Development 127, 4611-4617 (2000)
Printed in Great Britain © The Company of Biologists Limited 2000
DEV2597

Mediolateral somitic origin of ribs and dermis determined by quail-chick chimeras

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Accepted 4 August; published on WWW 9 October 2000

SUMMARY

Somites are transient mesodermal structures giving rise to all skeletal muscles of the body, the axial skeleton and the dermis of the back. Somites arise from successive segmentation of the presomitic mesoderm (PSM). They appear first as epithelial spheres that rapidly differentiate into a ventral mesenchyme, the sclerotome, and a dorsal epithelial dermomyotome. The sclerotome gives rise to vertebrae and ribs while the dermomyotome is the source of all skeletal muscles and the dorsal dermis. Quail-chick fate mapping and diI-labeling experiments have demonstrated that the epithelial somite can be further subdivided into a medial and a lateral moiety. These two subdomains are derived from different regions of the primitive streak and give rise to different sets of muscles. The lateral somitic cells migrate to form the musculature of the limbs and body wall, known as the hypaxial muscles, while the medial somite gives rise to the vertebrae and the associated epaxial muscles. The respective contribution of the medial and lateral somitic compartments to the other somitic derivatives, namely the dermis and the ribs has not been addressed and therefore remains unknown. We have created quail-chick chimeras of either the medial or lateral part of the PSM to examine the origin of the dorsal dermis and the ribs. We demonstrate that the whole dorsal dermis and the proximal ribs exclusively originates from the medial somitic compartment, whereas the distal ribs derive from the lateral compartment.

Key words: Dermomyotome, Dermatome, Dermis, Rib, Skin, Somite, Quail-chick chimeras

INTRODUCTION

Analyses of quail-chick chimeras in which whole somites of chick embryos are orthotopically substituted by their quail counterpart have shown that somites give rise to all skeletal muscles of the body (Christ and Ordahl, 1995), whereas head muscles derive essentially from the cephalic and prechordal plate mesoderm (Couly et al., 1992; Noden, 1983). Similar experiments have established the somitic contribution to the vertebrae and intervertebral disks that form the axial skeleton (Bagnall et al., 1988; Goldstein and Kalcheim, 1992). These types of experiment have also demonstrated the somitic origin of the ribs and of part of the scapula (Chevallier, 1975; Christ et al., 1974; Huang et al., 2000). In contrast, some other bones of the scapular and pelvic girdles, including the coracoid, the clavicle or the pelvic bone are of lateral plate origin (Chevallier, 1975). The somitic origin of the dermis of the back has also been evidenced using a similar approach (Mauger, 1972). In contrast, the dermis of the head and neck derives from neural crest cells (Couly and Le Douarin, 1988), while the dermis of the lateral and ventral body wall comes from the lateral plate (Christ et al., 1983).

Somites originally appear as epithelial structures but their ventral part rapidly loses its epithelial organization to form the sclerotome. The dorsal part of the somite retains its epithelial aspect and forms the dermomyotome. The fates of the dermomyotome and of the sclerotome have been followed by quail-chick chimera analyses (Huang et al., 2000). In these experiments, grafts of the dissected dermomyotome gave rise to skeletal muscles and to the dorsal dermis, whereas sclerotome transplants yielded the axial skeleton and the totality of the ribs. However, other series of transplants that involved the graft of three consecutive dermomyotomes, as well as dermomyotome ablations, have led to a different conclusion in favor of a dermomyotomal origin for the distal part of the rib (Kato and Aoyama, 1998).

Somites can be further subdivided into a medial and a lateral compartment on the basis of the origin of the cells and of the different fates of their derivatives. Quail-chick chimeras in which the medial or the lateral half of the epithelial somite

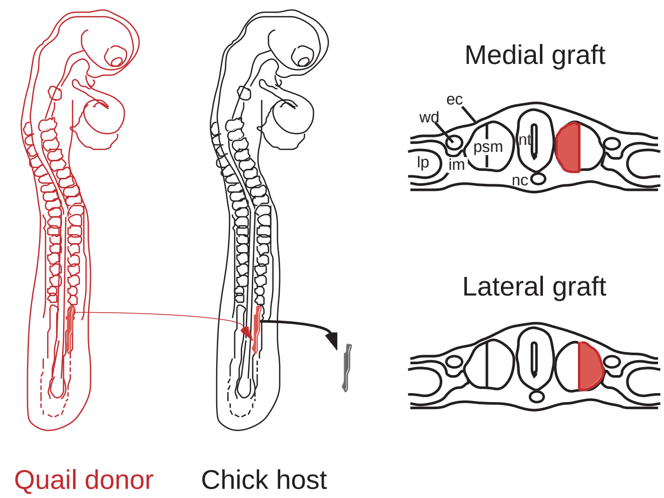

Fig. 1. Microsurgical procedure. The rostral medial or lateral unsegmented presomitic mesoderm (psm) from 12 to 20 somite chick host embryos was replaced by its quail counterpart. ec, ectoderm; im, intermediate mesoderm; lp, lateral plate; nc, notochord; nt, neural tube; psm, presomitic mesoderm; wd, wolfian duct.

from a chick host was replaced by its quail counterpart have demonstrated that they respectively give rise to the epaxial and the hypaxial muscles (Ordahl and Le Douarin, 1992). The epaxial muscles are formed by the translocation of cells of the epithelial dermomyotome into the myotome through a process that remains controversial (Cinnamon et al., 1999; Denetclaw and Ordahl, 2000; Denetclaw et al., 1997; Kalcheim et al., 1999). These cells ultimately give rise to the paraxial muscles associated with the vertebral column that are innervated by the dorsal ramus of the spinal nerve (Ordahl, 93). The hypaxial muscles derive from a set of migrating cells that arise from the lateral part of the dermomyotome and invade the somatopleural territory of the limb bud or of the body wall (Christ and Ordahl, 1995 and references therein). These limbs and body wall muscles are innervated by the ventral ramus of the spinal nerve. This subdivision of the somite into a medial and lateral compartment has been also evidenced by DiI-labeling experiments that demonstrated their distinct origins in the primitive streak (Psychoyos and Stern, 1996; Selleck and Stern, 1991).

Although the mediolateral origin of the muscles and the vertebrae has been well documented, nothing is known about the mediolateral origin of the dorsal dermis and the ribs. In this report, we have created quail-chick chimeras of the medial or lateral presomitic mesoderm (PSM) and analyzed the differentiation of the dermis and the ribs. Our results demonstrate that the dorsal dermis and the proximal ribs are exclusively of medial somitic origin, while the distal ribs derive from the lateral compartment.

MATERIALS AND METHODS

Orthotopic quail-chick chimeras

Fertile eggs from chick embryos (JA57 strain from Institut de Sélection Animale, Lyon, France) were incubated at 37°C until they reached stage 12 (Hamburger and Hamilton, 1992). All surgical experiments were performed in ovo. The somite staging system developed by Ordahl (1993) has been used for numbering somites, i.e. the somite number in roman numeral corresponds to the number of the somite starting from the last segmented somite (somite I). Grafts of the medial and lateral part of the presomitic mesoderm were performed as described in Ordahl and Le Douarin (1992). All graft experiments were performed at the level of the presomitic mesoderm and embryos were reincubated for different periods of time. Out of a total of 73 grafted embryos, 29 appeared morphologically normal and were retained for further analysis.

Immunohistochemistry

Chimeras were harvested between day 3 (E3) and day 10 (E10) of incubation and fixed in Bouin's fluid, paraffin embedded and sectioned at 7 μm. Adjacent sections were treated for immunohistochemistry to detect quail cell nuclei (QCPN anti-quail antibody; Developmental Studies Hybridoma Bank), and either early

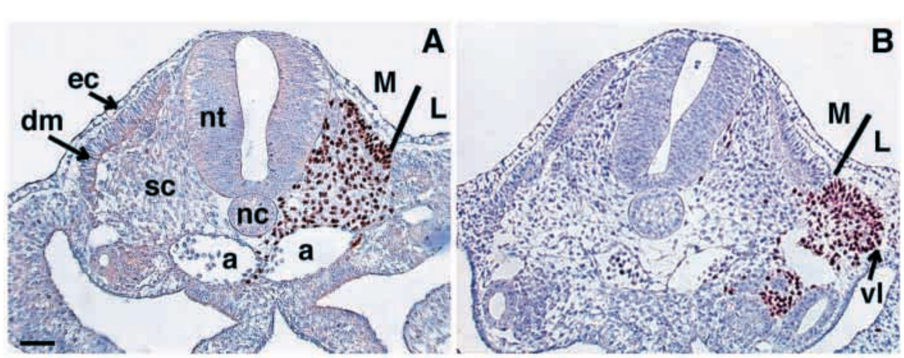

Fig. 2. The medial and lateral somitic compartments are already allocated in the presomitic mesoderm. Sections of medial (A) and lateral (B) PSM chimeras analyzed 15 hours after surgery. Sections were stained with QCPN antibody and revealed with the peroxidase reaction (brown), which labels the quail nuclei. (A) In differentiated somites derived from medial grafts, quail cells are found in the medial (M) half of the dermomyotome (dm) and sclerotome (sc), while the lateral (L) dermomyotome and its ventrolateral lip are made of chick cells. (B) In lateral grafts, the ventrolateral lip (vl) of the dermomyotome at thoracic levels, some lateral sclerotomal cells and part of the intermediate mesoderm are of quail origin. a, aorta; ec, ectoderm; nc, notochord; nt, neural tube. Scale bar: 25 μm.

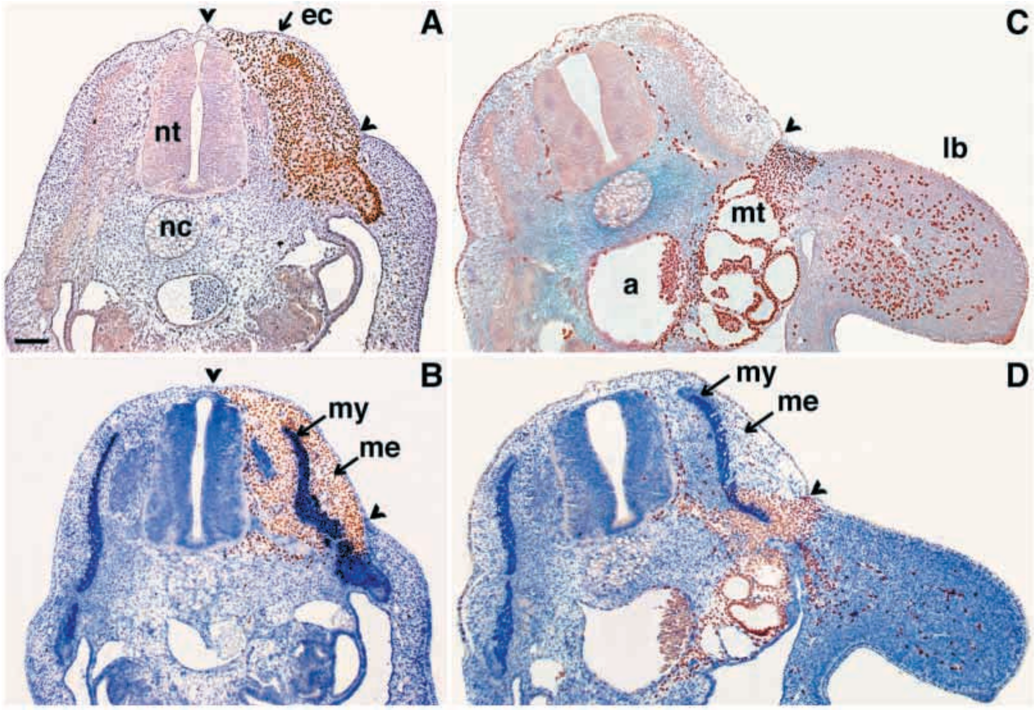

Fig. 3. The whole dorsal mesenchyme derives from the medial somitic compartment. Analysis of medial (A,B) and lateral (C,D) presomitic mesoderm chimeras, two days after the graft. Sections were stained with QCPN antibody (brown) to reveal quail nuclei and counterstained either with Alcian Blue (light blue), which labels the cartilage (A,C) or the 13F4 antibody (dark blue) to detect the mature muscle fibers (B,D). (A) In medial grafts analyzed at E4, quail cells gather under the ectoderm (ec) as a scarce mesenchyme over a differentiated myotome, as revealed by the 13F4 staining (B). The quail cells extend over the whole territory of the future dorsal dermis (between arrowheads). No quail cells are found in the mesenchyme in the hypaxial domain. (A,B) All epaxial derivatives including the dorsal mesenchyme underlying the ectoderm are of quail origin, but the hypaxial bud of the dermomyotome that will form the body wall muscles is also of quail origin, indicating that the graft extends into the lateral somitic domain. Nevertheless, the limit between the back and lateral predermal mesenchyme remains extremely sharp and is materialized by a constriction corresponding to a local thickening of the ectoderm. (C,D) In lateral grafts stained with QCPN and Alcian Blue (C), or with QCPN and 13F4 antibodies (D) scattered quail cells are detected in the limb bud and probably correspond to hypaxial limb muscle progenitors. In the graft shown in C, the limit between medial and lateral somitic domains is sharp and no somitic quail cells are detected in the medial somitic derivatives. In contrast, the most lateral part of the medial domain, including the myotome of the graft shown in D, also contains quail cells indicating that the graft extended slightly into the medial domain. The scattered cells observed around the neural tube and the nerves are probably endothelial cells that migrated from the graft. In both cases, the mesonephros is clearly derived from the graft as well as the most proximal dorsal limb mesenchyme. This indicates that the graft extended laterally into the intermediate mesoderm and lateral plate. Note that the boundary between medial and lateral predermal mesenchyme from somitic and lateral plate origin respectively is extremely sharp (arrowheads in C,D). a, aorta; lb, limb bud; me, subectodermal mesenchyme; mt, mesonephric tubules; my, myotome; nc, notochord; nt, neural tube. Scale bar: 50 μm.

myogenic or muscle cells (13F4 mAb antibody (Rong et al., 1987)), or stained with Alcian Blue to visualize cartilage cells (Pourquié et al., 1996).

RESULTS

Evidence for specification of the medial and lateral somitic compartments at the presomitic mesoderm level

In order to trace the origin of the medial and lateral somitic compartments in the PSM, we have produced quail-chick chimeras of either the medial (n=15) or the lateral part (n=14) of the PSM (Fig. 1). Five embryos in which the medial moiety of the chick rostral PSM was replaced by the corresponding quail tissue were analyzed one day after the operation (E3) to verify the quality of the grafting technique. In three cases the graft boundaries corresponded to the limit between medial and lateral somitic compartments. The medial half of the dermomyotome and of the sclerotome was of quail origin, while the lateral half was made up of chick cells (Fig. 2A). The chimeric dermomyotomes of quail and chick origin formed either a continuous epithelial structure, or two half dermomyotomes next to each other. In the remaining cases, either all or two-thirds of the dermomyotome and sclerotome of the somites at the operated level was of quail origin, suggesting that not only the medial but also most of the lateral PSM had been ablated in the host.

A converse experiment was performed in which the lateral moiety of the chick presomitic mesoderm was replaced by the corresponding quail tissue. To evaluate the limit of the grafts, five cases were analysed at E3. In three cases the lateral half to the lateral third of the somite were of quail origin (Fig. 2B). In one case, all the somite, medial and lateral, was made of quail cells, and in one case very few grafted cells were detected.

Therefore, the majority of the half-PSM grafts resulted in the generation of chimeric half somites in which either the medial or the lateral half was labeled by quail cells. These experiments confirm that cells of the medial and lateral somitic halves are already spatially segregated at the rostral level of the PSM, as originally suggested by DiI-labeling fate-mapping experiments (Psychoyos and Stern, 1996; Selleck and Stern, 1991).

Medial somitic origin of the entire dorsal dermis

In order to evaluate the respective contribution of the medial

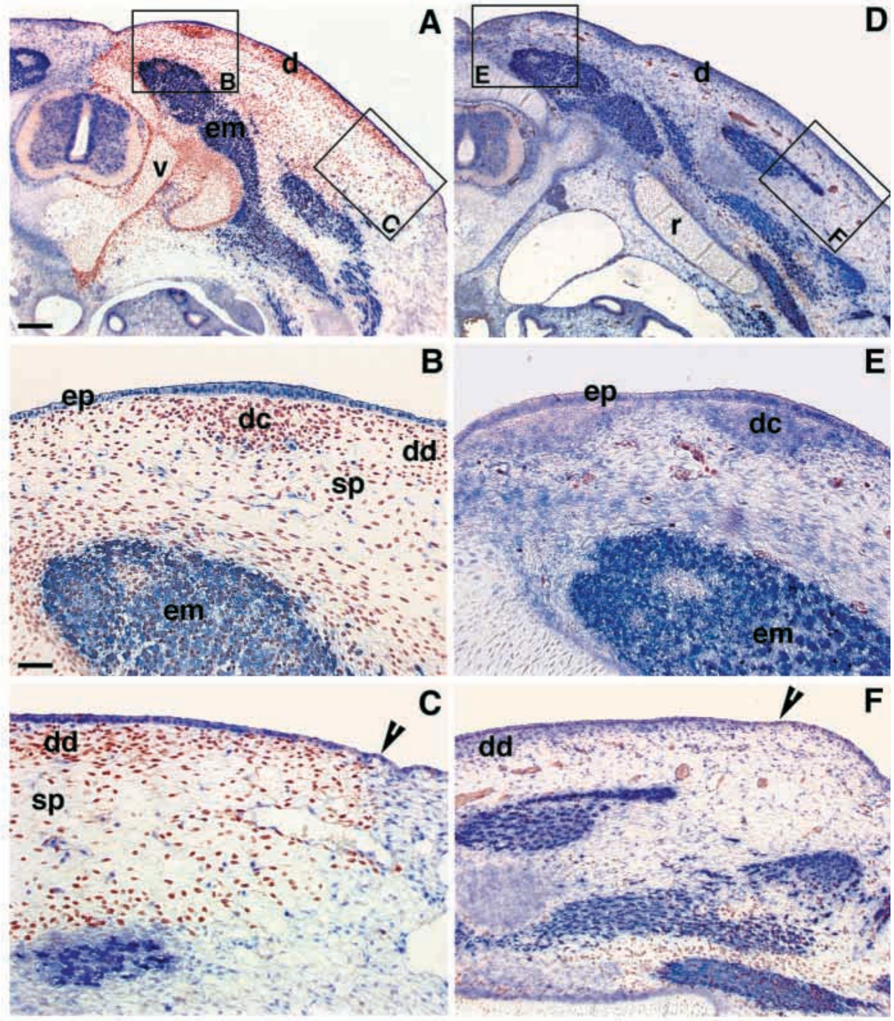

Fig. 4. Medial origin of the dorsal dermis. Sections of chimeric embryos at E7 (A-C) or E8 (D-F) double stained with QCPN to visualize the quail nuclei and the 13F4 antibody, which recognizes the muscle fibers. (A) In medial grafts, the dermis (d), epaxial muscles (em) and half of the vertebra (v) are comprised of quail cells. (B,C) Higher magnification of the panels boxed in A. (B) Detail of a dermal condensation (dc), which is exclusively composed of quail cells. Both the superficial dense dermis (dd) and a deep sparse dermis (sp) are also of quail origin. (C) Detail of the junction of the dorsal dermis with the dermis of lateral plate origin (arrowhead). The end of the dense superficial dermis corresponds to this boundary, which is sharp and physically identifiable as a notch in the ectoderm. Therefore, the dermis of all the future spinal pteryla is of quail origin, even in the most lateral region (C). (D-F) In contrast, when lateral quail presomitic mesoderm is grafted, the back dermis is entirely derived from the chick host. (D) A few quail cells are detected in the epaxial domain and are seen lining the blood vessels indicating that they correspond to endothelial cells. (E,F) Higher magnification of the regions boxed in D. (F) In these lateral grafts, hypaxial muscles are always made up of quail cells but the most lateral dense dermis is deprived of quail cells. dc, dermal condensation; ep, epidermis; r, ribs. Scale bars: 1 mm in A,D; 250 μm in B,C,E,F.

and lateral somitic compartments to the formation of the dorsal dermis, chimeras of medial and lateral PSM were created and allowed to develop up to E8. In the grafts of quail medial PSM, analysed two days after the operation (E4, n=3), the epithelial dermomyotome was no longer present and was replaced by a sparse mesenchyme localized between the ectoderm, and epaxial myotome and dorsal neural tube (Fig. 3A). This mesenchyme, which comprises presumptive dermal cells, was essentially composed of quail cells indicating its medial origin. Muscle cells of the myotome recognized by the 13F4 antibody (Fig. 3B), as well as the axial precartilaginous cells derived from the sclerotome, were also of quail origin. Laterally, quail cells were never found in the dermis beyond the back/flank border, which is distinguished by a local ectodermal thickening and a notch.

In grafts of quail lateral PSM analysed at E4 (n=2), the cell-sparse mesenchyme between the ectoderm, neural tube and epaxial myotome was composed of chick cells (Fig. 3C,D). In these grafts, scattered quail cells corresponding to the limb muscle progenitors known to derive from the lateral somite were observed in the limb bud. The mesonephros was always of quail origin, indicating that the quail intermediate mesoderm was included in the graft. In some cases a small domain of the proximal limb mesenchyme, including the future dermis, was also composed of quail cells, indicating that the graft extended into the medial-most part of the lateral plate (Fig. 3C,D). Remarkably, this domain never extended into the dorsal domain and exhibited a sharp boundary at the back/flank border.

Older chimeras were then analyzed to examine the differentiated dermis. A total of seven chick embryos in which the medial PSM was substituted by its quail counterpart were analyzed between 6 and 8 days of incubation. As already reported, vertebrae and epaxial muscles derived from the medial quail grafted tissue (Ordahl and Le Douarin, 1992). At E7 (Fig. 4A), a superficial dense dermis differentiated just beneath the ectoderm, on top of a sparse deep dermis that would not participate in feather development. In both kinds of dermis, almost all cells were of quail origin (Fig. 4B). Nevertheless, in rare instances, cells of the chick host intermingled with the quail-derived mesenchyme. These cells correspond to endothelial cells of invading vessels or Schwann cells of neural crest origin. At E7, the first dermal condensation surrounded by interfollicular dermis appeared. In the grafted embryos, both dermal regions were composed of quail cells (Fig. 4A). A sharp boundary (Fig. 4C) was seen between quail and chick fibroblasts in the dermis that demarcates the lateral edge of the dorsal feather field (spinal pteryla).

In grafts of the quail lateral PSM that were analyzed at E8 (n=7), all cells of the dense dermis of the back, as well as those

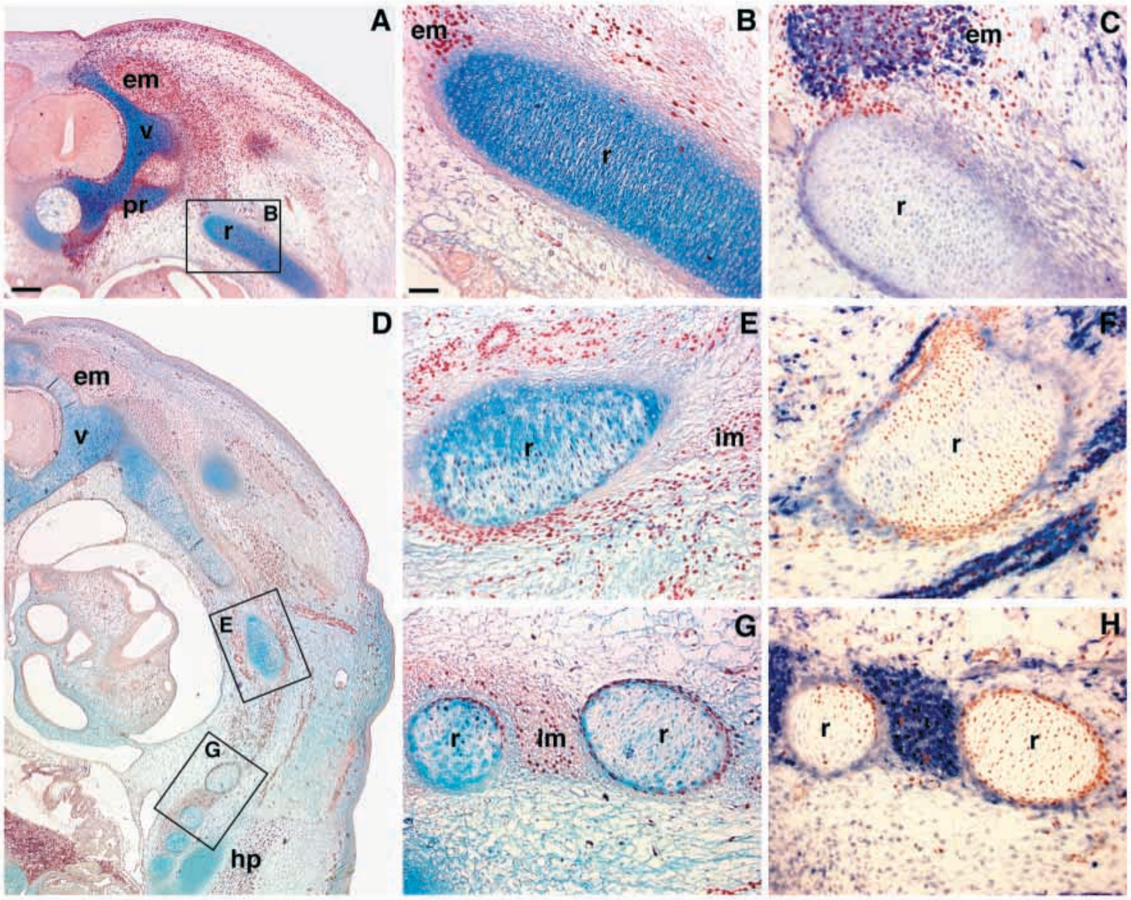

Fig. 5. Proximal ribs derive from the medial PSM while distal ribs originate from the lateral PSM. The medial (A-C) or lateral (D-H) unsegmented presomitic mesoderm was replaced by the corresponding quail tissues in chick hosts at the thoracic level and the embryos fixed at E7-E8. Sections were double-stained with QCPN to evidence the quail nuclei (brown) and with Alcian Blue to visualize cartilage (A,B,D,E,G) or with the 13F4 antibody which labels muscle fibers (dark blue) (C,F,H). (B,E,G) are adjacent sections of those shown in (C,F,H), respectively. (A-C) In grafts of medial quail PSM, the vertebra, the proximal rib (pr) the epaxial muscles and the dorsal dermis are exclusively composed of quail cells, whereas the distal ribs are made of chick cells. (B) Higher magnification of the region boxed in A showing the absence of quail cells in the distal part of the rib. (C) Adjacent section to the one shown in (A,B), an epaxial muscle containing quail cells is adjacent to the distal rib entirely composed of chick cells. (D-H) Grafts of quail lateral PSM. In these chimeras, quail cells are always found in the distal ribs and in intercostal muscles. Ribs can be partially (E,F) or completely (G,H) composed of quail cells. Note that Alcian Blue staining partially quenches QCPN staining in cartilage (compare adjacent sections B/C, E/F and G/H). em, epaxial muscles; hp, hypaxial muscles; im, intercostal muscles; r, ribs, v, vertebra. Scale bars: 1 mm in A,D; 250 μm in B,C,E,F.

forming the underlying connective tissue, epaxial muscles and vertebrae, were of chick origin (Fig. 4D-F). Most quail cells were found in the limbs and in the ventral muscles at trunk levels. In some cases, quail cells were also found in the lateral-most dorsal dermis, but they were always found in embryos that also had quail cells contributing to the epaxial muscles, indicating that the graft had extended into the medial domain. No quail cells were ever found in the dermis of the limbs or the body wall, indicating that the lateral somitic half does not give rise to any dermis. Therefore the lateral somitic half gives rise only to muscles and cartilage. Altogether, these experiments demonstrate a medial somitic origin for the dorsal dermis.

Mediolateral somite origin of the proximal and distal ribs

We subsequently analyzed the respective contribution of the medial and the lateral PSM to the ribs. Embryos in which medial chick PSM was substituted by its quail counterpart or vice-versa, at the thoracic level, were analyzed after E7. From this stage on, ribs can be unambiguously identified. Sections were processed with Alcian Blue staining, to visualize the cartilaginous condensation that identifies the ribs in the thoracic region, and with the 13F4 antibody, to label the differentiated muscles.

Out of four embryos analyzed at E7-E8 that received an orthotopic graft of medial quail PSM, only three were grafted at the thoracic level. In these chimeras, the proximal part of the rib, which is linked to the vertebra, was always found to be composed of quail cells (Fig. 5A). In contrast, the entire distal rib tissue was essentially composed of chick cells (Fig. 5A-C). A few quail cells were sometimes observed in the distal ribs (less than 10%), but they were always found in embryos that also had quail cells contributing to the intercostal muscles, which derive from the lateral somite, suggesting that the grafts slightly extended in the lateral compartment.

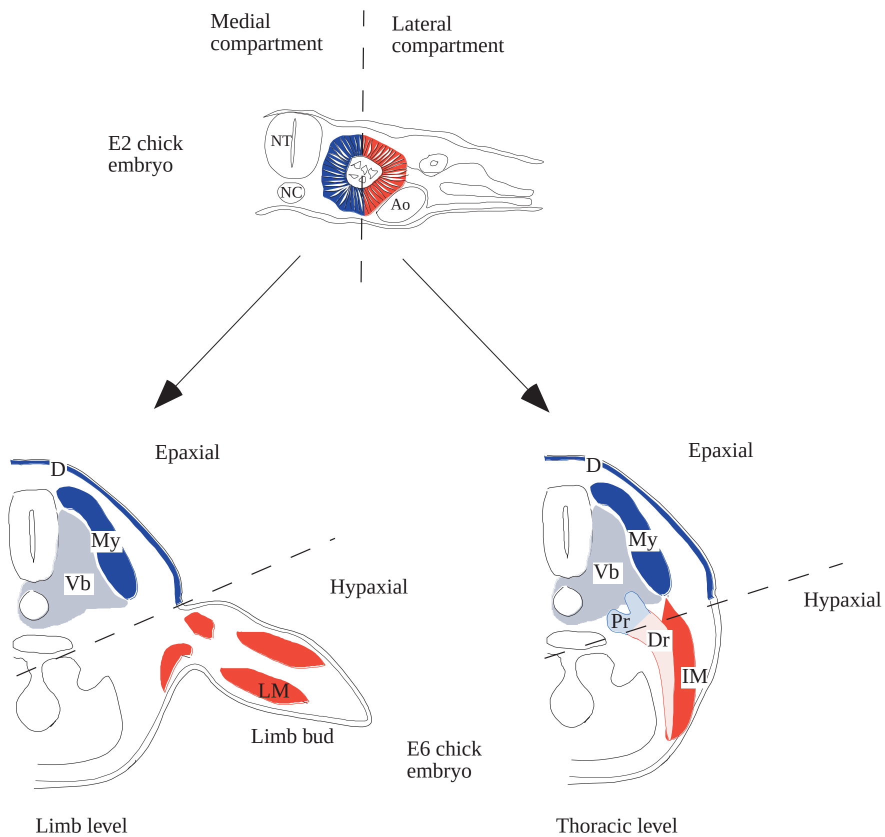

Fig. 6. The derivatives of the lateral and medial somitic compartment at the brachyal (left) and at the thoracic (right) levels. Ao, aorta; IM, intercostal muscles; LM, limb muscles; NC, notochord; NT, neural tube; Vb, vertebra; Dr, distal rib; Pr, proximal rib; D, dorsal dermis; My, myotome.

Three chick embryos in which the lateral PSM was replaced by its quail counterpart at the thoracic level were analysed at E7-E8. In the grafted region of chimeras, no quail cells were ever detected in the proximal ribs, whereas distal ribs were either entirely or partly composed of quail cells (Fig. 5D-H). In these animals, the body wall muscles were also found to be of quail origin in the grafted region. These experiments demonstrate that the proximal ribs derive from the medial somite, whereas the distal ribs originate from the lateral somite.

DISCUSSION

The avian somites can be subdivided into a medial and a lateral half, which respectively arise from distinct precursor populations distributed differentially along the primitive streak during gastrulation (Psychoyos and Stern, 1996; Selleck and Stern, 1991). Using quail-chick chimeras, it has been established that the medial somite gives rise to the sclerotome and to the epaxial muscles, while the lateral somite yields the limb and body wall muscles (Ordahl and Le Douarin, 1992). In these experiments, however, the origin of other somitic derivatives such as the dorsal dermis and the ribs was not addressed. We have reinvestigated this matter and shown that the entire dorsal dermis and the proximal ribs derive from the medial half of the somite, while the distal ribs originate from the lateral somite (Fig. 6).

We performed quail-chick chimeras of either medial or lateral PSM. An inherent difficulty of these microsurgery experiments is the absence of morphological demarcation between the medial and lateral domains of the paraxial mesoderm. Therefore, although great care was taken to separate the PSM into two equivalent halves, the mediolateral limit of the grafted tissues was found to vary significantly, owing to the technical approach. However, in all grafts of quail medial PSM, the epaxial muscles, the vertebrae, the proximal ribs and the dorsal dermis were always found to be mostly composed of quail cells. In contrast, in several chimeras of lateral PSM analyzed, some quail cells were also found in the dorsolateral dermis. However, these cells were always found in cases that also contributed quail cells to the epaxial muscles and the vertebrae. This suggests that the physical limit between the medial and lateral domains in the presomitic mesoderm is probably not precisely in the middle but slightly lateral. Our observations are thus in agreement with the limit proposed by Ordahl and Le Douarin (1992) between the epaxial and the hypaxial domain. Accordingly, expression of markers of the lateral somite, such as *Sim1*, defines a domain narrower than the medial domain (Pourquié et al., 1996).

The medial and lateral moieties of the somites are also characterized by their differential response to trophic factors derived from the neural tube and notochord (Teillet and Le Dourain, 1983; Rong, 1992). Ablation of the neural tube and the notochord has been shown to lead to a rapid death of the medial somitic derivatives, i.e. the epaxial muscles, the dorsal dermis and the vertebrae, whereas the muscles derived from the lateral compartment were found to develop normally (Mauger, 1972; Teillet et al., 1998; I. Olivera-Martinez et al., unpublished). Therefore, in terms of trophic dependence, and response to determining factors, the precursors of the dorsal dermis behave like cells derived from the medial somite, in agreement with the findings presented in this paper.

Intriguingly, in these ablation experiments, whole ribs were found to be absent, indicating that they also behave like medial somitic derivatives (although our experiments indicate a lateral origin for the distal rib). This suggests that sclerotome and dermomyotome exhibit different trophic requirements and that all sclerotomal cells are dependent on axial organ signals such as sonic hedgehog; only the medial dermomyotome shares this property.

We have also demonstrated that the proximal part of the rib originates from the medial part of the somite, whereas the distal one derives from the lateral compartment. These results are interesting in the light of the recent mutation analysis of the *Uncx4.1* gene in the mouse. In the *Uncx4.1* mutant, the proximal but not the distal rib is lacking, indicating that they are under different developmental control (Leitges et al., 2000; Mansouri et al., 2000). A distinct embryonic origin for the proximal and distal rib was recently proposed by Kato and Aoyama (1999), who have suggested that the proximal rib is of sclerotomal origin, whereas the distal rib is of dermomyotomal origin (based on quail-chick grafts and dermomyotome ablations). These results were, however, subsequently challenged by a study by Huang et al. (2000) that involved quail-chick sclerotome and dermomyotome grafts and indicates that both parts of the rib derive from the sclerotome. Our studies do not shed light on this controversy, since they do not reflect the dorsoventral origin of this structure in the somites. Although, together with these studies, our results suggest that the proximal part of the rib derives from the medial sclerotome, the origin of the distal rib origin (from the lateral dermomyotome or the lateral sclerotome) remains thus controversial. Nevertheless, our results strongly argue in favor of a distinct embryonic origin for the proximal and distal ribs.

We thank Dr N. Le Douarin for the use of her laboratory facilities, and an anonymous reviewer for his excellent suggestions on the proximal and distal rib origin. We also acknowledge Dr Kim Dale for her critical reading of the manuscript. This research was supported by the CNRS, the AFM and ARC (grant number 5269 to Dr D. Dhouailly). I. O.-M. is a recipient of an ARC fellowship.

REFERENCES

Bagnall, K. M., Higgins, S. J. and Sanders, E. J. (1988). The contribution made by a single somite to the vertebral column: experimental evidence in support of resegmentation using the chick-quail chimaera model. *Development* **103**, 69-85.

Chevallier, A. (1975). Role of the somitic mesoderm in the development of the rib cage of bird embryos. I. Origin of the sternal component and conditions for the development of the ribs. *J. Embryol. Exp. Morphol.* **33**, 291-311.

Christ, B., Jacob, H. J. and Jacob, M. (1974). Experimental studies on the development of the thoracic wall in chick embryos. *Experientia.* **30**, 1449-1451.

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

Christ, B. and Ordahl, C. P. (1995). Early stages of chick somite development. *Anat. Embryol.* **191**, 381-396.

Cinnamon, Y., Kahane, N. and Kalcheim, C. (1999). Characterization of the early development of specific hypaxial muscles from the ventrolateral myotome. *Development* **126**, 4305-4315.

Couly, G. and 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., 101-113.

Couly, G. F., Coltey, P. M. and Le Douarin, N. M. (1992). The developmental fate of the cephalic mesoderm in quail-chick chimeras. *Development.* **114**, 1-15.

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. J., Christ, B. and Ordahl, C. P. (1997). Location and growth of epaxial myotome precursor cells. *Development* **124**, 1601-1610.

Goldstein, R. S. and Kalcheim, C. (1992). Determination of epithelial half-somites in skeletal morphogenesis. *Development.* **116**, 441-445.

Hamburger, V. and Hamilton, H. L. (1992). A series of normal stages in the development of the chick embryo (1951). *Dev. Dyn.* **195**, 231-272.

Huang, R., Zhi, Q., Schmidt, C., Wilting, J., Brand-Saberi, B. and Christ, B. (2000). Sclerotomal origin of the ribs. *Development* **127**, 527-532.

Kalcheim, C., Cinnamon, Y. and Kahane, N. (1999). Myotome formation: a multistage process. *Cell Tissue Res.* **296**, 161-173.

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.

Leitges, M., Neidhardt, L., Haenig, B., Herrmann, B. G. and Kispert, A. (2000). The paired homeobox gene uncx4.1 specifies pedicles, transverse processes and proximal ribs of the vertebral column. *Development* **127**, 2259-2267.

Mansouri, A., Voss, A. K., Thomas, T., Yokota, Y. and Gruss, P. (2000). Uncx4.1 is required for the formation of the pedicles and proximal ribs and acts upstream of pax9. *Development* **127**, 2251-2258.

Mauger, A. (1972). 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**, 313-341.

Noden, D. M. (1983). The embryonic origins of avian cephalic and cervical muscles and associated connective tissues. *Am. J. Anat.* **168**, 257-276.

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

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**, 461-471.

Psychoyos, D. and Stern, C. D. (1996). Fates and migratory routes of primitive streak cells in the chick embryo. *Development.* **122**, 1523-1534.

Rong, P. M., Ziller, C., Pena-Melian, A. and Le Douarin, N. M. (1987). A monoclonal antibody specific for avian early myogenic cells and differentiated muscle. *Dev. Biol.* **122**, 338-353.

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**, 657-672.

Selleck, M. A. and Stern, C. D. (1991). Fate mapping and cell lineage analysis of Hensen's node in the chick embryo. *Development.* **112**, 615-626.

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**, 2019-2030.

Teillet, M. A. and Le Douarin, N. M. (1983). Consequences of neural tube and notochord excision on the development of the peripheral nervous system in the chick embryo. *Dev. Biol.* **98**, 192-211.