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Molecular mechanisms controlling dorsal dermis generation from the somitic dermomyotome

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ABSTRACT The initiation of the development of skin appendages (hair/feathers/scales) requires a signal from the competent dense dermis to the epidermis (Dhouailly, 1977). It is therefore essential to understand how to make a competent dermis. In recent years, a few studies have focused on the development of the dorsal dermis from the somitic dermomyotome. Our first aim in this review is to attempt to reconcile the available data on the origin of the dorsal dermis and summarize the present knowledge on the molecular mechanisms implicated in dermal lineage induction. Secondly, we open the discussion on the formation of a loose pre-dermal mesenchyme and more importantly of a dense dermis capable of participating in appendage development. To go further we draw a comparison between the chick and mouse systems to gain a new insight into how to initiate appendage morphogenesis and regulate the extent of hair/feather fields.

KEY WORDS: *chick, mouse, somite, Wnt*

Introduction

Two main types of dermis are present in birds and mammals at the onset of skin morphogenesis: a superficial dense dermis (overlying a deep sparse dermis) characteristic of future feather or hair fields, versus a superficial loose dermis in future bare skin regions. The next visible step of skin differentiation, consists of the redistribution of cells of the dense superficial dermis to form a regular array of local condensations (future dermal papillae) separated by a loose interfollicular dermis (F. Michon, personal communication). Those dermal condensations interact with the epidermis to form cutaneous appendages according to the species. The origin of the dermis has been traced by chick/quail chimerae: the head and neck dermis derives from neural crest (Couly and Le Douarin, 1988), while the lateral and ventral body wall dermis comes from lateral plate mesoderm (Christ *et al.*, 1983; Fliniaux *et al.*, 2004) and the dorsal trunk dermis is generated by the dermomyotome of the somites (Mauger, 1972), which also produces the progenitors of striated muscles and scapular blade (Aoyama and Asamoto, 1988; Christ and Ordahl, 1995; Huang and Christ, 2000; Huang *et al.*, 2000a; Huang *et al.*, 2000b).

The first sign of dermis development is the formation of a loose subectodermal mesenchyme between 3 (HH20) and 5 (HH26) days of incubation in chick and at an equivalent developmental stage, days 9.5 (E9.5) and 13 (E13) of gestation in mouse (Dhouailly *et al.*, 2004). Some of these cells will contribute to the formation of the dense dermis that is clearly visible in the future dorsal feather/hair field from HH29 (E6.5) in the chick and 12.5 in the mouse embryos. The only dermal marker available to date is *Dermo-1* (also known as *Twist-2*), a bHLH transcription factor. Chick *Dermo1* is expressed in superficial dermal cells in a decreasing gradient from the midline to the lateral trunk dermis from HH24 to HH29 (Scaal *et al.*, 2001). This gradient parallels the future order of chick dorsal dermis densification. *Dermo1* was first cloned in mice, where in contrast to the chick, it is detected initially in the lateral trunk at E11/E12 and it is not detected in the midline until E13 (Li *et al.*, 1995; Houzelstein *et al.*, 2000). This pattern can also be correlated with dermis differentiation in this species. However, *Dermo1* is not an exclusive marker of the somitic dermal lineage since it is also expressed in sclerotome and in limb mesenchyme (Li *et al.*, 1995; Scaal *et al.*, 2001).

The missing link between the somitic dermomyotome at E2/3 and dorsal dermis at E7 in chick has been examined by several studies these last years. In the light of abundant new information available on somitic differentiation, distinct populations of dermal

Abbreviations used in this paper: NC, notochord; NT, neural tube; PSM, presomitic mesoderm.

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cells deriving from the somites were uncovered in both mouse and chick, nevertheless, their contribution to dermis that participates in appendage differentiation is still controversial. Which dermomyotomal compartments contribute to the dorsal dermis and more importantly, to the dense dermis of the future feather fields? How are dermomyotomal cells instructed to become dermis and prevented from adopting an alternative fate? In what order is the subectodermal space populated by dermal progenitors and what controls their migration? How is dermal differentiation orchestrated? Finally, although mouse dorsal dermis development is less well documented, what comparisons can be drawn between birds and mammals that would allow us to better understand the process of formation of a dermis capable of inducing appendage differentiation? In our concluding remarks, we will take the opportunity to highlight those areas where further clarification is needed and that could form the basis for future fruitful research.

Contribution of medial and lateral dermomyotomal compartments to the dorsal dermis

Somites are metameric structures that segment sequentially from the presomitic mesoderm and convert into epithelial spheres (for a review see Pourquié, 2003). Their ventral aspect rapidly transforms into the so-called sclerotome, a mesenchyme that generates skeletal progenitors (Kato and Aoyama, 1998; Huang *et al.*, 2000a,b; Evans, 2003 and for reviews see: Christ and Ordahl, 1995; Ordahl *et al.*, 2000). The dorsal dermomyotome remains epithelial for a longer period and generates progressively mainly myogenic and dermal progenitors (for a review see Brand-Saberi and Christ, 2000). Somites can be additionally subdivided into medial and lateral compartments, on the grounds of their embryonic origin (Psychoyos and Stern 1996; Selleck and Stern, 1991; Eloy-Trinquet *et al.*, 2000; Freitas *et al.*, 2001), of the distinct fates of their derivatives (Ordahl and Le Douarin, 1992; Denetclaw and Ordahl, 2000; Huang and Christ, 2000; Olivera-Martinez *et al.*, 2000; Eloy-Trinquet and Nicolas, 2002) and in the information encoded for molecular segmentation and commitment to somite formation (Freitas *et al.*, 2001).

The precise origin of the dorsal dermis along the medio-lateral dermomyotome has been analysed recently by two different groups (Olivera-Martinez *et al.*, 2000, 2001, 2002 and Ben-Yair *et al.*, 2003). The replacement of the medial or lateral rostral presomitic mesoderm (PSM) by the equivalent quail tissues in a chick host was carried out at E2 (in 15 to 21 somite embryos) to trace the fate of the medial or lateral dermomyotome. Given that there is no morphological boundary between the two compartments some fluctuation is unavoidable on the medio/lateral extent of the transplanted tissues. Although, as observed one day later grafted cells were essentially in the expected territories, in agreement with previous DiI labelling fate mapping experiments (Selleck and Stern, 1991; Psychoyos and Stern, 1996) that suggested that cells were already allocated along the medio/lateral axis in the rostral unsegmented presomitic mesoderm. In order to evaluate the respective contribution of the medial and lateral somitic compartments to the formation of the two types of the upper dorsal dermis (dense and sparse), chimeras of medial and lateral presomitic mesoderm were allowed to develop further to E4 and especially to E8. In the grafts of quail medial presomitic mesoderm analyzed at E4, the vertebrae and epaxial myotome were of quail origin as were the remaining epithelial dorsomedial lip, the dissociated central dermomyotome and a loose subectodermal mesenchyme spanning from the midline to near the dorso/ventral frontier. This loose mesenchyme is located in the presumptive dorsal dermis territory thus indicating its medial origin. More significantly, at E8 the superficial dense dermis from the midline to the lateral border was of quail origin (Fig. 1A). In the most differentiated medial region, this dense dermis had begun redistributing to form dermal condensations associated to epidermal placodes, constituting feather primordia, while the lateral limit of the upper dense dermis with the loose upper sparse dermis correlates with the limit between quail and chick tissues (Fig. 1B). In the converse experiment, when the lateral chick rostral PSM was replaced by the equivalent quail tissue, at E4 a narrow ribbon of subectodermal mesenchyme around the D/V frontier was of quail origin (Olivera-Martinez *et al.*, 2000; and Ben-Yair *et al.*, 2003) in the vicinity of the ectodermal notch observed by several studies (Ordahl and Le Douarin 1992; Huang and Christ, 2000; Olivera-Martinez *et al.*, 2000; Nowicki *et al.*, 2003). At E7/8 quail cells were consistently not found in the lateral dense dermis unless the epaxial muscle was also labelled (Olivera-Martinez *et al.*, 2000). In the chick, *Sim1* expression characterizes the lateral somite and its expression

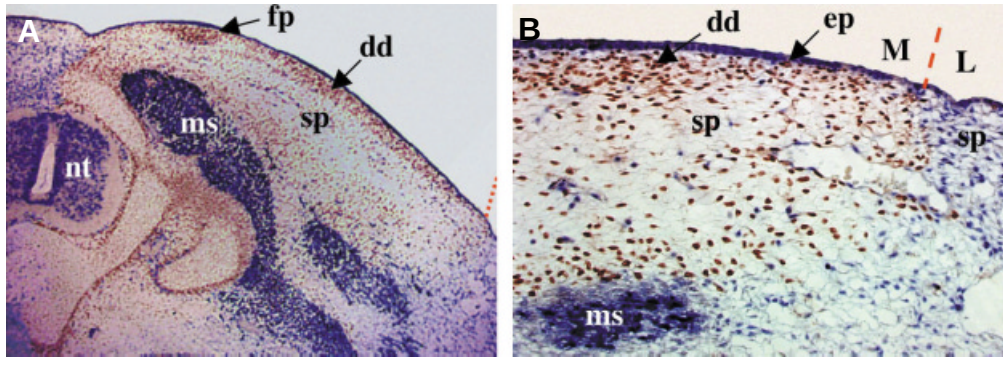


Fig. 1. In birds, the medial somitic compartment gives rise to the dense feather-forming dermis of the spinal pteryla and the lateral compartment to the sparse dermis of the marginal semi-apterium. *Quail/chick chimeras. Sections were stained with QCPN antibody and revealed with the peroxydase reaction (brown), which labels the quail nuclei. The myotome is shown by staining with the I3F4 antibody (dark blue).* **(A)** *At E7, in a medial quail graft in chick host, both the dense dermis (dd) and the deep sparse dermis (sp) are of quail origin. Note the redistribution of the dense dermis in the most medial region to form the dermal condensation of the first feather primordium (fp).* **(B)** *Higher magnification of the same section. Detail of the junction of the upper dense dermis of the spinal pteryla of medial somitic origin and the upper sparse dermis of lateral somitic origin. The red dashed line shows the limit between both the quail and chick nuclei and upper dense and sparse dermis. ep, epidermis; ms, muscles; nt, neural tube (from Olivera-Martinez* et al., *2000).*

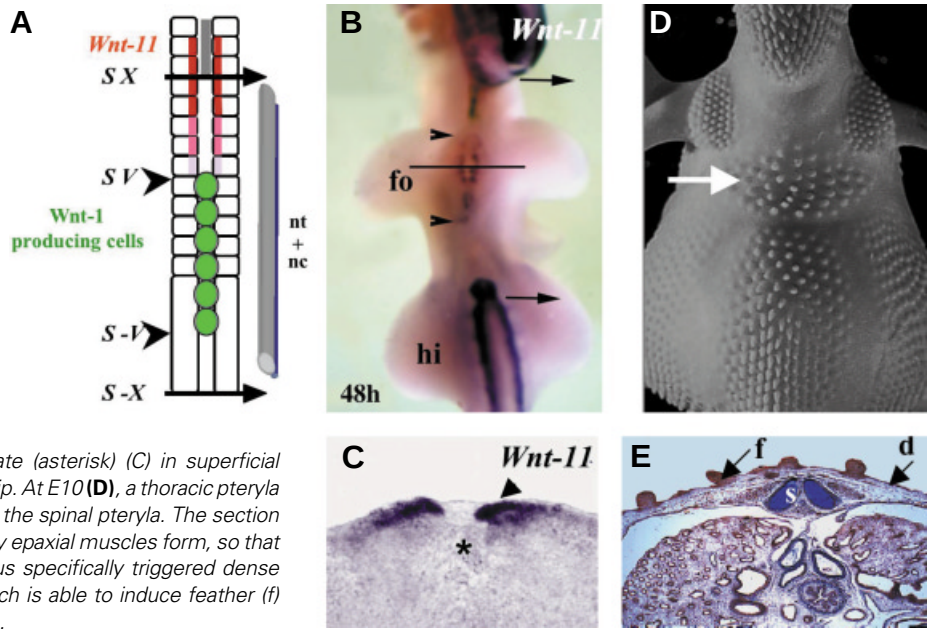


Fig. 2. Wnt-1 cells grafted in place of neural tube and notochord in chick embryo trigger *Wnt-11* expression in the medial dermomyotome and lead to the formation of a feathered dorsal skin. (A) *A fragment of neural tube (NT) plus notochord (NC) was excised at HH13 between somite X (SX) and the chordoneural hinge (arrows). Wnt-1 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 keep two ungrafted excised regions as controls. Embryos were fixed 48 hours (B,C) and 8 days (D,E) after the operation. Dorsal views and corresponding transversal section, hybridized with the* Wnt-11 *probe* **(B,C)**. *At E4,* Wnt-11 *was activated on both sides of the Wnt-1 cell aggregate (asterisk) (C) in superficial epithelial structures reminiscent of the dorsomedial lip. At E10* **(D)**, *a thoracic pteryla (arrow) formed in phase with unoperated regions of the spinal pteryla. The section* **(E)** *showed that neither axial skeleton nor almost any epaxial muscles form, so that scapulae (s) lie close together. The Wnt-1 graft thus specifically triggered dense dermis (d) formation in the mediodorsal region which is able to induce feather (f) morphogenesis (Olivera-Martinez* et al., *2001, 2002).*

has been shown to rely on BMP4 from the lateral plate (Pourquié *et al.*, 1996). At stage HH23 *Sim-1* is expressed in a narrow ribbon of mesenchymal cells at the epaxial/hypaxial border (Olivera-Martinez *et al.*, 2002). The lateral somite has been shown to generate the most lateral subectodermal mesenchyme at the limit of the dorso/ventral domain (Ben-Yair *et al.*, 2003). At E4 the expression of *cSim1* in this marginal subectodermal mesenchyme population supports its lateral origin (Olivera-Martinez *et al.*, 2002). Moreover, the location of *Sim1* positive cells in the subectodermal lateral mesenchyme correlates with the location of quail cells from the lateral presomitic mesoderm grafts in quail/chick chimerae at E4 days and at E8 to the loose dermis at the border of the dense dermis of the spinal pteryla (Olivera-Martinez *et al.*, 2000). Thus both Dhouailly's and Kalcheim's groups are convinced that dermal cells arose from the different parts of the dermomyotome and that the lateral dermomyotome generates the dermal cells localised near the boundary between the dorsal and ventral body domains (Olivera-Martinez *et al.*, 2000, 2002; Ben-Yair *et al*, 2003) up to the level of the lateral somitic frontier defined recently (Nowicki *et al.*, 2003). We propose that this lateral somite derived dermis will become a narrow skin region that will remain almost devoid of feathers, known as a semi-apteria and will not contribute to dense dermis of the spinal feather field. However, it is clear that a more detailed analysis by labelling only the lateral dermomyotome remains to be done to ascertain if the dense dermis is of medial and the loose dermis of lateral dermomyotomal origin. The rare feathers that arise in lateral semi-apteria do so independently of the dorsal field wave and therefore we do not consider this dermis, as well as the one of the midventral apterium as a proper (appendage forming) dermis (Dhouailly *et al.*, 2004). This idea is tantalizing because the known distinct origins and capabilities of these two somitic moieties would be correlated with two distinct dermal populations that either do or do not have full feather inducing capabilities.

Wnt-11 positive cells from the dorsomedial lip of the dermomyotome migrate and populate the dorsomedial subectodermal mesenchyme

The chick dorsomedial lip (DML) expresses *Wnt11* in rostral somites at stage HH14 and then its expression expands caudally but not further than somite IV (+/-1) (Tanda *et al.*, 1995; Marcelle *et al.*, 1997). Subsequently, DML cells downregulate *Wnt11*, turn on *MyoD* and translocate under the epithelial dermomyotome in a still controversial mechanism to give rise to the epaxial (dorsal) muscles (among others: Denetclaw *et al.*, 1997; Denetclaw and Ordahl, 2000; Kalcheim *et al.*, 1999; Ordahl *et al.*, 2001; Ordahl and Le Douarin, 1992). 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). Medial somitic cells depend on axial signals not only for their specification but also for their survival, as evidenced by the extensive cell death observed following the excision of neural tube and notochord, leading to the absence of many organs, including epaxial muscles, vertebrae, ribs (Rong *et al.*, 1992; Teillet *et al.*,1998) and the dermis of the dorsal feather field (Mauger, 1972; Olivera-Martinez *et al.*, 2001). Although survival of the medial somite relies mostly on notochord and floorplate derived Shh (Teillet *et al.*, 1998; Cann *et al.*, 1999; Marcelle *et al.*, 1999), dorsal neural tube Wnt1 can also allow the survival of some somitic cells (Olivera-Martinez *et al.*, 2001) most probably acting through the canonical Wnt pathway (among others: Schmidt *et al.*, 2000). Wnt1 has been shown to initiate *Wnt11* expression in the DML, which itself has been implicated not only in myotomal but also in dorsal dermis development (Marcelle *et al.*, 1997; Tanda *et al.*, 1995). The graft of Wnt1 producing cells in place of excised axial organs (neural tube plus notochord) (Fig. 2A) specifically restores the expression of *Wnt11* in the medial somite (Fig. 2 B,C) and strikingly the formation of a dense dorsal dermis, while no axial cartilage and almost no epaxial muscle form (Fig. 2 D,E). This

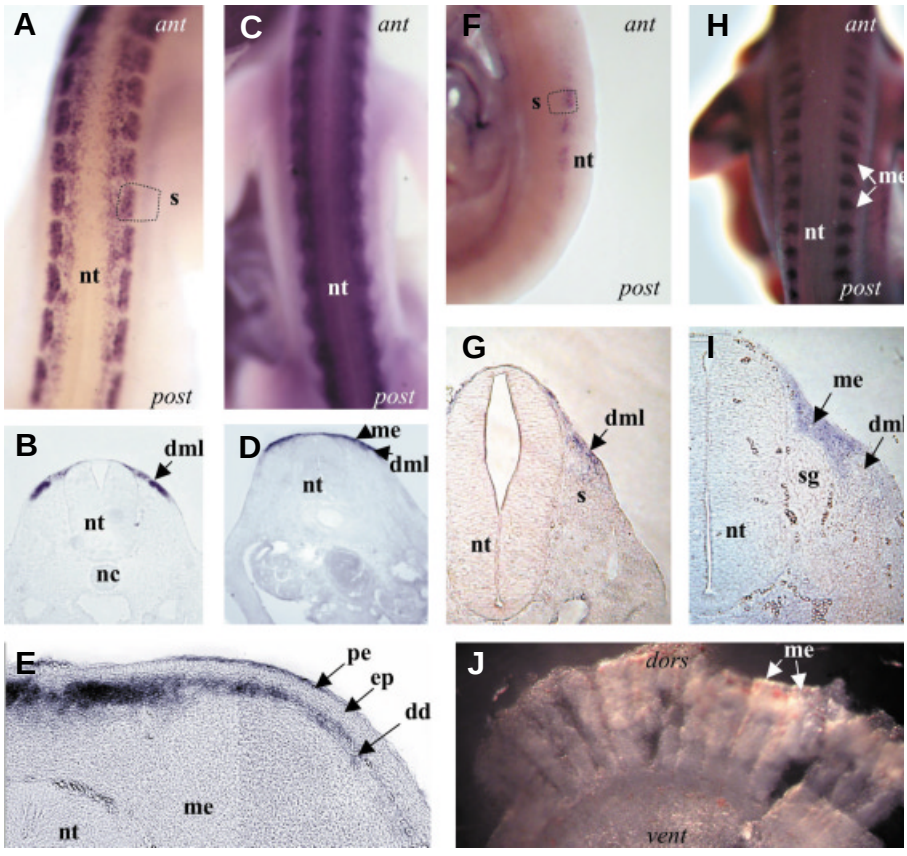


Fig. 3. In chick and mouse embryos, *Wnt-11* is expressed first in the dermomyotomal lip, then in the subectodermal dorsal mesenchyme. *(A-I) Dorsal views and corresponding transversal sections, hybridized with* cWnt-11 and mWnt-11 *probes. Rectangles outline a single somite (s). (J) Unfixed, living mesenchyme tissue just after dissociation.* **(A,B)** *In chick, at stage HH 18,* Wnt-11 *transcripts are detected in the dorsomedial lip (dml). Isolated* Wnt-11 *expressing cells are also detected dorsomedially to the lip, under the ectoderm. Note the increasing number of isolated* Wnt-11 *cells and their expansion over the neural tube from the posterior (post) to the anterior (ant) regions.* **(C,D)** *In chick, at stage HH 23,* Wnt-11 *transcripts are no longer detectable in the dorsomedial lip (dml), but* Wnt-11 *cells form a continuous subectodermal mesenchyme (me) located between the ectoderm, the neural tube and the dml.* **(E)** *In chick, at stage HH 28, the previous ectoderm had formed a palisade simple epidermis (ep), overlaid by a flat periderm (pe). Note the thickness of the mesenchyme over the neural tube (nt). The cells expressing* Wnt-11 *are located in the upper mesenchymal layer, where the dense dermis (dd) is differentiating.* **(F,G)** *In mouse at 9.5 days of gestation,* Wnt-11 *transcripts are detectable in the dorsomedial lip, as well as in isolated cells between the dml and the neural tube.* **(H,I)** *In the mouse at 11.5 days of gestation,* Wnt-11 *expressing cells form a segmented sub-ectodermal mesenchyme (me) on each side of the neural tube.* **(J)** *Mouse subectodermal mesenchyme of an E 11.5 embryo, after dissociation from its ectoderm. Note that the mesenchymal segments, which originate from the somites, are still well individualized and constitute the future dorsal dermis, connected to the homogeneous somatopleural ventral (vent) mesenchyme. Ant, anterior; dors, dorsal, nc, notocord, sg, spinal ganglion. Illustrations (A-D) are from Olivera-Martinez* et al. *(2002). (F-I) Experiments and photographs by S. Missier. (E-J) Experiments and photographs by J. Thélu.*

restored dermis possesses all the abilities which are characteristic (Dhouailly, 1977) of a competent dense dermis. It is able to induce the formation of a dorsal feather field in its overlying epidermis, with the feather buds arising in longitudinal rows, in a spatiotemporal sequence in accordance with the axial position of the Wnt-1 cell graft.

Later in development (HH18-19 at the forelimb level), chick *Wnt11* is detected not only in the DML, but also in isolated cells located between the DML and the dorsal neural tube, which at the beginning (in posterior regions) appear well grouped, reminding their somitic origin (Fig. 3 A,B).

These latter isolated Wnt11 expressing cells appear on the medial edge of somite XVI (+/-1). One day later (E4) (HH23), *Wnt11* labels the subectodermal cells of the dorsomedial mesenchyme (Fig. 3 C,D). More precisely, when looking at the dorsal side of the whole embryo, the isolated Wnt-11 expressing cells are not detected at the level of the last-formed somite (somite I to XVI). From somite XVI to rostral somite, the amount of isolated Wnt11 cells increases gradually, so that, at rostral level, they appear as a dense label (Fig. 3A). Conversely, the *Wnt-11* labelling in DML, decreases rostrally, so the DML appears at the end as negative within the *Wnt-11* positive mesenchyme. Briefly, from posterior to anterior position, Wnt-11 expressing cells seem to be progressively transferred from DML to the subectodermal mesenchyme. In order to test this idea, we did microsurgical experiments (see below). At stage HH 28 (E6), a thick mesenchyme is present between the dorsal neural tube and the ectoderm, only the upper part of which expresses *Wnt11* (Fig. 3E). In fact, at this stage the ectoderm had been transformed into a palissade epidermis, overlayed by a flat periderm (Fig. 3E).The upper mesenchyme, which express *Wnt-11* corresponds to the dense dermis. This has also been observed by another group (Chang *et al.*, 2004), who shows moreover that by stage HH30 (E7), the Wnt-11 expressing cells localize in the interbud dermis. In the mouse at 9.5 days of gestation, the somitic DML at the thoracic level expresses *Wnt11*, as well as a few cells between the dermomyotome and the neural tube (Fig. 3 F,G). At E11, *Wnt11* positive cells are located in the mesenchymal cells between what remains of the DML and the neural tube, in a clearly segmented pattern from the neck to the tail (Fig. 3 H,I). At E11.5, when the left and right part of the body wall were dissected on each side of the neural tube, then soaked in enzymatic solution, followed by a dissociation of the two tissues, the segmented structure of the mesenchyme adjacent to the lateral part of the neural tube is evident (Fig. 3J). One problem which remains to be solved is to determine whether the detaching cells from the dermomyotome express *Wnt-11* before expressing *Dermo-1*, in other words, what is the relative extent of the expression of these two genes at E11.5?

In order to test whether the *Wnt11* positive cells of the subectodermal space at HH22-23 in chick embryo have previously detached from the DML and migrated towards the midline, somites were transplanted from left to right to reverse the mediolateral axis

(somites VIII to XII in HH15-16 chick embyos) (Olivera-Martinez *et al.*, 2002). In other words, we dissected 5 contiguous somites (Fig. 4A) on the left side of a donor embryo, at a stage and antero-posterior axis level where it is known that *Wnt-11* expression has been induced by Wnt-1 from the neural tube, but where the isolated Wnt-11 cells are not yet present. We then transplanted this in place of the corresponding 5 somites on the right side of a same stage host embryo. Consequently, the medial somite abuts the intermediate and lateral plate mesoderm. Six hours after the transplantation the DML, now in a lateral position, still expresses *Wnt11,* showing that *Wnt11* expression has become independent of neural tube *Wnt1*. Remarkably ten hours later, *Wnt11* positive mesenchymal cells are detected between the central demomyotome and the ectoderm and 24 hours later they have colonized the subectodermal space from the transposed DML towards the midline, by progressing over the entire dermomyotome (Fig. 4 B,C). It is evident that the expression of a gene cannot be considered as a lineage tracer and other experiments, involving the labelling of the medial dermomyotome with a RCAS-GFP marker are in process.

Wnt11 is however a remarkable member of the Wnt family of signalling molecules, which is expressed particularly in cells that undergo an epithelial to mesenchymal transition to adopt a migratory behaviour. Its expression is maintained during the migration process as observed in the mesodermal cells exiting the primitive streak in Zebrafish and *Xenopus* (Heisenberg *et al.*, 2000 Tada and Smith, 2000). It is therefore likely that the *Wnt11* positive cells in the subectodermal mesenchyme derive from the DML. In vertebrates amniotes, we suggest that *Wnt-11* is expressed in the dorsal dermal lineage, as long as those cells are moving: first in order to reach the subectodermal space and then in the upper dense dermis which will reorganize to give rise to the dermal condensations and interbud dermis (Viallet *et al.*, 1998; F. Michon unpublished data). The importance of the *Wnt* gene family in skin morphogenesis has been demonstrated beautifully by the work of Elaine Fuchs laboratory (Das Gupta *et al.*, 2002). In chick embryo, the Wnt gene family has recently been shown to play a fundamental role in dermal organization (Chang *et al.*, 2004) via experiments involving the Wnt inhibitor Dickkopf-1 (Dkk-1). When dissociated chick dermal cells were transduced with RCAS-*Dkk-1* and then re-aggregated and overlaid by an epidermis, feather formation is inhibited. Dkk does not, however, differentiate between different Wnts and specific antagonists for each member of this large family do not exist yet. There is an apparent controversy between four different groups with respect to the most medial mesenchyme,that is above the neural tube. It has been proposed that it is not of dermomyotomal but of sclerotomal origin and will therefore give rise to dorsal vertebrae (Ben-Yair *et al.*, 2003). It is evident that the midline subectodermal mesenchyme at E4 in the chick embryo contains both cartilage, expressing *Msx1* and dermal progenitors (Monsoro-Burq *et al.*, 1994; 1996). In mouse the thin mediodorsal mesenchyme expressing *Msx1* at E12 was proposed to be part of the dermal progenitors (Houzelstein *et al.*, 2000). We suggest that two different migrations occur, one from the dermomyotome and the other from the sclerotome. These two migrations might be simultaneous or might occur at different times. Another possibility is that a primary dorsal mesenchyme might give rise to two different populations. Fundamentally both studies (Olivera-Martinez *et al.*, 2000; 2002; Ben-Yair *et al.*, 2003) agree that the medial dermomyotome generates the most axial progenitors of the dorsal dermis. It should be noted that in the thoracic region, there is not one initial feather row, but two, which delimit a semi-apterium between them (Dhouailly *et al.*, 2004). We suggest that the discrepancies in the midline extent of the somite-derived dermal cells observed by different groups are due to the observations not being done at the exactly the same level and might reflect the future pattern of dermis densification and may thus be very relevant in defining the exact position were the dermis is dense enough to initiate feather differentiation.

En-1 is expressed in the central dermomyotome, then later in the mediolateral subectodermal mesenchyme

Although the exact mechanism is still controversial, the DML is believed to be the source of new cells for the growth of the primary myotome and the dermomyotome epithelium, forming a central dermomyotomal compartment (Denetclaw *et al.*, 1997; Denetclaw and Ordahl, 2000; Denetclaw *et al.*, 2001; Ordahl *et al.*, 2001; Venters and Ordahl, 2002). In order to assess if this medially derived dermomyotome also contributed to dorsal dermal progenitors, we followed (Olivera-Martinez *et al.*, 2002) the expression of the central dermomyotomal marker *En1*. Our results

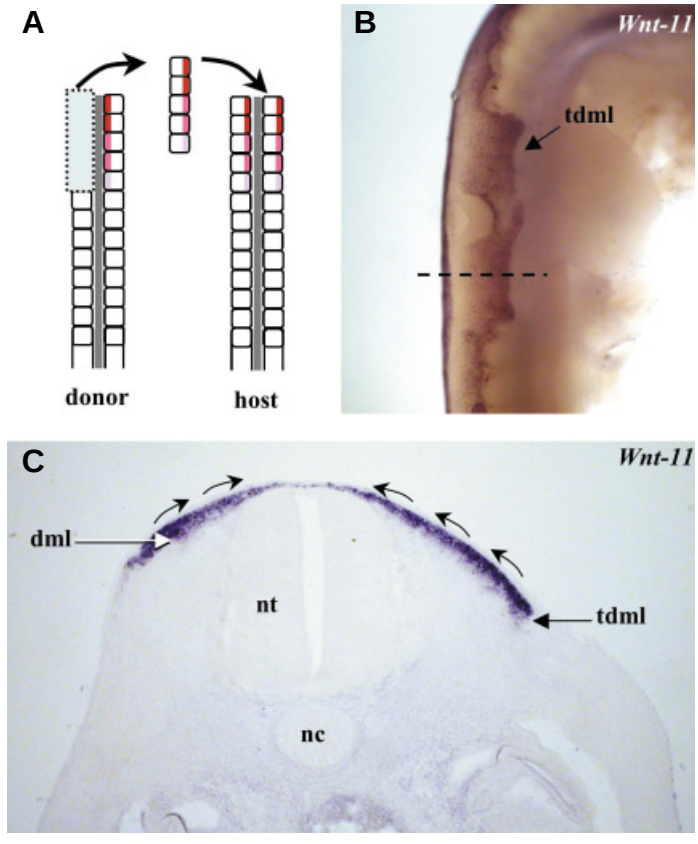


Fig. 4. Migratory behavior of cells from the chick somitic dorsomedial lip after the transplantation of 5 left-side somites, from a donor to the right side of a host embryo. (A) *Schematic view of the technique.* **(B,C)** *Hybridization with a* Wnt-11 *probe shows that after 24 hours,* Wnt-11 *cells have migrated from the transposed dorsomedial lip (tdml) over the entire dermomyotome towards the midline. This is seen in an external view of the right side (B) and in a transverse section (C). dml, dorsomedial lip; nc, notochord; nt, neural tube (Olivera-Martinez* et al., *2002).*

suggest that a second small population of subectodermal cell appears to be originally located in the central dermomyotomal domain (Fig. 5 A,C). This observation is in agreement with DiI labelling experiments showing the contribution of this central domain to the subectodermal mesenchyme (Ben Yair *et al.*, 2003). Some *En1* expressing cells delaminate under the ectoderm and give rise to subectodermal mesenchymal cells, following a desepithelialization process previously described (Christ and Ordahl, 1995) that might be under the control of NT-3 from the neural tube (Brill *et al.*, 1995). What, however, controls En-1 expression? We assayed for *En1* trancription in the presence of the notochord as a source of Shh, to allow medial somitic cell survival (Rong *et al.*, 1992; Teillet *et al.*, 1998, Marcelle *et al.*, 1999), but in absence of dorsal neural tube *Wnt1* (Olivera-Martinez *et al.*, 2001; 2002). In these experimental conditions, *En1* expression was properly initiated showing that *En1* onset does not require a neural tube factor. Next, we carried out the excision of the dorsal ectoderm on HH12-14 embryos and showed that *En-1* expression fails to be activated at the level of the excision 24 hours after the operation (Olivera-Martinez *et al.*, 2002), even though the ectoderm heals in an average of 16 hours (our data and Thévenet, 1969). Forty-eight hours later *En-1* expression reappears and although its domain of expression is reduced (Olivera-Martinez *et al.*, 2002), no abnormalities in the dorsal feather field can be detected 8 days later (Olivera-Martinez *et al.*, 2001). To prevent the contact of the ectoderm and the central dermomyotome for a longer period of time, we inserted a piece of teflon under the ectoderm, over the length of the three last-formed somites on the right side at stage HH 13-14 (Fig. 5E). The results showed that 24 hours later *En-1* expression was absent from the central dermomyotome of the somites located at the forelimb level, whereas *En-1* expression has been induced in the left side (control), as well as in the following somites (Fig. 5 F,G). Although, the desepithelialisation of the central dermomyotomal domain correlates with the downregulation of *Wnt6* in the overlying ectoderm (Schubert *et al.*, 2002; C. Marcelle, personal communication), the identity of the ectodermal signal that controls *En1* is still unknown.

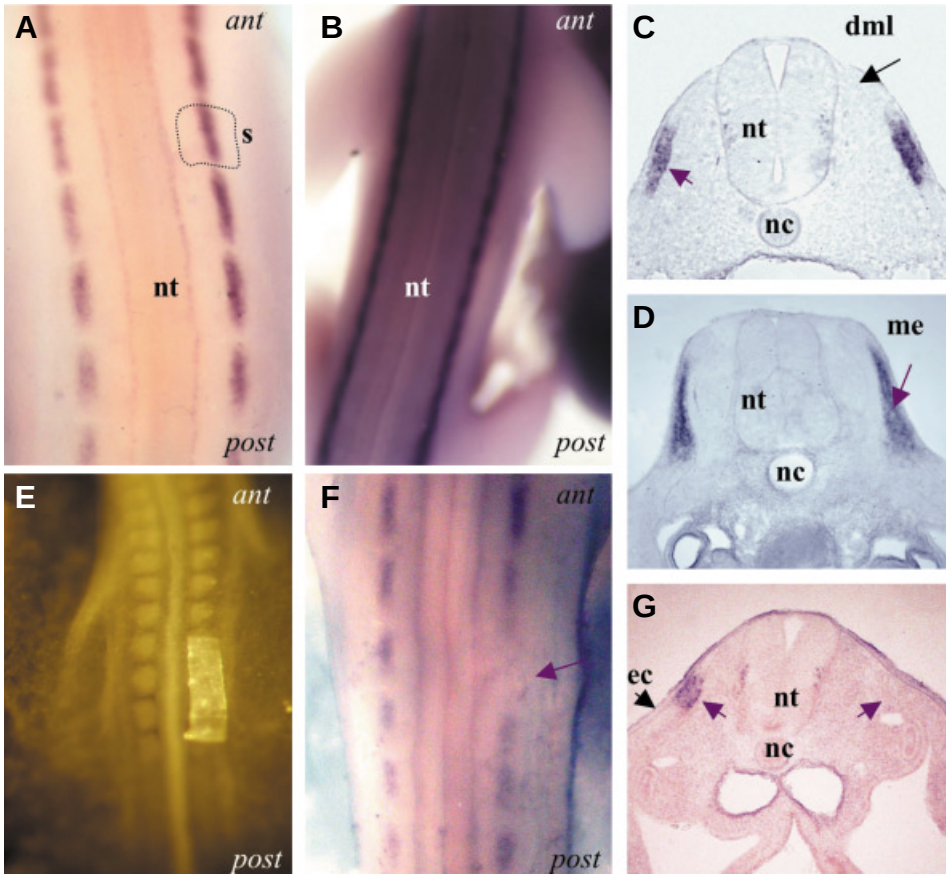


Fig. 5. In chick embryo, *En-1* expression in the central dermomyotome relies on an ectodermal signal. *Dorsal views and corresponding transverse sections, hybridized with an* En-1 *probe.* **(A,C)** *At stage HH 18,* En-1 *is expressed in a central compartment of the dermomyotome. Rectangle outlines a single somite (s) including the three regions, along the mediolateral axis.* **(B,D)** *At stage HH 23,* En-1 *is expressed in dorsolateral mesenchyme (me).* **(E-G)** *When a teflon piece is inserted between the ectoderm and the three last-formed somites on the right side at stage HH 13 (E) then, at stage HH 18, the somites at the forelimb level (arrow) did not express En-1 (F,G), in contrast to those of the un-operated left side. ant, anterior; dml, dorsomedial lip; ec, ectoderm;nc, notochord; nt, neural tube; post, posterior. Illustrations in (A-D) from Olivera-Martinez* et al. *(2002). (E-G) Experiments and photographs by S. Missier and J. Thélu.*

While Wnt-11 can be correlated with cell migration properties, what might be the role of En-1? This gene is known to be a transcriptional repressor. 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 *Wnt-11* is expressed) to the lateral dermis (where *En-1* is expressed). More precisely, the predermal cells which express *En-1* could be delayed in their acquisition of feather-inducing abilities, corresponding to the delay in their expression of *Dermo-1*.

Dorsal dermis differentiation in chick and mouse embryos

It should be noted that the pattern of skin appendage formation is notably dissimilar between chick and mouse. In chick, the first feather buds appear in a single row located at the midline of the dorsal feather field (i.e. spinal pteryla), over the neural tube. Subsequently, new rows emerge sequentially by pairs in both sides of this initial row in a wave of differentiation that stops at the edge of the dorsal feather field where a semi-glabrous (naked) region forms. In the mouse, by contrast, a large group of primary hair buds appear in concert, first in the lateral trunk, in the future hair pelage lateral field and then, two days later, the second group emerges in the midline skin (Dhouailly *et al.*, 2004). The difference in pattern formation of skin appendages in chick and mouse corre-

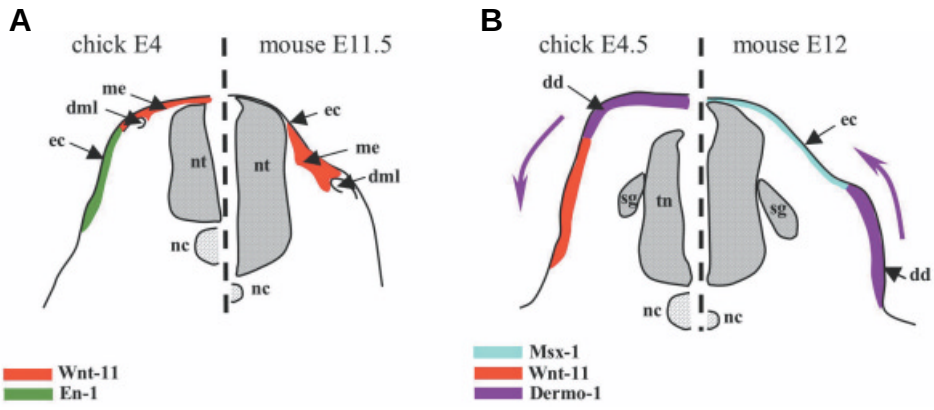
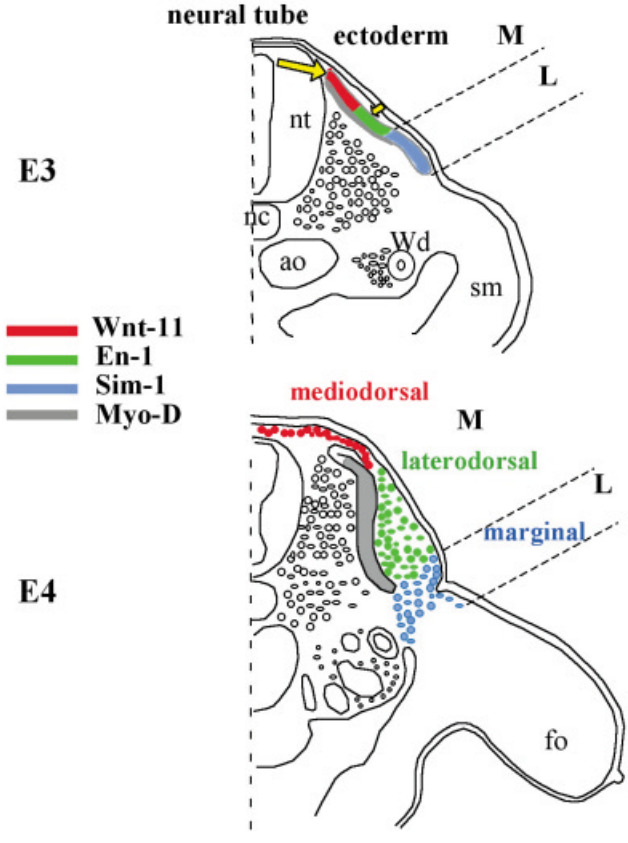


Fig. 6. Comparison of the different sub-ectodermal mesenchymal populations at corresponding developmental stages in chick and mouse embryos. (A) *At E4 in chick, the mediodorsal mesenchyme expresses* Wnt-11, *while the dorsolateral mesenchyme expresses* En-1. **(B)** *Twelve hours later, at E4.5,* Wnt-11 *is expressed in the dorsolateral mesenchyme, while the dorsomedial mesenchyme expresses* Dermo-1 *(Scaal* et al., *2001). In mouse, the mesenchymal cells which express* Wnt-11 *at 11.5 (A), appear to be the first to express* Dermo-1 *12 hours later (B). The wave of* Dermo-1 *expression correlates with the wave of dense dermis formation: in a medial-lateral wave in chick and in a latero-medial wave in mouse. Drawing by B. Peyrusse and S. Missier*

lates with the difference in the *Dermo-1* expression pattern. In chick, its transcripts are first detected at stage HH 24 in the mediodorsal sub-ectodermal mesenchyme (Scaal *et al.*, 2001), where the first feather buds appear. In contrast, in mouse, *Dermo-1* transcripts are first detected at E11 in the lateral part of the trunk (Houzelstein *et al.*, 2000; and S. Missier, personnal comunication).



At E13, *Dermo-1* expression is detected in the entire dorsal upper dermis (Houzelstein *et al.*, 2000; Li *et al.*, 1995), just before the emergence of the second group of hair primordia.

Two other arguments lead to suspect a crucial role of *Dermo-1* in dermis formation. This gene has been recently proposed (Sosic *et al.*, 2003), to be renamed *twist-2* based on its high homology with *Drosophila twist* (Wolf *et al.*, 1991) and mammalian *twist*. First, this gene is known to act as a repressor for *Myo D* transactivation (Gong and Li, 2001) and in this way might be able to stabilise dermis formation, by preventing cells to divert to muscle lineage. Second, *twist-2* null mutants (Sosic *et al.*, 2003), show a thin,loose dermis at E17, as well as dramatic cellular loss, by an extensive post natal apoptosis, which is especially apparent in the dermis. This apoptosis reinforce a phenotype already present in the embryo and results in the formation of sparse hair.

By grafts of mouse somites in chick hosts, it has been proposed that the subectodermal mesenchyme originating from the somites is composed of two distinct medial and lateral populations that express respectively *Msx-1* and *Dermo-1* (Houzelstein *et al.*, 2000). The same authors suggest that the most superficial dorsomedial mesenchyme downregulates *Msx-1* prior turning on *Dermo-1.* Other information arising from this study is that cell migration occurs from the medial somite to the ectoderm/ dorsal neural tube. Thus, the cells from the grafted mouse medial dermomyotome are able to respond to the Wnt-1 signal that comes from the chick neural tube and might be also directed by a still unknown ectodermal signal. The basic question of location of dermal progenitors within the mouse dermomyotome is, however, still poorly documented and not yet studied at an experimental level. Only a few facts are available. In our laboratory, we detected *Wnt-11* expression both in the medial dermomyotome and then in the dorsal mesenchyme in mouse embryos (see above). *En-1* expression has been detected in the dermomyotome, but only in tail somites (Davidson *et al.*, 1988). *Sim-1* is expressed in the mouse lateral dermomyotome (Ema *et al.*, 1996) and its expression domain is larger than that in chick.

Fig. 7. Regulation and localization of the thoracic dermal progenitors in chick embryo. *At* **(E3)**, *three distinct dermomyotomal compartments express, respectively,* Wnt-11, *in the most medial (activated by Wnt-1 from the dorsal neural tube (nt),* En-1 *in the central compartment, activated by a yet unknown signal from the ectoderm and* Sim-1 *in the lateral part. The* Wnt-11 *and* En-1 *expressing compartments form the medial dermomyotome. At* **(E4)**, *formation of dorsal subectodermal mesenchyme, which express* Wnt-11 *in the dorsomedial region,* En-1 *in the dorsolateral region and* Sim-1 *in the margin region. The latter forms the frontier with the mesenchyme originating from the somatopleural mesoderm (sm), at the intersection between the future dorsal and scapular pterylae. ao, aorta; fo, forelimb; nc, notochord; nt, neural tube; Wd, Wolffian duct. Modified from Olivera-Martinez* et al. *(2002). Drawing by B. Peyrusse.*

A preliminary diagram showing the similarities and differences in chick and mouse embryos on this question is presented (Fig. 6).

Concluding remarks

In chick it is now well established that there are three dermomyotomal domains, which give rise to three subectodermal mesenchymal domains (Fig. 7). The signal leading to *Wnt-11* expression in the most medial dermomyotomal part has been clearly established by different laboratories as relying on Wnt-1 expression from the dorsal neural tube. Likewise BMP4 from the lateral mesoderm is responsible for *Sim-1* expression in the lateral part. The function of *Wnt-11* expression is suspected to be linked to cell movements for dense dermis formation, as well as later for dermal organization and the formation of the dermal condensations, which are responsible for the induction of cutaneous appendages. Still unidentified ectodermal signals may regulate the dense dermis formation, as deduced from our studies of the chick mutant Ottawa naked (Olivera-Martinez *et al.*, 2004). Currently, we have not been able to identify the nature of the ectodermal signal responsible for *En-1* expression, but the dissociation of the cells belonging to the central dermomyotome appears to rely on the downregulation of *Wnt-6* expression in the ectoderm (C. Marcelle, personal communication).

What about the roles of *En-1* and *Sim-1?* Both are known to act as transcriptional repressors and we suggest that in the chick embryo *En-1* might act by retarding both *Wnt-11* and *Dermo-1* expression and thus the lateral wave of dense dermis formation. *Sim-1* might have even a stronger role, as the densification of the upper dermis of semi-apteria occurs with 2 days of delay. Finally, another problem that needs to be precisely addressed is the sequence of gene expression in the sub-ectodermal mesenchyme leading to the dorsal dense dermis formation, both in chick and in mouse.

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References

AOYAMA, H. and ASAMOTO, K. (1988). Determination of somite cells: independence of cell differentiation and morphogenesis. *Development* 104: 15-28.

BEN-YAIR R, KAHANE N, KALCHEIM C. (2003). Coherent development of dermomyotome and dermis from the entire mediolateral extent of the dorsal somite. *Development* 130: 4325-36.

BORYCKI, A. G. and EMERSON, C. P. (2000). Multiple tissue interactions and signal transduction pathways control somite myogenesis. *Curr Top Dev Biol* 48: 165-224.

BRAND-SABERI, B. and CHRIST, B. (2000). Evolution and development of distinct cell lineages derived from somites. *Curr Top Dev Biol* 48: 1-42.

BRILL, G., KAHANE, N., CARMELI, C., VON SCHACK, D., BARDE, Y. A. and KALCHEIM, C. (1995). Epithelial-mesenchymal conversion of dermatome progenitors requires neural tube-derived signals: characterization of the role of Neurotrophin-3. *Development* 121: 2583-94.

CANN, G. M., LEE, J. W. and STOCKDALE, F. E. (1999). Sonic hedgehog enhances somite cell viability and formation of primary slow muscle fibers in avian segmented mesoderm. *Anat Embryol (Berl)* 200: 239-252.

CHANG, C. H., JIANG, T. X., LIN, C. M., BURRUS, L. W., CHUONG, C. M. and WIDELITZ, R. (2004). Distinct Wnt members regulate the hierarchical morphogenesis of skin regions (spinal tract) and individual feathers. *Mech Dev* 121: 157-171.

CHRIST B, JACOB M, JACOB HJ. (1983). On the origin and development of the ventrolateral abdominal muscles in the avian embryo. An experimental and ultrastructural study. *Anat Embryol* (Berl). 166(1): 87-101.

CHRIST, B. and ORDAHL, C. P. (1995). Early stages of chick somite development. *Anat Embryol (Berl)* 191: 381-96.

COULY, G., LE DOUARIN, N. M. (1988). The fate map of the cephalic neural primordium at the presomitic to the 3-somite stage in the avian embryo. *Development* 103 (Suppl): 101-13.

DASGUPTA, R., RHEE, H. and FUCHS, E. (2002). A developmental conundrum: a stabilized form of beta-catenin lacking the transcriptional activation domain triggers features of hair cell fate in epidermal cells and epidermal cell fate in hair follicle cells. *J Cell Biol* 158: 331-344.

DAVIDSON, D., GRAHAM, E., SIME, C. and HILL, R. (1988). A gene with sequence similarity to *Drosophila* engrailed is expressed during the development of the neural tube and vertebrae in the mouse. *Development* 104: 305-16.

DENETCLAW, W. F., JR., CHRIST, B. and ORDAHL, C. P. (1997). Location and growth of epaxial myotome precursor cells. *Development* 124: 1601-10.

DENETCLAW, W. F. and ORDAHL, C. P. (2000). The growth of the dermomyotome and formation of early myotome lineages in thoracolumbar somites of chicken embryos. *Development* 127: 893-905.

DENETCLAW, W. F., JR., BERDOUGO, E., VENTERS, S. J. and ORDAHL, C. P. (2001). Morphogenetic cell movements in the middle region of the dermomyotome dorsomedial lip associated with patterning and growth of the primary epaxial myotome. *Development* 128: 1745-1755.

DHOUAILLY, D. (1977). Dermo-epidermal interactions during morphogenesis of cutaneous appendages in amniotes. In *Front. Matrix Biol.* (Ed. L. Robert), Creteil. 4: 86-121.

DHOUAILLY, D., OLIVERA-MARTINEZ, I., FLINIAUX, I., MISSIER, S., VIALLET, J.P. and THÉLU, J. (2004). Skin field formation: morphogenetic events. *Int. J. Dev. Biol.* 48: 85-91.

ELOY-TRINQUET, S., MATHIS, L. and NICOLAS, J. F. (2000). Retrospective tracing of the developmental lineage of the mouse myotome. *Curr Top Dev Biol* 47: 33-80.

ELOY-TRINQUET, S. and NICOLAS, J. F. (2002). Clonal separation and regionalisation during formation of the medial and lateral myotomes in the mouse embryo. *Development* 129: 111-122.

EMA, M., MORITA, M., IKAWA, S., TANAKA, M., MATSUDA, Y., GOTOH, O., SAIJOH, Y., FUJII, H., HAMADA, H., KIKUCHI, Y. and FUJII-KURIYAMA, Y. (1996). Two new members of the murine Sim gene family are transcriptional repressors and show different expression patterns during mouse embryogenesis. *Mol Cell Biol* 16: 5865-5875.

EVANS, D. J. (2003). Contribution of somitic cells to the avian ribs. *Dev Biol* 256, 114-126.

FLINIAUX, I., VIALLET, J.P. and DHOUAILLY, D. (2004). Ventral *vs.* dorsal chick dermal progenitor specification. *Int. J. Dev. Biol.* 48: 103-106.

FREITAS, C., RODRIGUES, S., CHARRIER, J. B., TEILLET, M. A. and PALMEIRIM, I. (2001). Evidence for medial/lateral specification and positional information within the presomitic mesoderm. *Development* 128: 5139-5147.

GOG XQ and LI, L., (2002) Dermo-1 a multifunctional basic loop-helix protein represses MyoD transactivation via the HLH domain, MEF2 interaction and chromatin deacetylation. *J. Biol. Chem.* 277: 12310-12317.

HEISENBERG, C. P., TADA, M., RAUCH, G. J., SAUDE, L., CONCHA, M. L., GEISLER, R., STEMPLE, D. L., SMITH, J. C. and WILSON, S. W. (2000). Silberblick/Wnt11 mediates convergent extension movements during zebrafish gastrulation. *Nature* 405: 76-81.

HOUZELSTEIN, D., CHERAUD, Y., AUDA-BOUCHER, G., FONTAINE-PERUS, J. and ROBERT, B. (2000). The expression of the homeobox gene Msx1 reveals two populations of dermal progenitor cells originating from the somites. *Development* 127: 2155-2164.

HUANG, R. and CHRIST, B. (2000). Origin of the epaxial and hypaxial myotome in avian embryos. *Anat. Embryol. (Berl.)* 369-74.

HUANG, R., ZHI, Q., SCHMIDT, C., WILTING, J., BRAND-SABERI, B. and CHRIST, B. (2000a). Sclerotomal origin of the ribs. *Development* 127: 527-532.

HUANG, R., ZHI, Q., PATEL, K., WILTING, J. and CHRIST, B. (2000b). Dual origin and segmental organisation of the avian scapula. *Development* 127: 3789-3794.

KALCHEIM, C., CINNAMON, Y. and KAHANE, N. (1999). Myotome formation: a multistage process. *Cell Tissue Res* 296: 161-73.

KATO, N. and AOYAMA, H. (1998). Dermomyotomal origin of the ribs as revealed by extirpation and transplantation experiments in chick and quail embryos. *Development* 125: 3437-3443.

LI, L., CSERJESI, P. and OLSON, E. N. (1995). Dermo-1: a novel twist-related bHLH protein expressed in the developing dermis. *Dev Biol* 172: 280-292.

MARCELLE, C., AHLGREN, S. and BRONNER-FRASER, M. (1999). *In vivo* regulation of somite differentiation and proliferation by Sonic Hedgehog. *Dev Biol* 214: 277-87.

MARCELLE, C., STARK, M. R. and BRONNER-FRASER, M. (1997). Coordinate actions of BMPs, Wnts, Shh and noggin mediate patterning of the dorsal somite. *Development* 124: 3955-63.

MAUGER, A. (1972a). The role of somitic mesoderm in the development of dorsal plumage in chick embryos. I. Origin, regulative capacity and determination of the plumage-forming mesoderm. *J Embryol Exp Morphol* 28: 313-41.

MAUGER, A. (1972b). Rôle du tube neural dans le développement du plumage dorsal de l'embryon de poulet. *Wilhelm Roux'Archiv* 170: 244-266.

MONSORO-BURQ, A. H., BONTOUX, M., TEILLET, M. A. and LE DOUARIN, N. M. (1994). Heterogeneity in the development of the vertebra. *Proc Natl Acad Sci USA* 91: 10435-10439.

MONSORO-BURQ, A. H., DUPREZ, D., WATANABE, Y., BONTOUX, M., VINCENT, C., BRICKELL, P. and LE DOUARIN, N. (1996). The role of bone morphogenetic proteins in vertebral development. *Development* 122: 3607-3616.

MONSORO-BURQ, A. H. and LE DOUARIN, N. (2000). Duality of molecular signaling involved in vertebral chondrogenesis. *Curr Top Dev Biol* 48: 43-75.

MUNSTERBERG, A. E., KITAJEWSKI, J., BUMCROT, D. A., MCMAHON, A. P. and LASSAR, A. B. (1995). Combinatorial signaling by Sonic hedgehog and Wnt family members induces myogenic bHLH gene expression in the somite. *Genes Dev* 9: 2911-22.

NOWICKI, J.L., TAKIMOTO, R. and BURKE, A.C. (2003). The lateral somitic frontier: dorso-ventral aspects of anterio-posterior regionalization in avian embryos. *Mech Dev.* 120: 227-40.

OLIVERA-MARTINEZ, I., COLTEY, M., DHOUAILLY, D. and POURQUIÉ, O. (2000). Mediolateral somitic origin of ribs and dermis determined by quail-chick chimeras. *Development* 127: 4611-4617.

OLIVERA-MARTINEZ, I., THÉLU, J., TEILLET, M. A. and DHOUAILLY, D. (2001). Dorsal dermis development depends on signal from the dorsal neural tube, which can be substituted by Wnt-1. *Mech Dev* 100: 233-44.

OLIVERA-MARTINEZ I, MISSIER S, FRABOULET S, THÉLU J, DHOUAILLY D. (2002). Differential regulation of the chick dorsal thoracic dermal progenitors from the medial dermomyotome. *Development* 129: 4763-72.

OLIVERA-MARTINEZ, I., VIALLET, J.P., MICHON, F., PEARTON, D. and DHOUAILLY, D. (2004). The different steps of skin formation in vertebrates. *Int. J. Dev. Biol.* 48: 107-115.

ORDAHL, C. P., BERDOUGO, E., VENTERS, S. J. and DENETCLAW, W. F. (2001). The dermomyotome dorsomedial lip drives growth and morphogenesis of the primary myotome and dermomyotome epithelium. *Development* 128: 1731-44.

ORDAHL, C. P. and LE DOUARIN, N. M. (1992). Two myogenic lineages within the developing somite. *Development* 114: 339-53.

ORDAHL, C. P., WILLIAMS, B. A. and DENETCLAW, W. (2000). Determination and morphogenesis in myogenic progenitor cells: an experimental embryological approach. *Curr Top Dev Biol* 48: 319-67.

POURQUIÉ, O., FAN, C. M., COLTEY, M., HIRSINGER, E., WATANABE, Y., BREANT, C., FRANCIS-WEST, P., BRICKELL, P., TESSIER-LAVIGNE, M. and LE DOUARIN, N. M. (1996). Lateral and axial signals involved in avian somite patterning: a role for BMP4. *Cell* 84: 461-71.

POURQUIÉ, O. (2003). Vertebrate somitogenesis: a novel paradigm for animal segmentation? *Int J Dev Biol* 47: 597-603.

PSYCHOYOS, D. and STERN, C.D. (1996). Fates and migratory routes of primitive streak cells in the chick embryo. *Development* 122: 1523-34.

RONG, P. M., TEILLET, M. A., ZILLER, C. and LE DOUARIN, N. M. (1992). The neural tube/notochord complex is necessary for vertebral but not limb and body wall striated muscle differentiation. *Development* 115: 657-72.

SCAAL M, FUCHTBAUER EM, BRAND-SABERI B. (2001). cDermo-1 expression indicates a role in avian skin development. *Anat. Embryol.* (Berl). 203(1): 1-7.

SCHMIDT, M., TANAKA, M. and MUNSTERBERG, A. (2000). Expression of (beta)-catenin in the developing chick myotome is regulated by myogenic signals. *Development* 127: 4105-4113.

SCHUBERT, F. R., MOOTOOSAMY, R. C., WALTERS, E. H., GRAHAM, A., TUMIOTTO, L., MUNSTERBERG, A. E., LUMSDEN, A. and DIETRICH, S. (2002). Wnt6 marks sites of epithelial transformations in the chick embryo. *Mech Dev* 114: 143-148.

SELLECK, M.A. and STERN, C.D. (1991). Fate mapping and cell lineage analysis of Hensen's node in the chick embryo. *Development* 112(2): 615-26.

SOSIC, D., RICHARDSON JA, YU, K., ORNITZ, DM and OLSON, EN, (2003) Twist regulates cytokine gene expression through a negative feedback loop that represses NF-κB activity *Cell, vol. 112, 169-180.*

TADA, M. and SMITH, J. C. (2000). Xwnt11 is a target of *Xenopus* Brachyury: regulation of gastrulation movements via Dishevelled, but not through the canonical Wnt pathway. *Development* 127: 2227-2238.

TANDA, N., OHUCHI, H., YOSHIOKA, H., NOJI, S. and NOHNO, T. (1995). A chicken Wnt gene, Wnt-11, is involved in dermal development. *Biochem Biophys Res Commun* 211: 123-9.

TEILLET, M., WATANABE, Y., JEFFS, P., DUPREZ, D., LAPOINTE, F. and LE DOUARIN, N. M. (1998). Sonic hedgehog is required for survival of both myogenic and chondrogenic somitic lineages. *Development* 125: 2019-30.

THÉVENET, A. (1969). Sur les modalités de la cicatrisation de l'ectoderme dorsal chez l'embryon de poulet au cours du 3ème jour d'incubation. *Ann. Embryol. Morphog.* 2: 71-85.

VENTERS, S. J. and ORDAHL, C. P. (2002). Persistent myogenic capacity of the dermomyotome dorsomedial lip and restriction of myogenic competence. *Development* 129: 3873-3885.

VIALLET, J. P., PRIN, F., OLIVERA-MARTINEZ, I., HIRSINGER, E., POURQUIÉ, O. and DHOUAILLY, D. (1998). Chick Delta-1 gene expression and the formation of the feather primordia. *Mech Dev* 72: 159-168.

WOLF, C., THISSE, C., STOETZEL, C, THISSE, P., GERLINGER, P. and PERRIN-SCHMITT, F (1991) The M twis gene of Mus is expressed in subset s of mesodermal cells and is closely related to the *Xenopus* X-twi and *Drosophila* twist genes. *Dev. Biol.* 143*: 363-373.*