and notochord, their expression being restored by Wnt1 cell grafts

In the absence of neural tube and notochord, a proper dorsal dermis, which is able to induce the formation of feathers, does not form (Mauger, 1972b; Olivera-Martinez et al., 2001). The graft of Wnt1-producing cells is sufficient to restore the survival and differentiation of the medial somitic cells towards the dermal lineage (Olivera-Martinez et al., 2001). In order to investigate whether *Wnt11* and *En1* expression are both restored in the paraxial mesoderm, the expression of dermomyotomal and myotomal markers was analysed between 6 and 48 hours after removal of the axial organs and graft of Wnt1 cells (Fig. 4A).

At the time of the operation, *Sim1* and *MyoD* were already expressed in the last formed somites, in contrast to *En1* and *Wnt11*. Results of excision of the neural tube and notochord were similar, regardless of whether they were followed by the graft of RatB1 control cells (data not shown). In the excised ungrafted regions, the domain of expression of *Sim1* extended medially, as expected (Fig. 4B,C), as early as 6 hours after the operation (data not shown). *MyoD* expression decreased until lost, here shown at 24 hours (Fig. 4E) as previously described (Teillet et al., 1998). Always in these excised ungrafted regions, *Wnt11* and *En1* were not detected after 24 (n=2 and 8), and 48 hours (n=3 and 4), respectively (Fig. 4G, I). By contrast, around the Wnt1 cell aggregates, *Sim1* expression was restricted to the lateral part of the somite, as in normal development, at all the stages analysed (n=10) (Fig. 4B,D). Some *MyoD*-positive cells were detected deeply in the somite near the Wnt1 cells, 12 hours (n=2) or 24 hours (n=5) after the operation (Fig. 4E,F). *Wnt11* was activated by the Wnt1 cells, as detected after 24 hours (n=2). Forty-eight hours after the operation (n=3), its expression was present in an epithelial structure reminiscent of the DML, in close contact with the graft and under the ectoderm (Fig. 4G,H). *En1* transcripts were expressed near the Wnt1 cells (n=9) (Fig. 4I,J).

The signal required to activate *En1* expression in the dermomyotome originates from the ectoderm and not from the neural tube

An intrinsic experimental problem is that many of the factors produced by the axial organs that act as patterning signals are necessary also for cell survival. Shh from the notochord and the floorplate, known as a ventral and myogenic factor (for a review, see Dockter, 2000), also allows survival of the medial somite (Teillet et al., 1998). Thus, we analysed the expression of *Wnt11* and *En1* in the presence of the notochord that allows medial somitic cells to survive, but in absence of the neural tube that expresses *Wnt1* (Rong et al., 1992; Teillet et al., 1998; Teillet and le Douarin, 1983).

Therefore, we excised only the neural tube, leaving the notochord in place (Fig. 5A). Forty-eight hours after the excision, somites segmented prior to the operation expressed *Wnt11* (*n*=3) and *En1* (*n*=3) (Fig. 5B,C,E,F). By contrast, only *En1* expression was initiated in the former presomitic mesoderm, which fused in a single mass of cells forming a ribbon under the ectoderm (*n*=3) (Fig. 5B,D,E,G). Consequently, *En1* onset does not require a neural tube factor and we set out to test if the ectoderm provided a signal involved in the activation of *En1* in the dermomyotome.

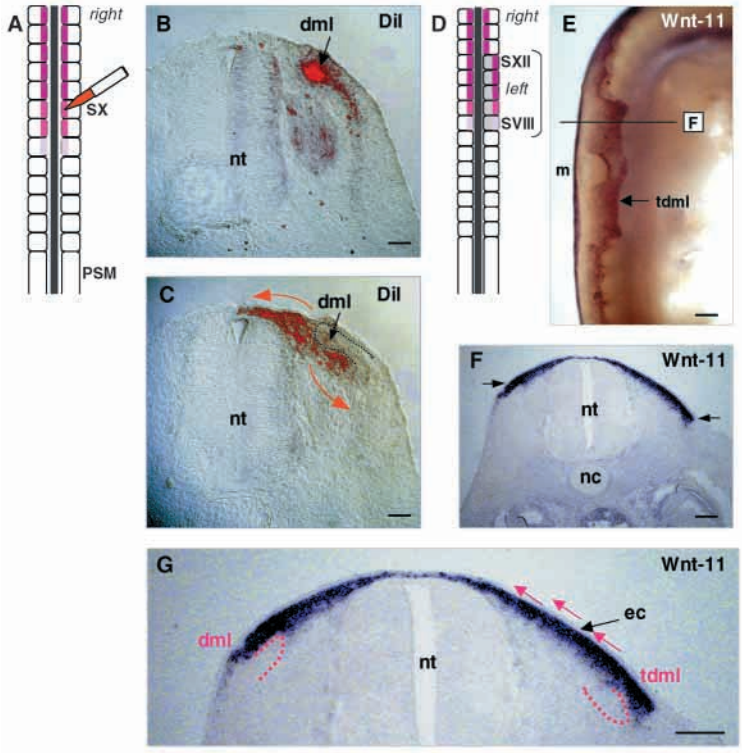

Fig. 3. Migratory behaviour of cells from the chick somitic dorsomedial lip. (A-C) DiI labelling of the dorsomedial lip (dml) of somite X (SX) (A, schematic) shows that dml cells (B, time zero, transverse section) give rise (C) to two distinct populations that migrate in opposite directions after 24 hours (red arrows). (D-F) Transplantation of five left-side somites, from a donor to the right side of a host embryo (D, schematic). (E-G) Hybridization with *Wnt11* probe shows that after 24 hours, the Wnt11 cells have migrated from the transposed dorsomedial lip (tdml) over the entire dermomyotome towards the midline (m). This is seen in an external view of the right side (E) and in a transversal section (F, arrows). A higher magnification (G) clearly shows the migration of the cells from the tdml under the ectoderm (red arrows). nc, notochord; nt, neural tube; PSM, presomitic mesoderm. Scale bars: 50 μm in B,C; 300 μm in E; 70 μm in F,G.

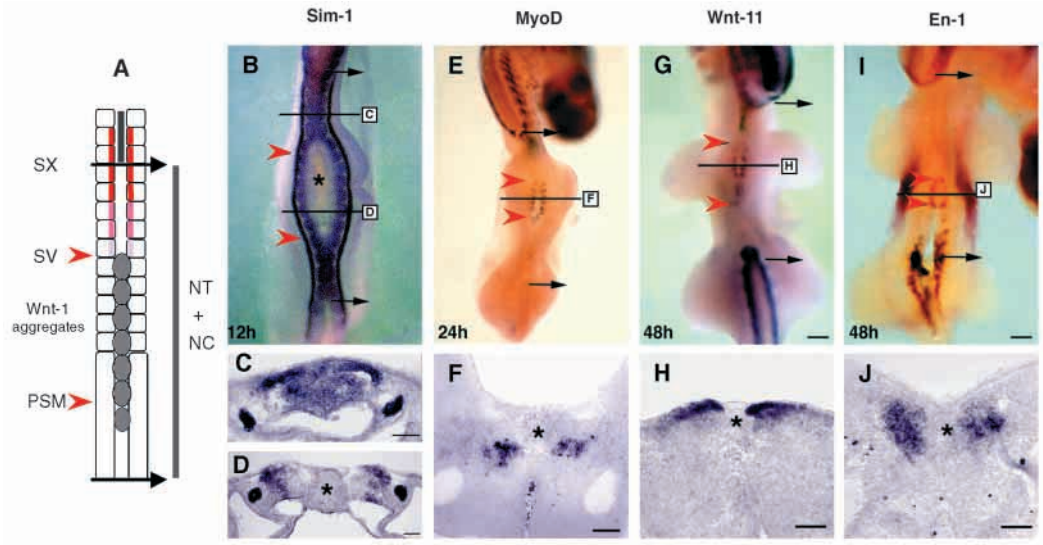

Fig. 4. Wnt1 cells grafted in place of neural tube and notochord inhibit *Sim1* and trigger both *Wnt11* and *En1* expression in the medial dermomyotome. (A) A fragment of neural tube (NT) plus notochord (NC) was excised at HH13 between somite X (SX) and the chordoneural hinge (arrows). Wnt1 cell aggregates were grafted from somite V (SV) to the unsegmented presomitic mesoderm (PSM) in a length equivalent to five presumptive somites (red arrowheads), in order to maintain two excised ungrafted regions as controls. Embryos were fixed 12 hours (B-D), 24 hours (E-F) or 48 hours (G-J) after the operation. Dorsal views and corresponding transversal section, hybridized with *Sim1* (B-D), *MyoD* (E, F), *Wnt11* (G, H) and *En1* (I, J) probes. (B-D) *Sim1* expression expanded medially in excised ungrafted regions (B,C), while its normal lateral domain of expression was restored around the Wnt1 cell aggregate (asterisk) (B,D). (E,F) *MyoD* was expressed on both sides of the Wnt1 cell aggregate, in a deep position (F). (G,H) *Wnt11* was activated on both sides of the Wnt1 cell aggregate (asterisk) in superficial epithelial structures reminiscent of the dorsomedial lip (H). (I,J) *En1* was detected around the graft, in an intermediate mesenchymal population (J). Scale bars: 50 μm.

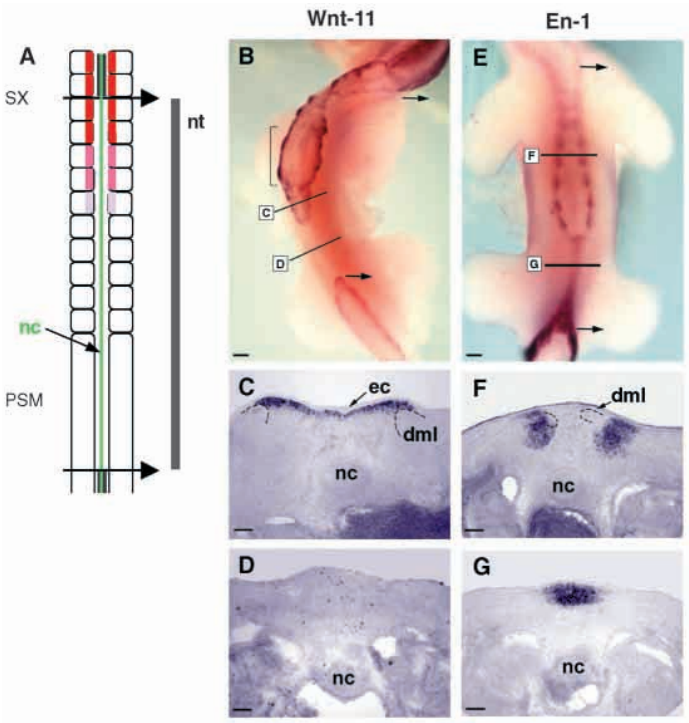

Fig. 5. After only neural tube removal, *En1* but not *Wnt11* is activated at the level of the presomitic mesoderm (PSM). (A) The neural tube (nt) was removed from somite X to the PSM (between the arrows) leaving the notochord (nc) in place. Embryos were fixed after 48 hours (in B, the bracket indicates a region where a ventral part of neural tube was accidentally left in place). Dorsal views and corresponding transverse sections of embryos hybridized with *Wnt11* (B-D) or *En1* (E-G) probes. (B,C,E,F) At the anterior level, where somites were already segmented, *Wnt11* (B,C) and *En1* (E,F) are expressed in distinct domains of the mesenchyme. (B,D,E,G) At the posterior level, where somites were still unsegmented, *Wnt11* is not activated (B,D), in contrast to *En1*, the expression domains of which are fused in a single medial band (E,G). ec, ectoderm; dml, dorsomedial lip. Scale bars: 50 μm.

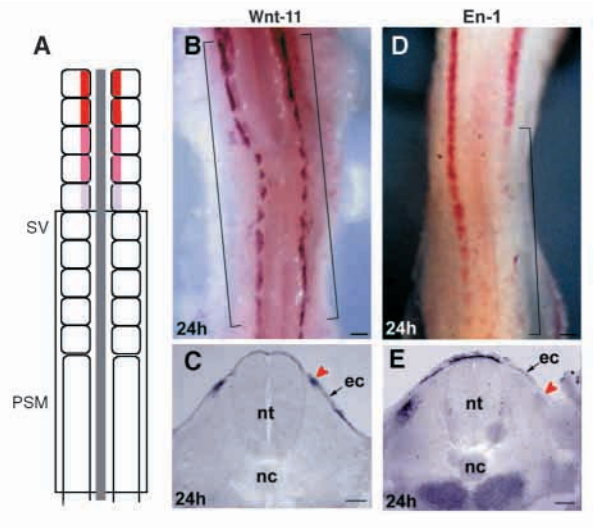

Fig. 6. The activation of *En1* but not of *Wnt11* depends on an ectodermal signal. (A) The ectoderm was removed bilaterally (or unilaterally, see text), over the dorsal region, from somite V (SV) to an equivalent length in the presomitic mesoderm (PSM). Dorsal views and corresponding transverse sections of the thoracic region, 24 hours after bilateral (B,C) or unilateral (D,E) ectodermal excision (indicated by the brackets), hybridized with *Wnt11* (B,C) or *En1* (D,E) probes. Note the complete healing of the ectoderm (ec), as shown in transverse sections (C,E). (B,C) *Wnt11* transcripts are detected (arrowhead) in all somites, although in the central region of the excision they are smaller than normal. (D,E) *En1* is absent (arrowhead) from the operated side, in contrast to the non-operated side. nc, notochord; nt, neural tube. Scale bars: 50 μm.

Bilateral (Fig. 6A) or unilateral excisions of the dorsal ectoderm were carried out on HH12-14 embryos (*n*=17). In these experimental conditions, the ectoderm heals in an average of 16 hours, as previously shown (Thévenet, 1969). This type of operation leads to smaller somites in the central excised region, where the ectoderm probably heals last (Fig. 6B). Nevertheless, *Wnt11* expression was activated at all axial levels, in all the recovered cases: 24 hours (Fig. 6B,C) (*n*=4), 36 hours (*n*=3) and 48 hours (*n*=4) later (data not shown). It confirms that *Wnt11* onset depends only on Wnt1 from the neural tube. By contrast, *En1* expression failed to be activated at excised levels 24 hours after the operation (*n*=3), although the ectoderm had already healed (Fig. 6D,E). Forty-eight hours later (*n*=3), En1 expression reappears (data not shown), and although its domain of expression is reduced, we already know that no abnormalities can be detected 8 days after a similar microsurgery in the dorsal feather field (Olivera-Martinez et al., 2001).

DISCUSSION

Our previous results have shown that (1) the dermal progenitors that form the dorsal feather field originate from the medial dermomyotome, and (2) in the absence of the neural tube and notochord, Wnt1 signalling is able to restore the formation of the dermal derivative (Olivera-Martinez et al., 2001). Here, in order to examine the specification in the dermomyotome of the dorsal dermal progenitors, we have analysed the expression of two potential *Wnt1* targets, *Wnt11* and *En1*. These two genes are expressed in distinct domains of the medial dermomyotomal compartment in cells that further colonize the subectodermal space. Although *Wnt11* is activated by *Wnt1* from the neural tube, this signal acts only as a survival factor for *En1* cells, the initiation of *En1* expression being dependant on the presence of the ectoderm.

Some of the *Wnt11* cells activated by neural tube Wnt1 migrate from the dorsomedial lip to form the dorsomedial dermis

The DML of the dermomyotome is a site of high mitotic activity (Borycki and Emerson, 2000; Denetclaw and Ordahl, 2000; Kalcheim et al., 1999; Ordahl et al., 2001), and expresses *Wnt11* from around somite IV (Marcelle et al., 1999; Tanda et al., 1995) (present work). While these cells are dividing, they are probably not committed to any lineage, although they express *Myf5* (Hacker and Guthrie, 1998; Hirsinger et al., 2000). Moreover, it has recently been shown (Kiefer and

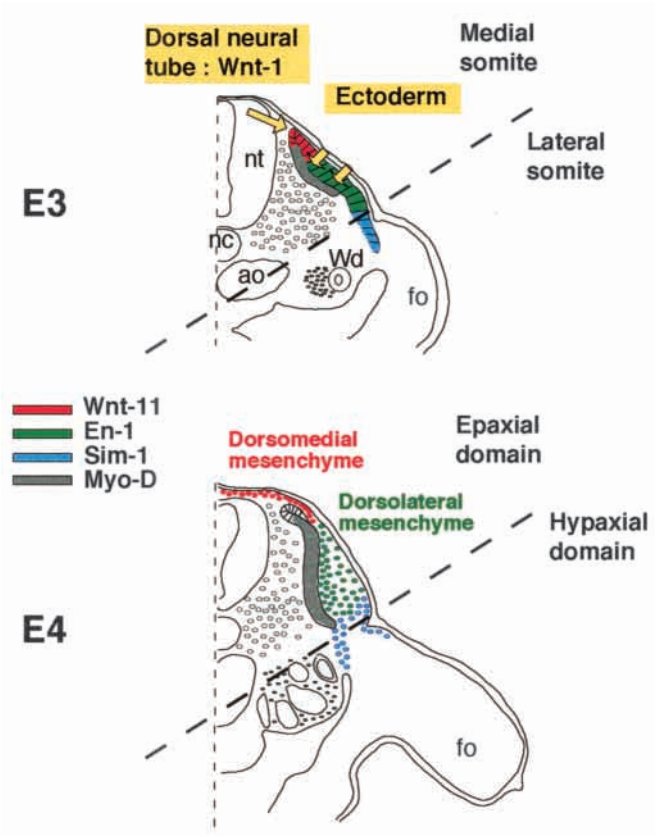

Fig. 7. Regulation and localization of the thoracic dermal progenitors in chick embryo. At E3, activation by the dorsal neural tube of *Wnt11* in the DML, then by the ectoderm of *En1* in the intervening space of the medial dermomyotome. At E4, formation by migration of the dorsomedial and by delamination of the dorsolateral mesenchyme, which continue to express respectively *Wnt11* and *En1*. It should be noted that a narrow third population that expresses *Sim1* forms the border between the epaxial and hypaxial domains. ao, aorta; fo, forelimb; nc, notochord; nt, neural tube; Wd, Wolffian duct.

Hauschka, 2001) that only a subset of cells that initially express Myf5 will upregulate its expression and differentiate as muscle. This subset translocates from the DML under the epithelial dermomyotome, does no more express *Wnt11*, begins to express *MyoD* and forms the epaxial myogenic progenitors (Cinnamon et al., 2001; Cinnamon et al., 1999; Denetclaw et al., 1997; Hirsinger et al., 2000; Ordahl et al., 2001; Ordahl and le Douarin, 1992; Ordahl et al., 2000). We show that beginning around somite XVI at stage HH18, *Wnt11* transcripts are also present in mesenchymal cells dorsomedially to the lip, prior to their detection in the subectodermal mesenchyme of the dorsomedial region. DiI labelling (our results) (Denetclaw et al., 1997), as well as chick/quail chimaeras of the DML (Ordahl et al., 2001), demonstrate that cells from this region can migrate towards the midline. As these dermal precursors continue to express *Wnt11* while migrating and when they have reached their target under the ectoderm, it is possible that Wnt11 coordinates the movement of dermal precursors in an autocrine or paracrine loop. Wnt11 has indeed been shown to be implicated in coordinating convergent extension movements during gastrulation in zebrafish (Heisenberg et al., 2000) and *Xenopus* (Tada and Smith, 2000) using a pathway that diverges from the canonical β-catenin pathway at the level of Disheveled. Interestingly, after the transplantation of differentiated somites from left to the right, *Wnt11* is still expressed by the DML that is now in a lateral location. More surprisingly, Wnt11-positive cells that detach from the DML still move towards the midline. These cells can thus either respond to attractive signals from the axial tissues, or have an affinity for extracellular materials present under the dorsomedial ectoderm and above the somite. Another possibility is that they are repelled or simply cannot move on the intermediate or lateral plate mesoderm. These possibilities still remain to be tested and the molecules involved identified.

The fact that *Wnt11* somitic expression relies on Wnt1 was already shown and confirmed by its ectopic activation in the lateral dermomyotome (Marcelle et al., 1997; Tanda et al., 1995). We show further that the absence of the ectoderm does not interfere with *Wnt11* onset in the dorsal dermal progenitors. The Wnt1 signal probably acts through the β-catenin pathway in this system (Schmidt et al., 2000). The fact that Wnt1 inhibits apoptosis through β-catenin (Chen and Chuong, 2000) is in agreement with the fact that Wnt1 could partially restore cell survival in the medial somite after removal of the neural tube and notochord (Olivera-Martinez et al., 2001). In particular, this would permit the survival of cells of the growing intervening space that will come to express *En1* under the control of the ectoderm.

Cells that detach from the highly mitotic DML undergo an epithelial-mesenchymal transition. The migration from the DML occurs over an extended period of time ranging from E2 to E5 at different levels of the anteroposterior axis in the chick embryo. Previous results have led to the conclusion that the epithelial-mesenchymal conversion of the dermomyotome requires a neurotrophin 3 (NT3) signal from the neural tube (Brill et al., 1995). However, in the absence of the neural tube, and thus in the absence of the NT3 signal, Wnt1 appears sufficient to restore the formation of a nearly normal dermis, suggesting that NT3 is not the only factor involved in the formation of the loose subectodermal mesenchyme. NT3 might be also one of the factors that allow the migration of the DML cells towards the dorsal neural tube. It would be interesting to study if NT3 controls the epithelial-mesenchymal transition by acting downstream of Wnt1 or by antagonizing ectodermal signals. Indeed, recent results show that the ectoderm is required to maintain the epithelial structure of the dermomyotome (Marcelle, personal communication). Wnt molecules, known to be expressed in the ectoderm from the mouse (Fan et al., 1997) as well as from the chick (Cauthen et al., 2001; Schubert et al., 2002; Wagner et al., 2000) may mediate this effect.

Some of the *En1* cells controlled by the ectoderm delaminate from the intervening space to form the dorsolateral dermis

En1 is expressed in a central dermomyotomal population, next to the *Wnt11*-expressing cells, that was originally called a dermatome (Davidson et al., 1988; Davis et al., 1991). Truly, it must still be considered to be dermomyotome as some of these cells give rise to myogenic cells (Hadchouel et al., 2000;

Ordahl et al., 2001; Tajbakhsh and Buckingham, 2000). As the *En1*-expressing region is distinct from the *Sim1* domain, and given that *Sim1* in the chick is considered as a marker of the lateral compartment (Pourquié et al., 1996), then we conclude that the *En1* domain can be allocated to the medial compartment of the dermomyotome. As the DML cells divide, they progressively generate an intervening space or central dermomyotome domain (Denetclaw and Ordahl, 2000; Ordahl et al., 2001) that corresponds to the *En1*-positive domain. As observed in other developmental systems such as the limb bud (Cygan et al., 1997; Laufer et al., 1997), the *En1*- and *Wnt11*-expressing cell populations do not appear to overlap. The cells that express *En1* in the central dermomyotome might previously have expressed *Wnt11*, but if so they have subsequently turned it off. Indeed, grafting of quail medial presomitic mesoderm in chick (Olivera-Martinez et al., 2000) leads to the formation of a dorsal dermal region that includes the *Wnt11* and the *En1* domains. Some of these *En1*-expressing cells constitute a second population of thoracic dermal precursors that undergo an epithelial-mesenchymal transition. These cells do not migrate, as they are already located at their final position and detach from the dermomyotome under the ectoderm to form the dorsolateral dermal region. This process might also be controlled by a NT3 signal from the dorsal neural tube (Brill et al., 1995).

Engrailed is known to be activated by Wg/Wnt proteins in various organisms, from *Drosophila* to vertebrates (Danielian and McMahon, 1996; Heemskerk et al., 1991; Ingham et al., 1988; Logan et al., 1997). We show here that the onset of *En1* expression in the somite does not depend on Wnt1 from the neural tube, but on an ectodermal signal. After the ectoderm excision, the absence of *En1* expression is transient, owing to the rapid healing of the ectoderm (our data) (Nodder and Martin, 1997; Thévenet, 1969) and does not lead to any perturbation of the dermis of the dorsal feather field as observed at 10 days (Olivera-Martinez et al., 2001). Wnt family members described in the ectoderm (Cauthen et al., 2001; Fan et al., 1997; Wagner et al., 2000) could be responsible for *En1* onset in the dermomyotome. Wnt6 was recently shown to be expressed throughout the chick dorsal ectoderm (Schubert et al., 2002), but its expression is strangely lowest above the zone of En1 expression in the central dermomyotome. Thus the identity of the ectodermal factor responsible for *En1* activation remains to be elucidated and is currently under examination. A second interesting issue that remains to be explored is the role of *En1* expression in this region. En1 is well known to be a repressor of transcription. It would be tempting to speculate that it could be acting as an inhibitor of dermal differentiation, given the fact that in the chick the initiation of skin differentiation proceeds in a gradient from the midline (where *Wnt11* is expressed) to the lateral dermis (where *En1* is expressed).

A dermatome does not exist as a discrete entity in the dermomyotome

Our data showing the role of the DML in the formation of dermis are in accordance with recent results (Ordahl et al., 2001) according to which this structure would give rise not only to epaxial muscles, as already well known, but also to dermal cells. Likewise, it is very likely that the En1 dermomyotomal domain contributes to lineages other than the dorsal dermis: in mice, this domain has been suggested to give rise to myotomal cells (Hadchouel et al., 2000; Ordahl et al., 2001; Tajbakhsh and Buckingham, 2000). *Wnt11* and *En1* are thus expressed in, at least, bi-potential progenitors. Taken together, these data imply that a discrete dermatome cannot be distinguished in the dermomyotome. Indeed, the dermal, as well as the muscle cell precursors can be identified only when they leave the same region of the epithelial dorsal somite, which thus deserves to be called a dermomyotome in its entirety.

Comparison between formation of the dorsal populations in chick and in mouse

In the chick embryo, dorsal dermis forms mainly by *Wnt11*-migrating cells that colonize the dorsomedial space under the ectoderm. In a lesser proportion, *En1* cells also contribute to the dermis that form the borders of the thoracic dorsal field. In this species, the first dense dorsal dermis expresses *Dermo 1* over the neural tube (Scaal et al., 2001) where the first rows of feathers differentiate. In chick, *Dermo 1* expression thus begins medially and then extends laterally, over both the territories previously labelled by *Wnt11* and *En1*, and even beyond, in the ventral dermis. By contrast, in mouse embryo, both the expression of *Dermo 1* (Li et al., 1995) and the formation of the first cutaneous appendages appear laterally, and only later extend over the neural tube. In chick, a narrow dorsomedial *Msx1* population was shown to be the origin of the spinous processes of the vertebra (Monsoro-Burq and le Douarin, 2000), while in mouse dorsomedial *Msx1*-expressing cells have been interpreted as a population of dermal cells, as it appears to originate from the dermomyotome (Houzelstein et al., 2000). An interesting comparison is a possible functional equivalence between Msx1 in mouse and En1 in the chick. As these proteins are known to be transcriptional repressors, they could indeed both prevent premature differentiation of the dermis, as already proposed in the case of *Msx1* in the mouse (Houzelstein et al., 2000). More precisely, the predermal cells that express *En1* could be delayed in the acquisition of their feather-inducing abilities, corresponding with the delay in their expression of *Dermo 1*. Anyway, defining the precise dermomyotomal origin and the migratory behaviour of the somitically derived dermis remains an unresolved issue in the mouse. Moreover, it cannot be easily resolved by mouse/chick chimaeras as the site of initiation of dorsal dermis formation is different.

Origin, induction and migration of the dorsal thoracic feather-forming dermis

The present findings, together with our previous work and that of others studying somite patterning, lead us to propose the following model for the origin and specification of the dorsal predermal mesenchyme in the chick thoracic region (Fig. 7). The dermal progenitors arise from the medial somite that forms under the influence of Wnt1 from the dorsal neural tube. Subsequently, Wnt1 activates *Wnt11* expression in the DML. While the DML divides, it receives different dorsal and/or ventral signals, and gives rise to at least three kinds of cells: (1) myogenic cells that translocate under the epithelial sheet and express *MyoD*; (2) dermal progenitors that detach from the lip, continue to express *Wnt11* and migrate towards the dorsal midline; and (3) cells of the growing dermomyotomal

intervening zone that begin to express *En1* under the ectodermal influence and form, among other derivatives, a second group of dermal progenitors. Moreover, a narrow ribbon of Sim1 expressing cells forms the border between the epaxial and hypaxial domains and may form a third and small group of dorsal dermal progenitors. This population could give rise to the semi-apteric (semi-glabrous) region between the dorsal and ventral feather pterylae (feather fields); this possibility is currently under examination.

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