Two myogenic lineages within the developing somite

CHARLES P. ORDAHL*† and NICOLE M. LE DOUARIN
Institut d'Embryologie Cellulaire et Moléculaire du CNRS et du College de France, 49 bis Avenue de la Belle Gabrielle, 94736 Nogent-sur-Marne-Cedex, France

*Present address: Department of Anatomy, University of California, San Francisco, San Francisco, CA 94116, USA
†Author for correspondence

Summary

It is well known that the muscles of the vertebrate body are derived from the somite. Precursor cells within the somite proper form the back or axial muscles while other precursor cells migrate away from the somite to populate the muscle of the limbs and ventral body wall. Although both types of muscle are generally thought of as arising from a common progenitor population, the myotome, recent evidence points to developmental differences in these two groups of muscles which may reflect different developmental lineages. To test the lineage hypothesis, we used microsurgery and the chick-quail nucleolar marker system to follow the developmental fate of the lateral and medial halves of somites at the wing level. The results showed that the structures of the mature somite (myotome and sclerotome) are derived virtually exclusively from cells residing in the medial half of the newly formed somite. On the other hand, virtually all of the cells residing in the lateral half of the newly formed somite are destined to leave the somite proper and populate the limb muscle and, probably, other somite-derived mesenchymal structures in the limb and ventral body wall. Switch-graft experiments show that the two halves of newly formed somites are largely interchangeable demonstrating that their ultimate developmental fate is position-dependent and that it becomes fixed as a result of extrinsic influences which act during later stages of somitogenesis. We conclude that at least two distinct myogenic lineages exist in the somite; one giving rise to the muscles of the back and the other giving rise to the limb musculature.

Key words: cell migration, chick-quail, determination, limb muscle, myotome, myfkins, sclerotome.

Introduction

The somites of the developing embryo represent the primitive metameric units of the vertebrate body (Keynes and Stern, 1988; Stern et al., 1988). Somites form in the segmental plate mesoderm immediately lateral to the developing neural tube. They give rise to the segmental vertebral column and, in the thoracic region, the ribs. Somites also contain the precursor cells destined to give rise to the dermis of the skin and virtually all of the skeletal musculature of the vertebrate body. Pre-somites, first discernible in the unsegmented mesoderm, pinch off at regular intervals from the anterior end of the segmental plate. Initially a solid ball of cells, each somite matures into a hollow ball composed of high columnar epithelium. Further changes follow leading to the formation of sclerotome, myotome and dermatome which give rise to cartilage, muscle and dermis, respectively (reviewed in Keynes and Stern, 1988; Stern et al., 1988).

In addition to the well-documented organizational changes outlined above, somites contain a suborganization which imposes segmental effects upon the nervous system as well as upon the mesodermal structures of the body. For example, the rostral half of each somite - but not its caudal half - is permissive for the outgrowth of spinal nerves, and the formation of the dorsal root ganglia (Keynes and Stern, 1984; Teillet et al., 1987; Kalcheim and Teillet, 1989; Bronner-Fraser and Stern, 1991). This segmental outgrowth of nerves is ultimately reflected in the body via the segmental innervation of specific muscle groups and areas of the skin. Although the rostral-caudal organization of the somite is not apparent to the eye, surgical procedures demonstrate that it exists as soon as somites bud off from the segmental plate.

Might similarly invisible suborganizations direct the development of other somite derived structures such as skeletal muscle? Although it is generally considered that all of the skeletal muscle arises from a common somitic progenitor pool, the myotome, there are at least two groups of skeletal muscle whose development show distinct differences. First, the muscles of the axial skeleton (back or axial muscles) arise from somite cells which differentiate in situ within the somite and between the sclerotomal organizing centers of the
vertebrae. A second group, the muscles of the limbs, arise from cells that migrate away from the somite and invade the lateral regions of the embryo (Christ et al., 1977; Chevallier et al., 1977). In addition to these migratory differences, these two groups of muscles show other developmental differences. For example, axial muscles differentiate initially as mononucleate myocytes (Keynes and Stern, 1988) while limb muscles are multinucleate (Rutz et al., 1982). Axial muscle precursor cells in the myotome express myogenic determination factors (Myf 5, MyoD1, etc) at, or before, the onset of myotome formation (Weintraub et al., 1991). Indeed, expression of such genes is presently the earliest marker of myotome formation (Ott et al., 1991). On the other hand, expression of myogenic determination genes in limb muscle cannot be detected until approximately 2 days later when the limb muscle masses begin to coalesce (Ott et al., 1991; de la Brousse and Emerson, 1990). Finally, recent experiments in this laboratory indicate that the early differentiation and survival of axial skeletal muscle is strongly influenced by the adjacent neural tube and notochord while the migration and subsequent differentiation of limb skeletal muscle is apparently independent of such influence (Teillet and Le Douarin, 1983; Rong et al., 1990).

Given such differences in the development in axial and limb musculature we wanted to determine if these two populations arise from a common pool of somitic muscle progenitor cells or from separate precursor populations. Our approach was to ask whether the axial and limb muscle progenitor cells could be separated by microsurgical means prior to the changes in somite organization outlined above. Newly formed somites of chick embryos were divided into lateral and medial halves and then host half-somites were replaced with appropriate counterparts derived from quail embryos. The distinctive nucleolar marker of the quail nuclei (Le Douarin, 1969, 1973) permitted the subsequent determination of the fate of the cells from the donor half-somite used to replace either the medial or lateral half of the chick somite. The results of these experiments indicate that the precursors of the axial and non-axial muscles do indeed arise from distinct regions in the developing somite. We also demonstrate that the commitment of these two myogenic lineages to their developmental fate is position-dependent and is established subsequent to somite formation. The developing axial organs appear to provide some of the cues for the differentiation of these two types of muscle. The implications of these results for current models of somitogenesis and muscle differentiation are discussed.

Materials and methods

The experiments reported here were performed using chick (Gallus gallus) and quail (Coturnix coturnix japonica) embryos from commercial sources which were incubated at 37°C for 2 days to stage 14-15 (Hamburger and Hamilton, 1951). Embryos possessing between 16 and 21 somites were chosen as donors (quails) or hosts (chick). Donor half-somites were excised from quail embryos in silicone-bottomed dishes using microscalpels forged from stainless steel sewing needles (Teillet and Le Douarin, 1983). Pancreatin (Sigma) was used to digest extracellular matrix to assist in the removal of somite fragments which were then transferred to Tyrode’s solution in holding dishes. Chick donor embryos were injected with black ink (Pelikan) between the yolk and embryo to aid in visualization and dissected in the same manner as quail donor embryos except that excised half-somites were discarded. Donor half-somites were transferred by micropipet into recipient embryos and manipulated into place using microscalpels.

In some experiments after removing the medial half-somites, the host neural tube and notochord were also excised at the level of the anterior limb buds essentially as described elsewhere (Teillet and Le Douarin, 1983). The space vacated by the neural tube/notochord was then filled by fragments of host shell membrane. Host medial half-somites were then replaced with corresponding quail half-somites as described above.

Those embryos that survived 4 days of postoperative incubation at 37°C (approximately 50% of operated embryos), were fixed in Carnoy’s fixative, embedded in paraffin, sectioned at 5 μm and stained by the Feulgen method to reveal the distinctive nucleolar marker of the quail nuclei (see Le Douarin, 1969, 1973). All such surviving embryos were included in the analysis presented here. Some slides were subsequently treated with the muscle-specific monoclonal antibody 13F4 (Rong et al., 1987) after removal of coverslips and rehydration followed by secondary staining with fluoresceinated goat anti-mouse antisera.

Basic experimental design

Previous work has shown that wing muscle arises from myogenic precursor cells that migrate from somites 15-21 of developing chick embryos (Christ et al., 1977; Beresford, 1983). To determine whether the lateral or medial halves of the somite are capable of contributing wing myogenic cells, the medial or lateral halves of somites 15-21 in 2 day old chick embryos were replaced with half-somites from the quail (see Fig. 1). In most cases two tandem half-somites were transplanted together. Table 1 lists the embryo ages and somites transplanted in the experiments reported here.

The timing of the transplant was considered to be of critical importance. A lag exists between the time a given somite is first formed (separated from the segmental plate) and the commencement of migration of muscle precursor cells to the limb region. Based on the results of Christ et al. (1977) this lag appears to be approximately the time required to form 5 subsequent somites. Given this information, we employed 2 criteria regarding the age of somites to be used in these experiments: First, that both donor and host embryos had formed at least 15, but no more than 21 somites. Second, that the half-somites transplanted or replaced were less than 3 somites “old” (that is that no more than 2 somites had formed posterior to the oldest somite chosen has a half-somite donor or host) (See Fig. 1 and Table 1). In two cases, 5 tandem lateral half-somites were transplanted and in one case the lateral half of the segmental plate was transplanted (see Table 1, experiments 6, 7 and 8).

The orientation of transplanted somites was determined by marking donor somites either with a carbon particle or by taking advantage of adventitious tissue or wounds, or both. Often the epithelium of the endoderm or aorta immediately underlying the somites was included in the graft as this assisted in orientation and in maintaining the connection between adjacent half-somites. Using these methods, it was possible in most cases to replace half-somites without
Somitic muscle lineages

Fig. 1. Medial and lateral half-somite replacements. (A) Diagrammatic representation of a 20-somite embryo. The first two somites (shaded) have disappeared by this stage (Beresford, 1983). Only the most recently formed (caudal-most) three somites were used either as donor or hosts. (B) Diagrammatic representation of the 5 half-somite replacement experiments reported here. Quail donor half-somites (left) and the site of implantation into chick hosts (right) are indicated in black. For experimental details see text.

Table 1. Half-somite transplantation experiments

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*The numbers indicated under "Age" of donor and host embryos is the number of formed somites in those embryos. The numbers listed under "Somites" indicate the somites actually used as donors or replaced in hosts.
changing their anterior-posterior or dorsal-ventral orientations. Fig. 2 shows a transverse section through a 19 somite embryo in which the medial halves of somites 17 and 18 on the embryo's right were replaced by medial half-somites from a quail embryo 3 hours earlier. It can be seen that the somite on the operated side is composed of two halves; the medial composed entirely of quail cells bearing the distinctive nucleolar marker; the lateral half-somite is composed entirely of chick cells. The alignment and orientation between the two halves, as well as the overall size and morphology of the chimeric somite, corresponds reasonably well to that of the unoperated chick somite on the contralateral side.

Results

Medial→medial half-somite replacements

To determine the fate of cells residing in the medial half of newly formed somites, the medial halves of two somites on the right side of chick embryos were replaced with corresponding medial halves of two quail somites (Experiment 1, Fig. 1B). In each case, conventional histology (Fig. 3A-C), and immunocytochemistry using the muscle-specific antibody 13F4 (Fig. 4A,B) demonstrated that quail cells extensively populate both myotomal and sclerotomal structures within the somite. Moreover, the somitic structures on the operated side appear normal in size and structure when compared to those on the unoperated side (not shown). We conclude, therefore, that the transplantation did not disrupt subsequent somite development and that the transplanted medial half-somite participated in somitogenesis in an essentially normal fashion.

The forelimb regions of each medial→medial chimera were then analyzed for the presence of nuclei containing the quail nucleolar marker. In every case, the forelimbs, and in particular forelimb muscle tissue, were devoid of quail nuclei (Fig. 3D, and Fig. 4C,D). Thus, the cells of the transplanted medial somites did not migrate to the developing limb buds to produce its musculature. Limbs on the operated side had apparently normal muscle morphology and size (not shown), suggesting that the transplantation, and the lack of migration of medial half-somite cells, did not affect limb bud muscle development.

Lateral→lateral half-somite replacements

In five experiments two tandem lateral half-somites at the forelimb level on the right side of chick hosts were replaced with corresponding lateral half-somites from quail embryos (Fig. 1B, exp. 2). Histological analysis showed that in each case the forelimbs were extensively populated with quail nuclei in apparent muscle regions (Fig. 5D,E). Staining with 13F4 confirmed that the quail nuclei were restricted to limb muscle tissue and that in such muscles substantial numbers of quail nuclei reside within cytoplasm which is 13F4-positive (Fig. 6C,D), indicating that these nuclei had already begun to differentiate. Not all cells with quail nuclei within the muscle tissue were 13F4-positive (Fig. 6D,C). This is to be expected because at day 6 of development a large proportion of myogenic cells remain as mitotically active, undifferentiated progenitor cells which do not yet express the 13F4 antigen. Quail cells were never observed in non-muscle tissues such as cartilage or limb mesenchyme. The possible contribution of transplanted cells to the endothelial lining of blood vessels (Beddington and Martin, 1989), however, was not rigorously investigated.

It is known that specific somites contribute muscle precursor cells to specific muscle groups in the limb (Beresford, 1983) and, consistent with this observation,
Fig. 3. Medial→medial half-somite replacement chimera. (A) Low power view of somitic region. Regions illustrated at high magnification in Panels B (myotome) and C (sclerotome) are indicated. Note both regions contain a preponderance of nuclei carrying the quail nucleolar marker. (D) Low power view of limb region. Muscle region illustrated at high magnification in Panel E is indicated. Note the absence of quail nuclei in this muscle.
Fig. 5. Lateral—lateral half-somite replacement chimera. (A) Low power view of somitic region. Regions illustrated at high magnification in Panels B (myotome) and C (sclerotome) are indicated. Note both regions contain few if any nuclei carrying the quail nucleolar marker. (D) Low power view of limb region. Muscle region illustrated at high magnification in Panel E is indicated. Note the abundance of quail nuclei in this muscle.
Fig. 4. Immunofluorescent staining of muscle in a medial→medial replacement chimera. (A) Myotome field stained with the antibody 13F4 followed by fluorescein-coupled anti-mouse antisera. (B) Same field as in A except with visible light. Note the bright red nucleolar marker of the quail nuclei within myotomal cells. (C) Limb muscle field stained with 13F4 as above. (D) Same field with visible light. Note the absence of quail nuclei in this field.

Fig. 6. Immunofluorescent staining of muscle in a lateral→lateral replacement chimera. (A) Myotome field stained with the antibody 13F4 followed by fluorescein-coupled anti-mouse antisera. (B) Same field as in A except with visible light. Note the absence of quail nuclei within myotomal cells. (C) Limb muscle field stained with 13F4. (D) Same field with visible light. Note the abundance of nuclei in this field bearing the bright red quail-specific nucleolar marker. A high proportion of these quail nuclei can be seen to reside in 13F4-positive cytoplasm.
Fig. 10. Immunofluorescent staining of limb muscle in heterotopic half-somite replacement chimeras. (A) Limb muscle field of a medial-lateral replacement chimera stained with 13F4 as in Fig. 4. (B) Same field as in A except with visible light. Note the abundance of quail nuclei. Arrows indicate quail nuclei within differentiated (13F4-positive) muscle cells. (C) Limb muscle field of one of three lateral-medial replacement chimeras stained with 13F4 as above. (D) Same field with visible light. Note the presence of a small number of quail nuclei within this muscle. Arrows indicate quail nuclei clearly surrounded by 13F4-positive cytoplasm. Quail nuclei were present only in one muscle of this chimera which was the only lateral-medial chimera to contain any quail nuclei within a limb muscle.
contributions to limb muscle from any two tandem lateral half-somites was found to be spatially restricted in the forelimb. However, simultaneous transplantation of up to 5 half-somites (Table 1, experiments II-6 and II-7) resulted in extensive numbers of quail nuclei in muscle tissue at all levels of the host forelimb (data not shown). Finally, in each experiment involving transplantation of 2 consecutive lateral half-somites virtually all of the limb muscle masses also contained chicken-derived muscle cells (Figs 5D,E; 6C,D) as expected from the multiple somitic origins of individual limb muscles (Beresford, 1983).

Surprisingly, the somitic structures of lateral→lateral half-somite replacement chimeras were essentially devoid of quail cells. Fig. 5A shows the somite region of one such chimeric embryo. Both the myotomal and sclerotomal regions are composed entirely of chick cells (Figs 5B,C; 6A,B). In some cases, however, a few quail nuclei could be seen scattered among the overwhelming majority of chick nuclei. In addition to the myotome and sclerotome, the mesenchyme regions dorsolateral to the somite were also free of quail nuclei.

The absence of quail cells from the somite regions of lateral→lateral transplants led us to determine the limits of their distribution. Fig. 7A shows the body-shoulder junction region of a lateral→lateral transplant recipient at 6 days of development (4 days after transplantation). A high magnification view of this region shows that a boundary exists between the distribution of quail and chick mesenchymal cells (Fig. 7B). This boundary, which is very sharp at the body-shoulder junction, extends medially to intersect the midline in the region of the notochord (see Discussion). Quail cells from lateral→lateral transplant experiments in general remained lateral and ventral to this boundary.

Quail cells from medial→medial half-somite replacements also respected this same boundary but were restricted to the region dorsal and medial to it (data not shown). In no case were quail cells from medial→medial transplants found ventral or lateral to the boundary. However, in localized regions of a few lateral→lateral transplant cases quail cells were observed on the dorsal/medial side of the boundary where they were seen to be incorporated into the ventral-most portions of the sclerotome and myotome. Such results suggest that in those cases the incision dividing lateral and medial somite halves probably deviated into the medial somite half and thus included a small contribution from the medial half-somite. This, and the fact that quail cells were never found ventral or lateral to this boundary after medial→medial half-somite transplants suggests that the true boundary between the lateral and medial somite “halves” may in fact be somewhat to the lateral side (see Discussion).

While these experiments were in progress, Selleck and Stern (1991) reported that the medial and lateral halves of the somite are derived from different precursor populations during gastrulation. To determine if the progenitors of the lateral and medial halves of the somite intermingle at the segmental plate stage the lateral half of the segmental plate of a 16-somite chick embryo was replaced with the corresponding half-segmental plate from a 15-somite quail embryo (Fig. 8). After four days of incubation, the myotome and sclerotome of the wing-level somites of this embryo were free of quail nuclei while virtually all of the wing musculature was extensively populated with quail nuclei (data not shown). Thus, even though this is a single experiment, its result corresponds well with those of the lateral→lateral half-somite transplants reported above and with the conclusion of Selleck and Stern (1991) that lateral and medial half-somite precursors segregate in the segmental plate prior to the formation of the somites.

**Heterotopic half-somite replacements**

The results of the orthotopic half-somite experiments described above indicate that cells from the medial half-somite are destined to give rise to the bulk of the mature somite; i.e. myotome and sclerotome. On the other hand, cells from the lateral half-somite appear to leave the somite proper, giving rise to the musculature of the limb and mesenchymal tissue, both lateral and ventral to the somite proper.

To assess the extent to which the different fates of the medial and lateral somite halves are properties intrinsic to each half, or result from extrinsic influences, a series of heterotopic half-somite replacement experiments were performed (Fig. 1B, experiments 3 and 4). The same timing/position considerations outlined above for the transplants were employed for the heterotopic experiments except that donor half-somites were always taken from the left side of donor embryos and then implanted into the right-hand side of host embryos. In this manner, the position of the donor somites could be altered without changing either their anteroposterior or dorsoventral orientation (see Fig. 1).

**Medial→lateral half-somite replacements**

In three experiments, chick lateral half-somites on the right side were replaced with medial half-somites from the left side of quail embryos. In each of these cases, numerous cells bearing quail nuclei were found in the fore-limb muscle regions (Fig. 9D,E). Staining with 13F4 confirmed that these quail cells were restricted to muscle tissue, and that many of the quail nuclei had already begun to differentiate and resided in multinucleate, 13F4-positive cytoplasm (Fig. 10A,B). Thus myogenic precursor cells migrated from the heterotopically transplanted medial half-somites to populate the muscle tissues of the fore-limb.

The myotome and sclerotome structures of medial→lateral somite chimeras were predominantly composed of chick cells as shown in Fig. 9A-C. However, the somitic regions of medial→lateral transplants generally showed a larger proportion of quail nuclei than those of lateral→lateral transplants, as discussed above. In a limited region of one embryo, quail cells extensively populated both the myotome and sclerotome (not shown). Therefore, while the majority of medial somite cells migrated to the limb when placed in a lateral
Fig. 7. The body-limb junction. (A) Low magnification view of the junction between the body and the developing wing of a lateral→lateral replacement chimera. Arrow indicates point of junction. (B) High magnification view of the mesenchyme immediately adjacent to the ectoderm in the region indicated in A. Note the sharp boundary between quail cells, on the right (limb) side and chick cells on the left.
Somitic muscle lineages

Excise
Chick
Lateral
Segmental
Plate

Replace Chick
Lateral Segmental
Plate with Quail

Fig. 8. Lateral half-segmental plate replacement.
Diagrammatic representation of the 2-step procedure used to replace the lateral half of a chick segmental plate with that of quail.

position, a number of such cells remained within the somite and participated in later stages of somitogenesis.

We conclude from these results that the developmental fate of most of the medial half-somite cells is not yet fixed at the time of the transplantation. These medial somite cells are capable of responding to the migratory cues which, presumably, arise from sources both lateral and extrinsic to the somite itself. The bulk of presumptive sclerotomal and dermatomal cells of the medial half-somite never expressed their potentialities when moved from a medial to lateral position. A minority of medial half-somite cells, however, appear not to respond to these putative cues and remain within the somite proper, behaving as typical medial half-somite cells.

Lateral→medial half-somite replacements
In each of three corresponding lateral→medial half-somite replacement chimeras both myotomal and sclerotomal structures were extensively populated by quail cells (Fig. 11A-C). Thus, cells from the lateral half of newly formed somites are not irreversibly committed to migrate to limb muscle beds but, when implanted into the medial half-somite position, are capable of forming both myotome and sclerotome, the latter being a novel differentiation end-point for these cells.

The forelimbs of two of these lateral→medial half-somite chimeras were free of quail nuclei (Fig. 11D,E), indicating that the lateral half-somite cells did not respond to the migratory cues after being placed in a medial position. However, a third chimera, while largely free of quail cells in the limb muscle, had a very small number of quail nuclei in a single ventral shoulder muscle predominantly composed of chick nuclei. Staining with the muscle-specific antibody 13F4 demonstrated that these few quail nuclei resided in myogenic cytoplasm (Fig. 11C,D). Thus, while the cells of the lateral half-somite, when placed in a medial position, show a drastically reduced capacity to populate forelimb musculature via migration some cells may be capable of responding to such cues even in ectopic positions (see Discussion).

Interestingly, the medial somitic regions of the lateral→medial half-somite transplants often contained renal tubule-like structures (Fig. 11A). Renal precursor cells probably adhere to the lateral margins of transplanted lateral half-somites. When such transplants are made orthotopically, the adventitious renal precursor cells participate in renal development in the normal location (not shown). However, when such lateral half-somites are transplanted into a medial position these renal precursor cells appear to undergo differentiation in situ, possibly due to non-instructive induction by the adjacent neural tube which is known to be competent to induce mesonephric differentiation (Croisille et al., 1976).

Medial half-somite replacements in the absence of neural tube and notochord
The results above indicate that external forces influence the fate of the lateral and medial halves of the developing somites. The neural tube and notochord are sources of major external influences upon somite development. Removal of the neural tube and notochord lead to disintegration of the somite and complete disappearance of myotomal musculature (Teillet and Le Douarin, 1983). Interestingly, in such embryos the limb musculature is normal (Rong et al., 1990). Thus, there is a substantial difference between the precursors of myotomal muscle and limb muscle in their response to the presence or absence of the neural tube and notochord.

To determine if myotomal muscle precursors might be capable of migrating to the limb in the absence of the neural tube and notochord, a series of experiments were conducted in which medial→medial half-somite replacements were performed on embryos in which the neural tube and notochord had been removed in the forelimb region. After 4 days of development, four of these embryos were analyzed histologically for the presence of quail cells either in the limb muscle or somite areas.

As expected from previous studies, somites were completely absent from those regions of embryos from which the neural tube and notochord had been excised. In addition, no quail cells were found in the developing limbs or in the somite areas of these embryos (data not shown). We tentatively conclude, therefore, that removal of the neural tube and notochord does not free the medial somite cells to migrate into the developing limb bud. We assume that cells of the transplanted medial half-somite undergo cell death, as is known to be
Fig. 9. Medial→lateral half-somite replacement chimera. (A) Low power view of somitic region. Regions illustrated at high magnification in Panels B (myotome) and C (sclerotome) are indicated. (D) Low power view of limb region. Muscle region illustrated at high magnification in Panel E is indicated. Note the extensive contribution of quail cells to this muscle.
Fig. 11. Lateral→medial half-somite replacement chimera. (A) Low power view of somitic region composed of quail nuclei. Regions illustrated at high magnification in Panels B (myotome) and C (sclerotome) are indicated. (D) Low power view of limb region. Muscle region composed entirely of chick cells illustrated at high magnification in Panel E is indicated. Arrows in A indicate quail-derived renal tubule-like structures within sclerotome (see text).
the fate of a substantial proportion of somite cells in the absence of the neural tube and notochord (Teillet and Le Douarin, 1983).

Discussion

Muscle lineages and somitogenesis

The experiments described here were undertaken to determine if the myogenic precursor cells of the somite represent two distinct precursor cell populations having different origins, fates and properties. The approach that we took was surgically to divide newly formed somites longitudinally, and then to replace either the lateral or medial halves with appropriate half-somites derived from donor quail embryos. Since the cells of the Japanese quail bear a distinctive nucleolar marker (Le Douarin, 1973) the subsequent fate of the donor half-somite cells could be determined histologically. The results of these experiments demonstrated that two muscle precursor populations could be distinguished in newly formed somites. The precursors of limb muscle arise exclusively in the lateral half of the somite, whereas the precursors of myotome (axial or back muscles) arise exclusively from the medial half of the somite (Fig. 12).

These results confirm and extend those reported by Christ and Jacob and co-workers who concluded that cells that appear to be exiting the ventrolateral edge of the somite at later stages are the migrating limb muscle precursor cells (Christ et al., 1978). That conclusion is consistent with the results presented here which further demonstrate that the limb muscle precursor cells are already restricted to the lateral half of the somite when it first buds off from the segmental plate. Surprisingly, the results presented here indicate that virtually all of the cells in the lateral half of the newly formed somite are destined to migrate away from the somite proper. It is not yet known what fraction of these lateral half-somite cells are myogenic precursors.

A series of elegant studies by the same group also indicate that the myotome is first recognizable at the anteromedial edge of the somite (Christ et al., 1978; Kaehn et al., 1988). That conclusion is also consistent with the results presented here which show, in addition, that the cells destined to give rise to the mature myotome, and indeed all of the structures of the somite proper, are localized within the medial half of the newly formed somite. The generally accepted view of somitogenesis is that the ventromedial sector of the somite becomes mesenchymal and migrates around the neural tube and notochord to become the sclerotome (reviewed by Keynes and Stern, 1988). The present results would indicate that, in addition to the migration of sclerotome cells, virtually the entire lateral half of the somite is also destined to migrate to the limb and outer body wall regions. Moreover, chick-quail experiments involving replacement of the dorsal half of the somite indicate that the myotomal precursors are localized within the dorsal half of the somite (Christ et al., 1978). Taking the results of these previous studies and those of the present study together, suggest that the myotome per se is derived solely from precursor cells located within the dorsomedial quadrant of the somite at the time of somite formation (see Fig. 12). Such a conclusion is not only consistent with previous work on older somites but is also consistent with the first expression of genes involved in myogenic determination (see below).

Are the two different muscle precursor lineages found here an accident of the division of the somite along its sagittal plane or are they distinct cell lineages with different developmental origins? While the present work was in progress, Selleck and Stern reported that the lateral and medial halves of the somite are derived from different lineages during gastrulation (Selleck and Stern, 1991). They found that the progenitors of the

Fig. 12. Summary of fates of lateral and medial halves of somites at the limb level. (A) Newly formed somite at 2 days of development. Lateral (dark shading) and medial (light shading) halves are separated by a line (a-b). Ventral quadrant of medial half is lightly shaded while dorsal quadrant of the medial half is unshaded. (B) Somite of 6 day embryo shows distribution of cells shaded according to A. Darkly shaded structures arise from the lateral half of the somite. The unshaded myotome arises from the dorsomedial quadrant of the somite while the lightly shaded sclerotome arises from the ventromedial quadrant (composite of results reported here and in Christ et al., 1978). The line a-b has effectively rotated clockwise. The zone of occasional mixing indicated probably results from difficulty in precisely defining the incision of the ventral part of the somite along the median plane.
medial half of the somite are derived from the lateral portion of Hensen's node, while the progenitors of the lateral half of the somite are derived from cells located a considerable distance posterior to Hensen's node, in the primitive streak (Selleck and Stern, 1991). Their photographs of embryos in which the medial or lateral half-somite precursors were labeled with fluorescent dye suggest that the lateral and medial half-somite lineages do not intermingle within the segmental plate (Fig. 4 in Selleck and Stern, 1991). After replacing the lateral half of a chick segmental plate with that of a quail the resulting chimera had quail-derived wing muscles but chick-derived somitic structures (see Fig. 8 and Results). Thus, the chick-quail grafting experiments reported here support the conclusion of Selleck and Stern that the precursors to the lateral and medial halves of the somite are derived from distinct lineages established at the time of gastrulation and which segregate in the segmental plate prior to somite formation.

Myogenic lineage determination

If different lineages for lateral and medial half-somite precursor cells arise early in development, to what extent are their different developmental fates predetermined? It is known that some parts of the somite are endowed with specific properties in relation to their position along the anterior-posterior embryonic axis. For example, it was shown that the anterior half of the somite is uniquely capable of supporting both motor nerve outgrowth from the developing neural tube and the immigration of neural crest cells for the formation of the dorsal root ganglion (Keynes and Stern, 1984; Kalcheim and Teillet, 1989; Teillet et al., 1987).

To determine if the fates of the precursors of axial and limb muscle might be predetermined, we performed switch grafts, in which the mediolateral positions of the half-somite donors were reversed. By implanting donor half-somites from the left side to the right side of the host, this transplantation could be made without changing either the dorsoventral or anteroposterior axis. When medial half-somites were placed in a lateral position a substantial fraction of the limb muscles were populated by donor somite cells. Thus, cells normally destined not to migrate, but to differentiate into muscle (myotome) and cartilage (sclerotome) in situ, were induced to migrate to the limb and form muscle after being placed in a lateral position. Conversely, when lateral half-somites were placed in a medial position the vast majority of cells remained within the somite proper and participated in the formation of the sclerotome as well as the myotome. These results clearly suggest that, despite their segregation into distinct lineages at the time of gastrulation, the developmental potential of the cells within the medial and lateral halves of the somite are virtually equivalent when the somite is formed and that prospective limb myogenic precursor cells can alter their fate and differentiate into cartilage.

Both switch-graft experiments, however, also suggested that some of the cells in the lateral and medial half-somite may become committed to their fates during maturation of the somite. For example, when the medial half-somite was placed in a lateral position, a greater proportion of donor cells participated in mature somite formation than was observed when the lateral half-somite was placed in a lateral position. Conversely, in one of three experiments in which the lateral half-somite was placed in a medial position, a small number of donor cells were found to be present in one limb muscle, a result never obtained in nine medial—medial transplant experiments. Such results suggest the possibility that the developmental fate of the lateral and medial halves of the somite may become fixed at later times in response to influences that arise from sources outside the somite itself. While further work will be necessary to establish the time course of this putative fate commitment process, it is noteworthy that it takes place much later than that of rostral-caudal determination which is already established at the time of somite formation (Stern and Keynes, 1987).

Myogenic determination gene expression

Recent studies of the developmental expression of the gene family of inter-related myogenic determination factors (ie. myoD, myogenin, herculin, myf, qmf, etc) demonstrate that the expression of such genes is the earliest known marker of skeletal muscle development (Ott et al., 1991). Since forced expression of any one of these genes can trigger myogenesis in a wide variety of non-muscle cell types it is likely that these genes are either directly, or indirectly, involved in the determination events that lead an uncommitted embryonic mesodermal precursor cell to become committed to myogenic differentiation (Weintraub et al., 1991). Since these genes are apparently equivalent in their myogenic potency, and since there are differences in their timing of expression between species, we will refer to this family of related genes collectively as “myfkins” (Ordahl, 1992), which takes into account their kinship and the systematic nomenclature of this family first proposed by Arnold and co-workers (Braun et al., 1989, 1990).

What is the timing of myfkin expression in the two lineages leading to the development of somitic and limb muscle? Elegant studies recently published by Buckingham and co-workers (Ott et al., 1991) show that the earliest detectable expression of myfkins (in the developing mouse) is in the dorsal-medial quadrant of the somite prior to the formation of the dermomyotome. It seems clear therefore, that myfkin expression identifies myotomal precursor cells a few hours prior to the onset of their terminal differentiation. It also seems equally clear from the experiments reported here, that these early myfkin-expressing cells in the medial half of the somite cannot be precursors of the limb musculature.

In limb muscle precursor cells, the first unequivocal detection of myfkin mRNA is in the coalescing dorsal and ventral muscle masses of the limb two days after the precursor cells have left the somite (de la Brouse and Emerson, 1990, Ott et al., 1991). Myfkin expression has
not been detected in the migratory precursor cells. The question of whether myfkin expression occurs in the limb precursor cells before they leave the somite is less clear. Low level myfkin expression has been detected in the lateral portions of older somites (Ott et al., 1991), but it is not yet known whether or not the limb precursors have left the somite by that time. Further work will be necessary to comprehensively determine the time course of myfkin expression in the limb myogenic precursor cells.

The timing of myfkin expression in limb precursor cells, whatever it is found to be, will present intriguing problems regarding the relationships between myfkins and myogenic determination. If, for example, limb precursors express myfkins only transiently in the somite, by what mechanism do they maintain myogenic commitment during their migratory phase? Conversely, if limb muscle precursors have never expressed myfkins prior to the onset of terminal differentiation in the limb bud, what gene(s) are responsible for their commitment during the two days they are in transit? There are indications that another gene (myd) may act upstream of myfkins in the myogenic commitment cascade (Pinney et al., 1988). On the other hand, some classical experiments suggest that the commitment of somitic precursors in the limb may be more plastic than is generally assumed (Kiény et al., 1981). Thus, the migratory precursor cells of the limb musculature present new questions which may open windows upon the cellular and molecular mechanisms of myogenic commitment.

Concluding remarks

The experiments reported here indicate that the lateral and medial halves of the somite have distinctly different developmental fates which, among other things, give rise to two different skeletal muscle lineages within the vertebrate embryo. Although early-on the two somite halves are apparently interchangeable, during subsequent development heritable differences arise that raise questions about the intrinsic and extrinsic forces that govern somitogenesis.

Based upon previous work and the experiments reported here, the fate and eventual commitment of somite cells can be considered to be determined by their position in relation to three axes of the somite:

(1) **Position along the anteroposterior axis** which confers specific properties to the rostral and caudal halves of the somite which, in turn, impose segmentation effects upon the development of the adjacent nervous system.

(2) **Position along the mediolateral axis** which determines which somite cells will migrate out to form extrasomatic structures, such as wing muscle, and those that will remain in situ to form the sclerotome, myotome and dermomyotome.

(3) **Position in the dorsoventral axis** which, at least for the medial half-somite, determines the commitment to formation of sclerotome and dermomyotome.

Although all three forms of commitment ultimately affect the morphogenesis of somitic and non-somitic structures within the developing embryo, only the first appears to be an intrinsic property of the somite at the time of its formation. The latter two are imposed upon the somite from forces that arise from outside the somite. Thus, the somite can be viewed as a reciprocating structure in development, alternately receiving and imposing differentiative and morphogenetic information between itself and its immediate environment.

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