

Dermomyotomal origin of the ribs as revealed by extirpation and transplantation experiments in chick and quail embryos

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SUMMARY

To elucidate role of the dermomyotome in the formation of the axial skeleton, we performed extirpation and transplantation experiments on the dermomyotomes in chick and quail embryos. When the thoracic dermomyotomes of chick embryos were removed, the intercostal muscles and the distal ribs were deficient, while the proximal ribs were more or less normal. Quail tissues including the dermomyotome, the ectoderm and the medial edge of lateral plate, were transplanted to replace chick dermomyotomes. In these chimeras, the ribs, which would be deficient without the back-transplantation, were recovered. The cells of the recovered part of the ribs as well as the intercostal muscles were derived from the quail transplants. These findings suggest that the distal rib originated from the dermomyotomes and not the sclerotome as previously believed. To localize the origin of the distal rib further, we removed restricted regions of the

dermomyotomes along the mediolateral and the rostrocaudal axis. The more lateral the part of the dermomyotomes that we removed, the more distal the part of the ribs affected. On the contrary, when the rostral and caudal edges of the dermomyotomes were removed, only the vertebral ribs showed extensive deficiencies while removal of the middle part between the edges caused less deficiency. The sternal ribs were not deficient in either case, but were extensively affected when the entire lateral edge of dermomyotomes was included in the region removed. We conclude that the lateral edges of the dermomyotomes are the primordia of the sternal ribs, and the rostral and/or caudal edges of the medial part of dermomyotomes are the primordia of the distal part and not of the proximal part of the vertebral ribs.

Key words: Rib, Dermomyotome, Origin, Chick, Quail, Chimera

INTRODUCTION

The ribs show clear regionalization along the rostrocaudal axis in birds and mammals. While they are independent long bones in the thoracic region, in the other regions they appear as small parts of the vertebrae. Such distinct morphological differences in the ribs along the rostrocaudal axis facilitates investigation of the mechanism of the regional differentiation of axial skeleton and is important in understanding the rostrocaudal regionalization of vertebrates.

The vertebrate somite in the early stage is composed of an epithelial vesicle within which somitocoel cells form mesenchyme. Soon the ventral part of the epithelial tissue begins to de-epithelialize and combines with somitocoel cells to form mesenchymal tissue, the sclerotome, which has been considered to be the primordium of the axial skeleton, both the vertebrae and the ribs. The remaining epithelial tissue in a somite is called dermomyotome; this differentiates into the myotome, which gives rise to the muscles of limbs and trunk, and into the dorsal dermis. Thus the somite is subdivided into two distinct compartments, a dorsal part, which develops into

muscle and dermis, and a ventral part, which produces the axial skeleton (for reviews, see Tam and Trainor, 1994; Christ and Ordahl, 1995; Christ et al., 1998).

Although the intercostal muscle and the ribs have been considered to be derived from separate primordia, there seems to be a relationship between these two tissues in their morphogenesis. The formation of the distal ribs, which constitute the main part of rib (see Fig. 1), was shown to need expression of *myf5*, a transcriptional factor gene for muscle differentiation, by a knockout mouse experiment (Braun et al., 1992). The observed very close anatomical relationship between the distal ribs and the intercostal muscle in avian embryos has led us to examine the interaction of these two tissues in morphogenesis.

To elucidate role of the dermomyotome in the formation of the ribs, we performed extirpation and transplantation of the dermomyotomes in avian embryos. Surprisingly, the results of these experiments strongly suggest that the distal ribs are not derived from the sclerotome but from the dermomyotome. We will discuss the mechanism of rib formation in relation to the development of intercostal muscles.

MATERIALS AND METHODS

Embryos

Fertilized eggs of the White Leghorn chick and the Japanese quail were purchased from local farms and incubated at 38°C. The long axis of the hen's egg was always kept in a horizontal position during incubation.

Extirpation and transplantation

All operations were performed on chick embryos incubated for 3 days (the 30- to 35-somite stage, HH stage 17-18; Hamburger and Hamilton, 1951) *in ovo*. After removal of about 3 ml of the albumen, a window about 3 cm in diameter was made on the upper surface of the eggshell, through which the embryos were observed. The embryo was operated on under a dissecting microscope illuminated by a double-light guide. In order to improve the contrast between the embryo and the yolk, a small amount of India ink diluted with Tyrode's solution was injected under the embryo, and the number of somites of the embryo was counted for staging. Tyrode's solution was dropped onto the vitelline membrane overlying the embryo and the membrane was torn with a tungsten needle to expose the embryo. For the extirpation experiments, three consecutive thoracic dermatomyotomes at around somite stage X (Ordahl, 1993), with the overlying epidermal ectoderm, were entirely or partially removed using a tungsten needle and a microscalpel made from a sewing needle. In some cases, to confirm the removal, a vitelline membrane was inserted between the dermatomyotomes and sclerotomes during the operation and the embryo fixed immediately. After the operation, the window of the eggshell was sealed with Sekisui OPP tape and the egg further incubated at 38°C for 7 days.

For the isochronic and isotopic transplantation, we used chick and quail embryos that were at the same stages as for the extirpation experiment stated above. The host chick embryos were deprived of three consecutive thoracic dermatomyotomes and their overlying ectoderm as stated above. The grafts were prepared from the quail embryo using tungsten needles and comprised dermatomyotome and ectoderm, and also part of lateral plate with a width of half a dermatomyotome to prevent the graft from rolling up. The isolated quail tissue was transplanted isotopically into chick host embryo and fixed with sharpened tungsten needles with a diameter of 10 µm. After the operation, the window of the eggshell was sealed with Sekisui OPP tape and further incubated at 38°C for 7 days.

Histology

7 days after the operation (HH stage 35-37), the embryos were taken out of the egg and fixed with Carnoy fixative overnight before the visceral organs were removed.

For observation of cartilaginous skeleton in the whole-mount preparation, the embryos were stained with Alcian blue 8GX in 70% ethanol and 1% HCl overnight (Simons and Van Horn, 1971). They were destained in 70% ethanol and 1% HCl and dehydrated through an ethanol series and cleared with methyl salicylate.

To identify the quail cells in the chimeras that received transplanted quail tissues, some embryos were embedded in paraffin after fixation and removal of the visceral organs. On 7 µm serial sections, quail cells were identified after Feulgen's staining by their characteristic nuclear marker (Le Douarin, 1969, 1973). The sections were stained with Alcian blue 8GX to visualize cartilaginous tissue.

The embryos in which a vitelline membrane was inserted between the dermatomyotome and the sclerotome were fixed with 4% paraformaldehyde in phosphate-buffered saline (pH 7.4) immediately after the operation. They were embedded in paraffin and serially sectioned to a thickness of 7 µm before being stained with hematoxylin/eosin.

RESULTS

Nomenclature: subdivision of the ribs in avian

Fig. 1 shows our division of the rib into the proximal, the distal vertebral and the sternal rib.

Removal of the thoracic dermatomyotomes

We removed surgically three consecutive thoracic dermatomyotomes, which were at around somite stage X (Ordahl, 1993), with the overlying epidermal ectoderm, from chick embryos at the 30- to 35-somite stage (HH stage 17-18; Hamburger and Hamilton, 1951) using a microscalpel (Fig. 2A,B). We observed the skeletons of the embryos ($n=8$) 7 days after the operation. All the embryos that we examined lacked some part of the ribs on the experimental side (Fig. 2C). The number of malformed ribs varies from one to four in different embryos so we analyzed the most affected rib. If the lengths of the proximal, distal vertebral and sternal rib on the operated side were less than half those on the opposite unoperated side, they were regarded as deficient. Fig. 2E shows the frequency of deficiency in each part of the rib caused by the dermatomyotome removal. All the distal vertebral ribs and 75% of the sternal ribs showed deficiency, while the proximal ribs were not affected at all.

To confirm whether the dermatomyotome was removed without any contamination of other tissues and whether any part of the dermatomyotome was left in the embryos, we inserted the vitelline membrane between the dermatomyotomes and the sclerotomes in some embryos during the operation and fixed them immediately. In most embryos ($n=11$), the surgically removed dermatomyotomes did not carry the fragment of the sclerotome (Fig. 3) while, in two embryos, a small clump of mesenchymal cells attached to the removed dermatomyotome. In Fig. 3, the sclerotome as well as the ectoderm and the dermatomyotome appear to be smaller than in the control, which may be due to shrinkage on histological preparation. In three embryos, the myotomes, derivatives of the dermatomyotomes, remained on the embryos and, in two embryos, there remained

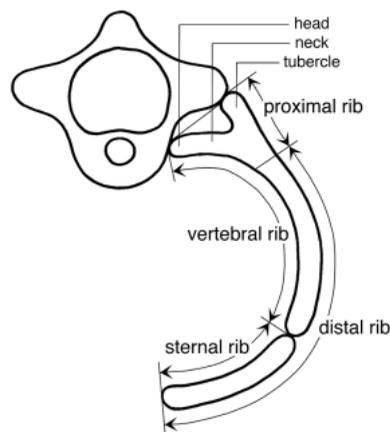


Fig. 1. Diagram of the axial skeleton at the third to seventh thoracic vertebra levels of a chick embryo at HH stage 35-37. Caudal side view. Two components of the avian ribs are the vertebral rib and the sternal rib. The ribs are also divided into the proximal rib including the head, the neck and the tubercle of rib, and the distal rib. Upper is dorsal.

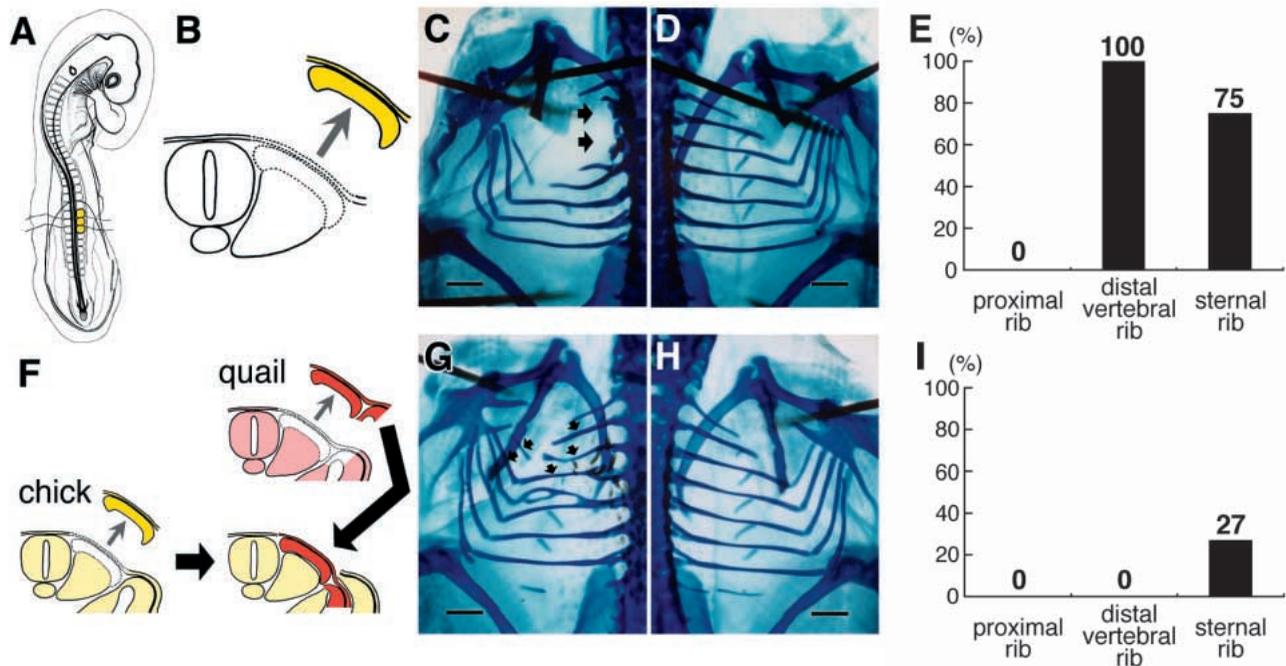


Fig. 2. Extirpation (A-E) and transplantation (F-I) of the thoracic dermomyotomes. (A) Illustration of a chick embryo at the 30- to 35-somite stage (HH stage 17-18). Dorsal view. Three consecutive thoracic dermomyotomes (yellow) were removed. (B) Cross section diagram of the extirpation. Upper is dorsal. Dermomyotomes were removed together with the overlying epidermal ectoderm. (C,D) Ventral view of the axial skeleton in the embryo 7 days after the extirpation. Stained with Alcian blue. Upper is rostral. Deficient distal ribs (C, arrows) on the experimental right side and normal ribs on the control side (D). (E) Incidence of deficiency in each part of the rib after the dermomyotome removal. The ordinate represents the incidence of embryos with deficient rib. If the proximal, distal vertebral, and sternal rib on the operated side were evidently less than half of those on the opposite unoperated side, we regarded them as deficient. (F) Diagram of the transplantation. Dermomyotomes and the overlying ectoderm of a chick embryo at the 30- to 35-somite stage were replaced with the corresponding piece of a quail embryo. The grafts were composed of not only the dermomyotome with the ectoderm, but also a part of the lateral plate with a width of half a dermomyotome to prevent the graft from rolling up. (G,H) Ventral view of the axial skeleton in the embryo 7 days after the transplantation. Stained with Alcian blue. On the experimental side (G) ribs are not deficient comparing those on the control side (H). (I) Incidence of deficiency in each part of the rib after the dermomyotome transplantation. The ordinate represents the incidence of the deficiency. (C,D,G,H) Scale bars, 1 mm.

a small piece of the lateral edge of the dermomyotomes. Thus in most cases, we removed only the dermomyotomes, though not always completely, and left the sclerotome in situ.

These findings suggest that the dermomyotome and/or the epidermis overlying somites is necessary for the development of distal rib but not for the proximal rib morphogenesis.

Does the dermomyotome include the distal rib primordium? – labeling experiments on the thoracic dermomyotome using chick-quail chimeras

The above findings could be interpreted in two ways. First, the dermomyotome that was removed surgically might include the primordium of the distal rib; second, the dermomyotomes and their overlying ectoderm might signal the sclerotome to generate the distal ribs. To establish which is the case, we replaced the dermomyotome and the overlying ectoderm of a chick embryo at the 30- to 35-somite stage with the corresponding piece of a quail embryo, which contained some additional tissues for the reason stated below (Fig. 2F), and examined whether the resultant distal rib, which would be lost without the graft, was derived from the host or the graft (Le Douarin, 1969, 1973). In this experiment, the graft included not only the dermomyotome and the ectoderm but also the intermediate mesoderm and a small part of the lateral

mesoderm, since the dermomyotomes with or without the ectoderm curved so intensely soon after the isolation from quail embryos that we could not transplant them into chick embryos in the normal position. With the mesoderm lateral to the dermomyotome, the graft remained flat, which allowed us to transplant it easily.

One week after the transplantation, 11 chimeric embryos out of 15 showed no deficiency in the ribs (Fig. 2G), while four showed deficiency in the sternal ribs (Fig. 2I). Thus the deficiency of the distal rib caused by the removal of the dermomyotome was recovered by the back transplantation. To elucidate the origin of the recovered ribs, we made sequential sagittal sections of these embryos and identified the quail cells after Feulgen's staining ($n=2$; Fig. 4). Quail cells were found in the ribs (Fig. 4C) as well as in the intercostal and some other trunk muscles (Fig. 4B). Quail cells occupied the entire cross section of the ribs, indicating a part of the rib was made exclusively with the derivatives of transplants containing dermomyotome. A reconstruction from the serial sections (Fig. 5) showed the distribution pattern of the quail cells. In this figure, the parts of the rib and the intercostal muscles made exclusively of quail cells are coloured blue and red, respectively. Descendants of the transplants formed the entire intercostal muscle through the proximodistal axis and the distal

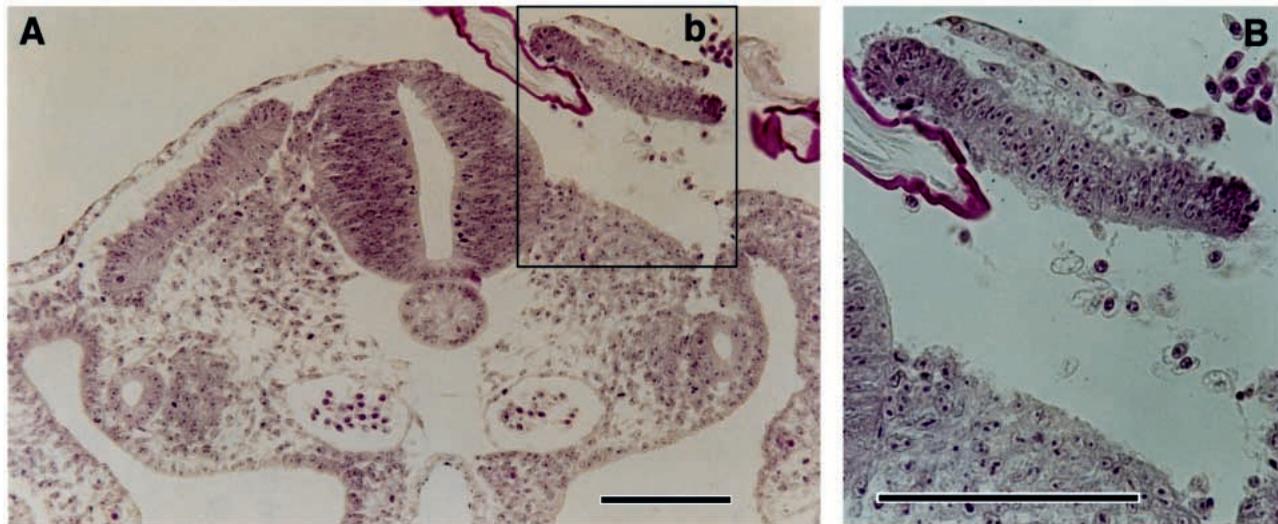


Fig. 3. (A) Transverse section of the embryo with the vitelline membrane inserted underneath the dermomyotome separated from the sclerotome. The embryo was fixed immediately after the operation. (B) Higher magnification of the surgically removed dermomyotome (boxed area in A) was shown to bear little sclerotomal tissue. Scale bars, 100 μm .

rib including the sternal rib. Rostrocaudally, both tissues made of quail cells completely overlapped.

The interspecific grafting experiments demonstrate that the primordium of the distal rib was located in the graft. Since the distal rib as well as the proximal rib has been shown to originate in the somite (Chevallier, 1975), the primordium of the distal rib must be in the dermomyotome, the derivative of somite, and not in the mesoderm more lateral to the dermomyotome. It is surprising that the distal rib is the derivative of the dermomyotome, since the entire rib has been considered to be derived from the sclerotome.

Where is the primordium of the rib? – extirpation experiments of the restricted part of dermomyotomes

To identify the location of the primordium of the rib within the dermomyotome, we removed a different part of the dermomyotome in 3-day chick embryos at about the 30-somite stage and tried to correlate the resultant deficiency of the rib to each part of the dermomyotome removed. We compared the most deficient rib on the operated side with the rib on the opposite unoperated side. Since the proximal rib was not affected at all by any type of operation, we compared quantitatively the length of the vertebral and the sternal ribs on the operated side with those on the unoperated side. As the length of vertebral rib, we measured from the tip of tubercle to the distal end, where they articulated with the sternal ribs.

Mediolateral localization of the distal rib primordium in the dermomyotome

To determine the position of the distal rib primordium in the mediolateral direction, we removed the medial two thirds and the lateral third sections of three successive dermomyotomes as a rectangle with the long axis parallel to the rostrocaudal axis of the embryo (Fig. 6).

When the medial two thirds of the three thoracic dermomyotomes were removed surgically ($n=12$), the vertebral

ribs shortened to 51% (s.d.=14%) of the control, while the sternal ribs were scarcely affected, 92% (s.d.=21%) of the control. Removal of the lateral third of the dermomyotomes ($n=9$) caused shortening of only the sternal rib (64%; s.d.=30%) and scarcely affected the vertebral rib formation (92%; s.d.=7%). Removal of the medial two thirds of the dermomyotomes resulted in significantly shorter vertebral ribs than removal of the lateral third of the dermomyotomes ($P<0.001$, Welch's *t*-test). On the contrary, the sternal ribs were significantly shorter after removal of the lateral third of the dermomyotome compared with removal of the medial two thirds ($P<0.05$, Student's *t*-test).

Thus the part of the distal rib that was deficient corresponded to the part of the dermomyotome removed along the mediolateral axis. These findings suggest that the primordium of distal rib extends all over the dermomyotome in terms of the mediolateral axis and the vertebral rib is derived from the medial part of the dermomyotome and the sternal rib from the lateral part.

Rostrocaudal localization of the distal rib primordium in the dermomyotome

To elucidate the distribution of distal rib primordium in the rostrocaudal direction, the rostral and caudal edges or middle parts between them were removed from each of three successive dermomyotomes (Fig. 6).

When we removed part of the thoracic dermomyotomes in the vicinity of the area of contact between two adjacent dermomyotomes, that is, the caudal edge of one dermomyotome and the rostral edge of another ($n=21$), the lengths of vertebral rib and sternal rib on the experimental side were 51% (s.d.=16%) and 90% (s.d.=28%) of those on the control side, respectively. The vertebral rib shortened significantly ($P<0.001$, paired *t*-test), while the sternal rib did not ($P>0.05$, paired *t*-test).

On the contrary, when we removed the middle part of the dermomyotome leaving the caudal and the rostral edges intact

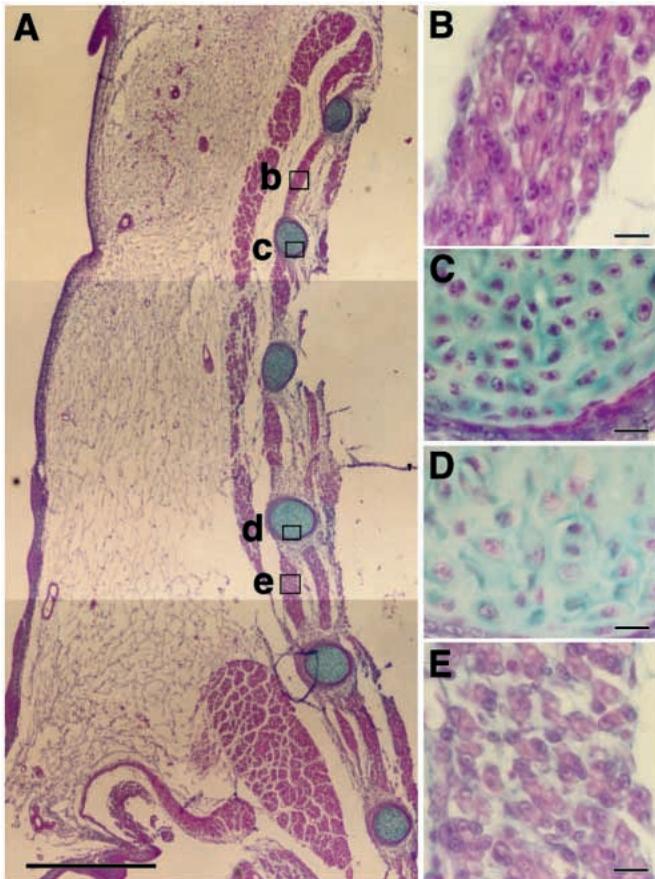


Fig. 4. (A) Transverse section of the thoracic wall through distal vertebral rib of the operated chimeric embryo. Quail cells were distinguished from chick cells by their conspicuous nucleoli after Feulgen's reaction. Tissues were stained with Alcian blue to identify the cartilaginous tissue. Scale bar, 500 μm . (B-E) Higher magnification of rectangles in A marked with b-e, respectively. (B) Quail cells derived from the graft in the intercostal muscle. (C) Quail cells in the cartilaginous rib, which was stained with Alcian blue. (D) Chick cells in the rib. E: Chick cells in the intercostal muscle. Scale bars, 20 μm .

($n=30$), little deficiency was found in any part of the rib. The lengths of the vertebral and the sternal rib on the operated side were 80% (s.d.=12%) and 90% (s.d.=8%) of the controls, respectively. The vertebral rib shortened significantly ($P<0.001$, paired t -test) even in this case, but not as much as in embryos deprived of the rostral and caudal edges of dermomyotomes ($P<0.001$, Student's t -test).

Thus, the extirpation of the rostral and caudal edges of dermomyotome or the middle part of dermomyotome leaving the two edges, caused a deficiency of vertebral rib and hardly affected the sternal rib formation. Moreover, the removal of the rostral and caudal edges of dermomyotome shortened the vertebral rib more than the removal of the middle part between them.

The extirpation experiments showed overall that the vertebral rib clearly shortened both in the case of the removal of the medial two thirds of dermomyotome and of the removal of its rostral and caudal edges. On the contrary, the sternal rib shortened only when the lateral third of the dermomyotomes

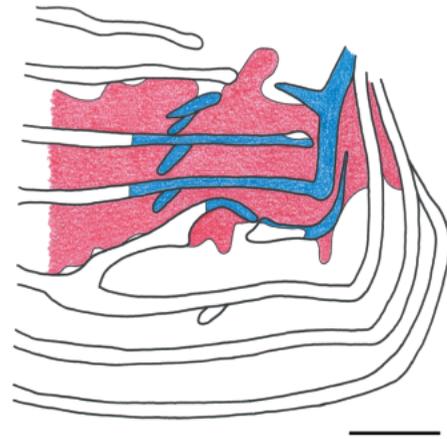


Fig. 5. Illustration of the ribs and the intercostal muscles in the chick-quail chimera in which the quail dermomyotome was transplanted. Reconstruction from every twentieth 7 μm serial section. Ribs and intercostal muscles composed of quail cells are coloured blue and red, respectively. The area occupied by the ribs made of the quail cells were included in the area of intercostal muscles of the quail cells. Scale bar, 1 mm.

were removed. It was significantly shorter than in the case of the removal of the medial two thirds of dermomyotomes, of their rostral and caudal edges ($P<0.05$, Student's t -test) and of their middle part along the rostrocaudal axis ($P<0.05$, Student's t -test).

DISCUSSION

Distal rib development and the dermomyotome

When we removed thoracic dermomyotomes in 3-day chick embryos, deficiencies in the distal ribs occurred with high frequency but the proximal ribs were almost normal 7 days after the operation. Back graft of the quail tissue containing the dermomyotome rescued the deficiency. The fact that the recovered ribs were composed of quail cells derived from the graft indicates that the deficiency was caused by a loss of the primordia of the distal ribs and not by a loss of induction factor for rib formation, i.e., the distal rib must be derived from the dermomyotome and not from the sclerotome. This result is surprising, since the axial skeleton has been believed to be entirely derived from the sclerotome (for reviews, see Tam and Trainor, 1994; Christ and Ordahl, 1995; Brand-Saberi et al., 1996).

In this chimeric back transplantation, the grafts contained not only the dermomyotomes but also the epidermis, the intermediate mesoderm and the lateral plate to maintain shape. Although the lateral plate was once considered to be the origin of the sternal rib (Straus and Rawles, 1953), it has now been well demonstrated that the sternal rib as well as the vertebral rib originates in the thoracic somites by various marking experiments (Seno, 1961; Pinot, 1969; Chevallier, 1975). Thus, the rib is considered to be derived from the somites. Therefore, in the chimeras with the transplants of dermomyotomes, the ribs composed of the quail cells must originate from the dermomyotomes of the graft and not from the epidermis or the lateral plate mesoderm transplanted with the dermomyotomes.

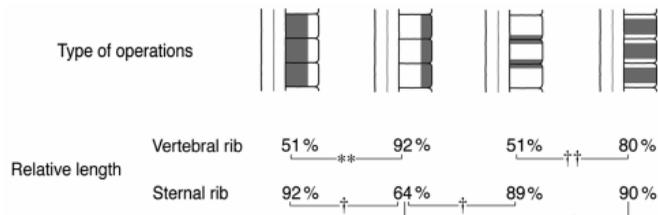


Fig. 6. Partial deprivation of the dermomyotome and a deficiency of the rib. The removed part of the dermomyotome with the ectoderm is represented by shading in dorsal view diagrams. Left is medial. Beneath the diagrams are represented percentages of the length of ribs most affected on the operated side to those on the opposite unoperated side. Values are compared using Welch's *t*-test (* $P < 0.05$; ** $P < 0.001$) and Student's *t*-test († $P < 0.05$; †† $P < 0.001$). The vertebral ribs were deficient in the embryo deprived of the medial part of the dermomyotome and the sternal ribs were deficient when the lateral edges of the dermomyotome were extirpated. The vertebral rib is much shorter after removal of the rostral and caudal edges of dermomyotome than after removal of the middle part between them.

We used only a sharpened tungsten needle and a microscalpel for the extirpation of the dermomyotomes, and did not employ an enzymatic processing method. Nevertheless the sclerotomes did not remain attached to the operated dermomyotomes except for a few cases, in which a very small clump of mesenchyme was seen. It could be argued that these small amounts of mesenchymal tissue are the origin of the distal ribs. However, it is unlikely because the incidence of the recovery was very high.

Our results show that the distal ribs are derived from the dermomyotome and/or, possibly, a small amount of tissue adjacent to the dermomyotome, and not from the sclerotome.

Proximal and distal ribs

The rib can be divided into two parts, the proximal rib containing the head, the neck and the tubercle of rib, and the distal rib, containing the more distal vertebral rib and the entire sternal rib. Our result indicates that the distal ribs are derived from the dermomyotome, while the proximal rib as well as the vertebra derive from the sclerotome.

Recently, it has been suggested that, in both the origin and mechanism of development, the proximal and distal rib differ. Using chick-quail chimeric embryos, the somitocoel cells (Huang et al., 1994, 1996) or the caudal half of the somite (H. A. and K. Asamoto, unpublished data) were shown to be the origin of the proximal rib. The mechanisms controlling morphogenesis of the proximal and the distal rib are also suggested to be different. *Myf5* knockout mice lacked the distal ribs, while their vertebrae and proximal ribs formed (Braun et al., 1992). On the contrary, both in knockout mice of *Sonic hedgehog* (*Shh*) (Chiang et al., 1996) and in *Danforth's short tail* mutant mice (Gluecksohn-Schoenheimer, 1945), which could not form complete notochord, the proximal ribs were deficient with malformation of vertebrae. Furthermore, Koseki et al. (1993) found that *undulated* mutant mice showed both deficiency in the proximal rib and lack of *Pax1*, which expresses in the sclerotome specifically in normal embryos (Deutsch et al., 1988).

Thus, these results together show that the development of the distal ribs depends on *myf5*, the transcriptional factor for

muscle cell differentiation from the dermomyotome, while the proximal ribs develop from somitocoel cells or caudal-half somites depending on the *Shh* produced by the axial structures. Our present result showing that the removal of dermomyotome caused deficiency in the distal ribs and not the proximal ribs and vertebrae, and that transplanted dermomyotome gave rise to the distal rib, accord with these studies and suggest that the origin of distal ribs is different from those of the proximal ribs and the vertebrae. Furthermore, the fact that the dermomyotome is the common origin of muscles and the distal ribs suggests intimate interactions of the intercostal muscles and ribs in their morphogenesis, which was suggested by *myf5* knockout mice experiment.

The somitic compartments forming the rib

Next, we determined which part of the dermomyotome gives rise to the distal rib. Our partial removal experiments showed a higher frequency of deficiencies in the distal vertebral ribs on removal of the medial part of dermomyotomes than of the lateral part, and of the rostral and caudal edges than the middle part (Fig. 7). These results suggest that the primordium of the distal vertebral rib is in the rostral and/or caudal edge of the medial dermomyotome. Marking the intersomitic area by carbon particles (Seno, 1961) showed that medially injected carbon particles labeled only a proximal part of the distal rib, while laterally the entire rib was marked. Our fate map for the distal vertebral rib is consistent with his results.

On the contrary, the sternal rib was deficient only when the lateral edge of the dermomyotome was extirpated. Since the removal of a part of it caused little effect on the sternal rib formation, we could not localize further the origin of the sternal rib. When a piece of metal foil was inserted into the medial end of the somatopleure, the sternal ribs did not form (Sweeney and Watterson, 1969; H. A. and H. Koseki, unpublished data). Injection of carbon particles into the lateral plate labeled only the sternal rib (Seno, 1961). These experiments suggest that the sternal ribs are derived from a cell population emigrating laterally along the parietal peritoneum. *Pax3* is strongly expressed in the dermomyotomal lateral edge and in laterally

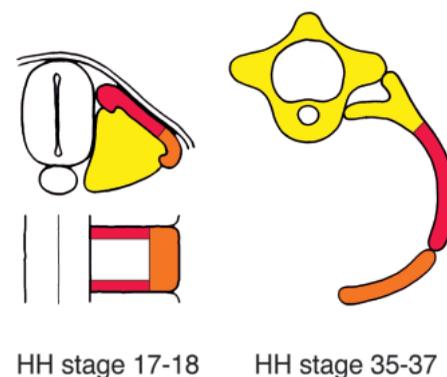


Fig. 7. Developmental fate of the mesodermal tissue in the formation of the axial skeleton. Colouring indicates corresponding part of the embryos at two different stages. In the dermomyotome, the medial part of rostral and/or caudal edges produces the distal vertebral rib (red), while the lateral edge of the dermomyotome gives rise to the sternal rib (orange). The sclerotome produces the proximal rib and the vertebra (yellow). Upper left, transverse section; lower left, dorsal view.

emigrating descendent cells until they begin to differentiate into muscle cells (Goulding et al., 1993). The expression of *MFH1* (Winnier et al., 1997) was found to be specific to the thoracic dermomyotomal lateral edge and to the anlage of rib (H. Koseki and H. A., unpublished data). Considering these findings, the myotomal lateral edges are likely to be the origin of both the sternal ribs and the hypaxial muscles, although we need further experiments to trace the fate of dermomyotomal lateral edge.

Therefore, we tentatively concluded that the origin of the distal vertebral rib was the rostral and caudal edges and of the sternal rib was the lateral edge of the dermomyotome (Fig. 7).

The mechanism of the distal rib morphogenesis

Our findings that the dermomyotome is the origin of both the intercostal muscles and the distal ribs are very important in elucidating the morphogenesis of the rib in relation to the development of the intercostal muscles. *Myf5* knockout mice showed delay in the muscle development and deficiency in the distal ribs (Braun et al., 1992). Recently, the development of intercostal muscles was shown to be controlled by *myf5* and not by *myoD* (Kablar et al., 1997). *Myf5* might control the development of the distal rib through the development of intercostal muscles. If common stem cells for the distal rib cells and the hypaxial muscle cells are present in the dermomyotome, they might produce cartilage and muscle primordial cells simultaneously, resulting in the spontaneous establishment of an anatomical relationship between the distal rib and the intercostal muscles. Even if such stem cells of the rib and the hypaxial muscles are separated in the early stages of somite development, both primordium should be close together and the myoblast precursor cells might migrate accompanying the cartilage precursor cells by mechanisms such as specific intercellular adhesion, chemotaxis, etc. To identify the hypothetical common stem cells, cell lineage should be traced in the somite or its derivatives with such as a dye injection. Thus, our findings on the origin of the distal rib can lead us to propose new ideas on the morphogenesis of the ribs in relation to the development of intercostal muscles.

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