INTRODUCTION

In vertebrates, skeletal muscle is derived from somites that develop by segmentation of the paraxial mesoderm. In the mouse, somites begin to form at 8 days post coitum (dpc) and develop caudally over a period of several days (Tam, 1981). Initially, they take the form of epithelial balls of cells with no discernible morphological heterogeneity (Rugh, 1968; Tam and Beddington, 1986). Early morphological change occurs when cells in the ventromedial part of the somite lose their epithelial arrangement and form a loosely arranged mesenchyme from which sclerotome cells are derived. Cells in the dorsal part of the somite retain their epithelial organization and form the dermomyotome. This stage of somitogenesis coincides with the migration of myogenic progenitor cells from somites into limb buds at 11 dpc. The sclerotomes finally participate in axial structures such as vertebrae and ribs. The dermomyotomes subsequently develop into two structures, an outer dermatome cell layer that contributes to the skin and an inner myotome cell layer which differentiates into myotomal muscles from the outset of axial musculature (Rugh, 1968).

The events that govern the development of the somite and the generation of different cell types within this structure are not yet totally understood. However, embryonic manipulations in the chick embryo have provided numerous insights into the development of avian somite (Chevallier et al., 1977; Christ et al., 1977). They demonstrate that early somite development is determined by the interaction of somitic cells with adjacent tissues (Aoyama and Asamoto, 1988; Ordahl and Le Douarin, 1992).

Through the use of experiments manipulating somites in the chick embryo, particular attention was given to the specification of the myogenic lineage in the somite. Like the cellular events, the molecular mechanisms underlying myogenic specification in the embryonic somite are still poorly understood, and different factors appear to be involved in myogenic determination. Studies of the temporal and spatial appearance of myogenic regulatory factor (MRF) genes demonstrate that they constitute the earliest known markers of myotome precursor cells in the somite. In the mouse, the first myogenic factor detected is Myf 5 at 8.5 dpc (Ott et al., 1991), which appears in the dorsal medial quadrant of the somite prior to formation of the dermomyotome. A few hours later, myogenin is clearly detectable in the myotome, at the same time as the initial appearance of α-cardiac actin. MRF4 is also detected at this time, but transiently (Hinterberger et al., 1991; Bober et al., 1991). MyoD expression occurs in the myotome 2 days after initiation of muscle differentiation (Sassoon et al., 1989).

However, the control of myogenic specification by MRFs alone remains unclear since mice carrying null mutation in MRFs Myf5 or MyoD apparently have normal skeletal muscles. Mice lacking a functional MyoD gene are viable and fertile and exhibit no morphological and physiological abnormalities in skeletal muscles (Rudnicki et al., 1992). Similarly, newborn mice lacking a functional Myf5 gene display no defects in musculature (Braun et al., 1992), whereas newborn

SUMMARY

Chimeras were prepared by transplanting somites from 9-day post-coitum mouse embryos or somitic dermomyotomes from 10-day post-coitum mouse embryos into 2-day-old chick embryos at different axial levels. Mouse somitic cells then differentiated in ovo in dermis, cartilage and skeletal muscle as they normally do in the course of development and were able to migrate into chick host limb. To trace the behavior of somitic myogenic stem cells more closely, somites arising from mice bearing a transgene of the desmin gene linked to a reporter gene coding for Escherichia coli β-galactosidase (lacZ) were grafted in ovo. Interestingly, the transgene was rapidly expressed in myotomal muscles derived from implants. In the limb muscle mass, positive cells were found several days after implantation. Activation of desmin nls lacZ also occurred in in vitro cultures of somite-derived cells. Our experimental method facilitates investigation of the mechanisms of mammalian development, allowing the normal fate of implanted mouse cells to be studied and providing suitable conditions for identification of descendants of genetically modified cells.

Key words: in ovo transplantation, mouse, somite graft, desmin, transgenic mouse, chimera

INTRODUCTION

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Through the use of experiments manipulating somites in the chick embryo, particular attention was given to the specification of the myogenic lineage in the somite. Like the cellular events, the molecular mechanisms underlying myogenic specification in the embryonic somite are still poorly understood, and different factors appear to be involved in myogenic determination. Studies of the temporal and spatial appearance of myogenic regulatory factor (MRF) genes demonstrate that they constitute the earliest known markers of myotome precursor cells in the somite. In the mouse, the first myogenic factor detected is Myf 5 at 8.5 dpc (Ott et al., 1991), which appears in the dorsal medial quadrant of the somite prior to formation of the dermomyotome. A few hours later, myogenin is clearly detectable in the myotome, at the same time as the initial appearance of α-cardiac actin. MRF4 is also detected at this time, but transiently (Hinterberger et al., 1991; Bober et al., 1991). MyoD expression occurs in the myotome 2 days after initiation of muscle differentiation (Sassoon et al., 1989).

However, the control of myogenic specification by MRFs alone remains unclear since mice carrying null mutation in MRFs Myf5 or MyoD apparently have normal skeletal muscles. Mice lacking a functional MyoD gene are viable and fertile and exhibit no morphological and physiological abnormalities in skeletal muscles (Rudnicki et al., 1992). Similarly, newborn mice lacking a functional Myf5 gene display no defects in musculature (Braun et al., 1992), whereas newborn

Mouse chick chimera: a new model to study the in ovo developmental potentialities of mammalian somites

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mice deficient for the two myogenic factors (Myf5 and MyoD) appear to be devoid of all skeletal muscles (Rudnicki et al., 1993). These results suggest that these different factors could be jointly involved in the determination of myoblasts. However, other findings provide evidence that myogenin, another member of the basic helix-loop-helix-gene family, is essential for muscle development. Myogenin gene disruption results in perinatal lethality because of muscle deficiency (Hasty et al., 1993; Nabeshima et al., 1993). Thus, myogenin seems to be required for skeletal muscle development in vivo, a function in sharp contrast with that of Myf5 and MyoD.

Once a cell has been determined to be a myogenic lineage, numerous myofibrillar proteins are known to be coordinately induced during skeletal myogenic differentiation. MRFs are believed to play an important role in the transcriptional regulation of most specific genes coding for skeletal muscle proteins (Emerson, 1990; Olson, 1992; Ordahl, 1992), including contractile proteins whose distinctive expression pattern characterizes muscle development. In the myotome, differences occur in the timing and extent of encoding gene expression of contractile protein (Buckingham and Tajbaksh, 1993). The first myosin heavy chain transcripts are detectable only one day after cardiac actin (Lyons et al., 1990). Desmin, another muscle cytoskeletal protein whose gene belongs to the family of intermediate filament proteins (Lazarides and Hubbard, 1976; Small and Sobieszek, 1977; Giesler and Weber, 1982), is expressed before skeletal muscle actin and myosin heavy chain (Babai et al., 1990; Mayo et al., 1992). This component is the major subunit of intermediate filament expressed by skeletal, cardiac and smooth muscle cells in both embryonic and adult tissues. Its expression has been previously studied in chick developing somites (Borman and Yorde, 1994). Borman et al. (1994) examined whether each somite had an inherent capacity to support myogenesis evidenced by desmin accumulation. They found that the ability to activate desmin expression is not the same for all somites observed. To consider how desmin expression is regulated in vivo and to trace the embryonic fate of myogenic cells, Li et al. (1993) obtained stable lines of transgenic mice harboring reporter genes coding for E. coli lacZ linked to the 1 Kb DNA 5' regulatory sequence of the desmin gene. Whole-mount staining for lacZ activity revealed high levels of expression of this transgene by 9 dpc in rostral-most somites (Li et al., 1993). The rostrocaudal progression of transgene expression was parallel to the pattern of somite maturation and concordant with the time course for expression of endogenous desmin. lacZ-positive cells were observed in the limb bud from 11 dpc.

As much understanding of mammalian development is based on findings in avian embryogenesis, it is important to trace the pattern of somitic mesoderm cells in other vertebrates than birds. The aim of this paper was to determine whether the development of somitic derivatives in mammals occurs according to the events that govern those implemented in birds. Although microsurgery can be performed with relative ease in avian embryos, manipulative techniques are rather difficult in mammalian embryos. For this reason, we attempted to create a chimeric mouse/chick model in which mouse somites are implanted into a chick host. We used a stable desmin lacZ transgenic mouse line to obtain data about the behavior of muscle stem cells in transplanted somites. To provide more precise analysis of the development of muscular somitic derivatives, chick somite dorsal halves from which myogenic stem cells originate were replaced by mouse somitic dermomyotomes. Our findings indicate the ability of mouse somitic cells to differentiate when confronted with a chick environment.

**MATERIALS AND METHODS**

Experiments were performed using JA 657 chicks and Swiss mice from commercial sources. Desmin lacZ transgenic mice were obtained according to the method of Li et al. (1993). Embryos were staged according to Hamburger and Hamilton (1951) for the chick and Rugh (1968) for the mouse. Pregnant mice from transgenic lines or the Swiss strain were killed by cervical dislocation just before removal of embryos at 9 (15-20 somites) and 10 (25-30 somites) dpc (day of vaginal plug = day 0 of gestation). Host embryos formed at least 18 but not more than 20 somites at the time of surgery (stages 12-14 of H. and H.). Several experimental series were carried out.

**Somite transplantation**

Donor somites were excised from 9 dpc mouse embryos (Fig. 1). Pantrecin (Gibco) diluted 1/10 in Tyrode’s solution was used in the removal of somites, which were then transferred into Tyrode’s solution to which 10% fetal calf serum was added. In stage 12-14 of H and H chick embryo (18-20 somites), the vitelline membrane was opened and the last 4-6 somites removed unilaterally. Donor somites were then transferred by micropipette into chick embryos. The orientation of transplanted somites was determined with respect to adventitious tissue. In most cases, somites could be replaced without changing their orientation.

It is known that wing muscles arise from myogenic precursor cells migrating from somites 15-21 (Christ et al., 1977; Beresford, 1983). Accordingly, somites at this level in 2-day-old chick embryos were replaced by either 15-20 (truncal) or 5-10 (brachial) somites from 9 dpc mouse embryos to determine whether somites arising from different levels were capable of contributing to wing myogenic cells.

![Fig. 1. Somite replacement.](image)

(A) Diagrammatic representation of a 9 dpc mouse embryo with isolation of somites 16-20. (B) Somites 16-20 were unilaterally excised in 20-somite chick host embryos and truncated mouse somites (16-20) were grafted into the space left by the ablation.
In ovo mouse somite transplantation

**Dermomyotome transplantation**

The mouse dermomyotome transplantation procedure was carried out according to the previously described method used in avian embryos (Auda-Boucher and Fontaine-Pérus, 1994). The axial portions of mouse embryos were cut out at 10 dpc. Pancreatin (Gibco) was used to facilitate dermomyotome removal, and mid-portions of the first ten cranial dermomyotomes were then excised and stored in Tyrode’s solution to which 10% fetal calf serum was added. Vitelline membrane was opened in stage 12-14 H and H chick embryo, and the dorsal halves of the last five somites, together with the overlying ectoderm, were removed unilaterally. Mid-portions of mouse dermomyotome were then grafted into the space left by the ablation. Host embryos formed at least 18 but not more than 20 somite stages at the time of surgery. In most cases, 4 or 6 dorsal halves of host somites were replaced with 4 or 6 dermomyotome explants. The dorsoventral positioning of the grafted tissue was not disturbed.

**In vitro culture**

After isolation of 9 dpc transgenic mouse embryos, their truncal parts were dissected and immersed in a pancreatin solution (Gibco). All somites on both sides of the neural tube were isolated. The last five pairs of somites were explanted separately from the others. Around 100 somites of each category were seeded per 35 mm collagen-coated Petri dish. For cultures, Dulbecco’s minimal essential medium was used, supplemented with 20% fetal calf serum and 2% chick embryonic extract. Cultures were routinely grown in a 5% CO2, 95% air atmosphere from 2 to 7 days.

**Histological procedure**

Eggs were sealed and returned to the incubator. Host embryos were killed between day 2 and 12 after surgery and fixed at 2°C in Carnoy’s solution before being embedded in paraffin. Serial transverse sections were cut out and treated by Feulgen and Rossenbeck (1924) staining. This procedure allowed the identification of implanted mouse cells (Moscona, 1957) among the chick cells because of differences in staining of chick and mouse nuclei (Fig. 2a,b). In the chick, chromatin forms a network evenly dispersed throughout the nucleus during interphase, whereas in the mouse nucleus, it appears condensed in one or several dense heterochromatic masses. Due to this different arrangement of chromatin, it is possible to distinguish cells belonging to each species. Although the distinction is less acute than in the quail/chick system, chick nuclei stain weakly in pink and mouse nuclei have strong purple staining.

When immunofluorescence techniques were used, the species origin of the immunoreactive cells was identified by staining the sections with either acridine orange (Fontaine-Pérus et al., 1985), (Fig. 2c) or bis-benzimide reagent (diluted 1/1000, Hoechst No. 33258), (Fig. 2d).

**Histochemical procedure for β-galactosidase**

Embryos were fixed in 4% paraformaldehyde in phosphate-buffered saline solution (PBS) and frozen in liquid nitrogen-cooled isopentane. Sections (7 μm thick) were collected on gelatin-coated slides and treated for β-galactosidase detection. The staining solution contained 2 mM 5-bromo-4-chloro-3-indolyl-b-D-galactoside (X-gal; United States Biochemicals, Cleveland, OH), 5 mM potassium ferricyanide, 5 mM potassium ferrocyanide and 2 mM MgCl2 in PBS. Staining was performed for 4 hours at 30°C. In some cases, whole embryos were fixed in 4% paraformaldehyde in PBS and stained in toto for β-galactosidase.

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**Fig. 2.** Feulgen-Rossenbeck-stained section of mouse myotomal cells in chick chimera at day 5 of incubation. Mouse (a) and chick (b) nuclei are easy to distinguish because of differences in DNA staining which make mouse nuclei appear purple and chick nuclei pink (×960). Due to differences in DNA distribution, mouse nuclei (arrows) appear more brightly fluorescent than chick nuclei (arrowheads) after (c) acridine orange (×960) and (d) Hoechst staining (×810).
Immunohistochemical detection for muscle protein
Sections first stained for β-galactosidase were incubated to determine the myosin or desmin content of myogenic cells. Myosin was detected using either monoclonal anti-fast myosin (Sigma, clone MY32) diluted 1:1,000 in PBS applied 1 hour at 37°C or antibody NN6, a polyclonal antibody shown to be specific for neonatal myosin heavy chain (Laframboise et al., 1991; a generous gift from Dr. G. Butler Browne, UFR Biomédicale des St Pères, Paris). After washes in PBS, sections were treated for 1 hour at room temperature respectively with fluorescein-conjugated goat anti-mouse IgG diluted 1:100 or fluorescein-conjugated anti-rabbit Ig diluted 1:100. Desmin detection was carried out using rabbit polyclonal antibody to desmin (Bio Makor, code 1085, diluted 1:50) and revealed with fluorescein-conjugated anti-rabbit Ig diluted 1:100. In some cases, the sections first treated for muscle protein detection were immunostained with a neurofilament monoclonal antibody (monoclonal antineurofilament 68·10^3 M_r, Clone NR4, Sigma) to visualize the chick neural components.

RESULTS
In ovo development of mouse somites
Swiss strain (Table 1)
Surgery was performed on 180 chick embryos in which the last 4-6 somites of 18- to 20-somite stages were replaced unilaterally with either brachial (5-10 somitic level) or truncal (15-20 somitic level) somites from 9 dpc Swiss mouse embryos. Somites were extirpated on the right side, and the unoperated left side served as controls. 85 embryos died and 95 developed normally. One day after surgery, histological examination showed that the grafted mouse somites appeared well inserted into the host. Initially formed as epithelial balls, they developed into dermatome, myotome and sclerotome (Fig. 3a,b). They grew in symmetry with somites of the nonoperated side but sometimes appeared more ventrally located. No visible signs of death were noted. Implanted mouse cells survived and thrived, and considerable mitosis occurred, as evidenced by Feulgen-Rossenbeck staining (Fig. 3b). At this stage, antimyosin and anti-desmin antibodies stained almost all of the myotomal cells (Fig. 3c). At graft level, after 2 days of postoperative incubation, the myotome layer from the mouse extended dorsolaterally within the mesenchyme of the chick body wall (Fig. 4a-c). Dermatome lost its epithelial structure and gave rise to dispersed mouse cells located beneath the chick ectoderm (Fig. 4d), whereas the ventral portions of grafted somites gave rise to sclerotome. At this level, chick crest cells normally migrated, constituting dorsal root and sympathetic ganglia (see Fig. 3c). Curiously, neural crest located in the mouse somites invaded the host dorsal root ganglion at the graft site, indicating that mouse neural crest cells contained in implanted somites emigrate from the graft to invade neural crest-derived structures such as peripheral ganglia (Fig. 5a). During further development, mouse
myotomal cells contributed to paravertebral muscles (Fig. 5a,b), whereas mouse sclerotomal cells more often participated in host vertebrae (Fig. 6a,b) and sometimes gave rise to additional vertebral pieces (Fig. 6c). Cells constitutive of dorsal and lateral paravertebral muscles were clearly evidenced by their myosin and desmin content. As expected, the connective tissue and the tendons of these muscles were composed of chick cells since these structures arose from the host adjacent somatopleure (Chevallier, 1977; Chevallier et al., 1978). Chick motor axons grew perfectly in these conditions (see Fig. 3c) and, as development proceeded, contacted the grafted mouse cells (Jarno et al., unpublished data).

It is well-known that specific somites contribute to specific limb muscle groups (Beresford, 1983; Chevallier et al., 1977; Christ et al., 1977) by migrating out from the lateral portion of the somite myotome (Christ et al., 1978; Ordahl and Le Douarin, 1992). Mouse somites, whatever their origin level, when grafted into the chick at the brachial level, gave rise to dispersed or grouped cells in the chick wing bud in 20 cases examined from day 2 to 3 post-surgery. During further development, the chimeric embryos were examined to determine the different limb muscles invaded by mouse cells. In the seven cases observed 6 days post-surgery, transplantation resulted in an extensive number of mouse nuclei in triceps muscle of the host forelimb in which mouse myotubes were found in a high proportion. Mouse cells were also observed in intercostal muscles examined 6 and 8 days post-surgery (Fig. 7a-c) and easily distinguished from chick muscle cells (Fig. 7d). These experiments clearly demonstrate that mouse somitic cells were inserted into the chick environment. Their further differentiation occurred, with dorsal and ventral derivatives, i.e., dermomyotome and sclerotome, forming and differentiating respectively into dermis, muscle and vertebrae. Moreover, some mouse somitic-derived cells were capable of migrating into chick host limb and thorax muscles.

The results of these experiments were largely improved by the use of the trangenic desmin nls lacZ mouse (Li et al., 1993), which provides a highly sensitive means of studying the behavior of myogenic cells in somites. We isolated somites arising from 9 dpc transgenic mouse embryos and grafted them at the brachial level of a chick host. In a first series (18 cases), the selected mouse somites were characterized at surgery by a high level of lacZ activity visualized by intense blue staining. However, in another series (6 cases), the grafted somites were without blue staining at graft time.

**Desmin nls lacZ mouse**

The temporal and spatial pattern of desmin transcription was previously analyzed in stable lines of transgenic mice harboring a lacZ reporter gene linked to region 75 to 115 of the human desmin gene (Li et al., 1993). In toto staining for lacZ activity revealed transgene activity from 8 dpc in some particular neuromuscular regions and in the conduction system of the heart rudiment. By 9 dpc, transgene expression was found in the rostral-most somites (Fig. 8a) and, by 10 dpc, within all somites. In somites, staining was restricted to myotomes, allowing mapping of the cells activating desmin transcription, and served as a marker of the embryonic development of skeletal muscles (Fig. 8b).

In 18 experiments, somites from the forelimb level of 18–20 dpc mouse embryos were unilaterally replaced by the anterior-most somites (5–10 somitic level) of 9 dpc desmin lacZ transgenic mice. 2 days postsurgery, in toto examination and histological analysis showed that the grafted somite level was detectable by Xgal blue staining (Fig. 9a,b), which corresponded to the nuclear location of the reporter gene. During subsequent development, the paravertebral muscles located at the graft site expressed lacZ transgene and exhibited blue staining. No lacZ-positive cells were observed in the migration zone between the somite myotome and the limb bud. However, in the cases observed at day 6 post-surgery, reactive cells were readily detectable in muscles located in the proximal region of the right limb bud. At this age, myoblasts had fused and formed primary myotubes, accumulating fibrillar desmin and embryonic fast myosin which was well organized into striated structures (Fig. 9c,d).

Using the same experimental procedure, either the truncal somites (15–20 somitic level) of 9 dpc transgenic mouse or the four posterior-most somites of 8.5 dpc transgenic mouse were implanted into the chick host. Although grafted somites did not express the transgene at the time of surgery, in toto examination of grafted embryo one day later, after X-gal treatment, clearly showed blue staining confined to myotomal cells at the graft site (Fig. 10a). They subsequently differentiated into myotubes accumulating myofibrillar proteins, as observed in the 3 cases examined 6 days post-surgery. These results demonstrate that specified desmin precursor cells were present in somite myotomes before transgene activation.

**In ovo development of mouse somitic dermomyotomes**

**Swiss strain** (Table 1)

In another experiment, dorsal halves of the last somites from 18- to 20-somite chick embryos were cut out and replaced on the right side with somitic dermomyotomes of 10 dpc mouse embryos. Surgery was performed on 35 chick embryos and 20 were examined. The dermomyotomes from which the grafts of this series were cut out comprised several types of cells: myoblasts in their proliferative phase, myotubes and dermal precursor cells. The grafted tissue perfectly inserted into the host. Macroscopic examination showed no alterations in bilateral symmetry in the development of the operated

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**Table 1. Number of cases examined**

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In ovo mouse somite transplantation    1709
Fig. 4. Transverse section of a 4.5-day-old chick host embryo implanted at the 20-somite stage with brachial 9 dpc mouse somites. (a) The grafted side (framed area) has developed in symmetry with the nonoperated side (×60). (b) Myotome (M) has formed in the graft (×120). (c) High-power view of the framed area in b. Mouse myotomal cells (M) are easily distinguishable from surrounding chick cells (C) (×960). (d) High magnification of zone 1 in b. Dermatome (D) has lost its epithelial structure and mouse nuclei (arrowheads) are easily distinguished from chick nuclei (arrows) (×960). Feulgen-Rossenbeck staining.
Fig. 5. Transverse section of 6-day-old chick host embryo grafted at the 18-somite stage, at brachial level, with 9 dpc mouse somites. (a) Graft (G). Neural crest cells located in the grafted somites have infiltrated the host dorsal root ganglion (arrows) (×260). (b) Mouse myotome-derived cells (M) participate in host axial muscle formation (×380). Feulgen-Rossenbeck staining.

Fig. 6. (a) Transverse section of a 6-day-old chick host embryo grafted at the 18-somite stage, at brachial level, with 9 dpc mouse somites. Mouse sclerotomal-derived cells (S, framed area) participated in the formation of chick host vertebrae (×240), (b) High-power view of zone 1 in a showing mouse nuclei (arrowheads) in host vertebra which are distinct from chick nuclei (arrows) (×960). (c) Same experiment as in a. Examination of the chick host at day 8 of incubation. An additional, heavily stained vertebral piece (V) has differentiated from graft cells (×240). Feulgen-Rossenbeck staining.
Fig. 7. Transverse section of an 8-day-old chick embryo implanted at the 18-somite stage, at brachial level, with 9 dpc brachial mouse somites. (a) Low-power view of the host thoracic region, lung (L), (×90). Graft-derived cells have infiltrated the intercostal muscles. (b,c) High-power view of zone 1 and 2 in a, respectively. These muscles are made up of mouse myotubes. (d) High-power view of zone 3 in a. This muscle was entirely composed of chick myotubes (×960). Feulgen-Rossenbeck staining.
In ovo mouse somite transplantation. Grafted somitic structures appeared to be normal in size compared to those of the unoperated side. Transplantation did not disrupt subsequent somite development, and mouse dermomyotome participated in chick somitogenesis (Fig. 10b,c). The distribution of mouse cells was determined after a subsequent 3- to 10-day incubation period. Conventional histology 3 days postsurgery showed mouse cells in both dermal and myotomal structures within the somites. Mouse cells were never observed in sclerotome at graft level and, in further development, vertebrae appeared to be entirely composed of chick cells. Whereas mouse dermomyotome implantation took place partly at the wing prospective level, a few mouse cells were observed in the host limb bud, in which they were confined in myogenic areas.

Desmin lacZ transgenic mouse
When grafted dermomyotomes arose from desmin lacZ transgenic mouse, myotomal cells were clearly discernible by the blue staining of their nuclei. In the 10 cases observed 6-10 days after implantation, axial muscles at graft level were composed of mouse cells simultaneously characterized by X-gal staining and the accumulation of myofibrillar proteins. In one case out of 10 examined 10 days postsurgery, not only the paravertebral muscles but also an intercostal muscle at the implantation level were composed of lacZ-positive cells (Fig. 10d). In another case observed at day 8, the triceps muscle of the right host limb was evidenced by X-gal blue staining, which corresponded to the nuclear location of the reporter gene.

In vitro culture of desmin lacZ transgenic mouse somites
Activation of desmin transgene was studied in primary myogenic cultures from either the most anterior (i.e., blue-stained) or posterior (i.e., without blue stain) somites of 9 dpc mouse embryos. Myogenesis occurred in somites enzymatically isolated, and numerous flat and well-spread myoblastic cells attached to the substratum within a few hours after plating. Within a short time, they became slightly elongated and, then, after 3 days of culture, developed into very thin, short myotubes with 2 or 3 nuclei. As myotube maturation proceeded, these cells became aligned, indicating the beginning of fusion.

When explants arose from the anterior-most somites of 9 dpc mouse embryos, all myogenic cells were rapidly characterized by blue staining of their nuclei after X-gal treatment. When explants came from the most posterior somites of 9 dpc mouse embryos, transgene activity was evidenced after 2 days of culture following X-gal staining (Fig. 10e). The myoblasts and in vitro differentiated myotubes accumulated myofibrillar proteins.

DISCUSSION
Avian embryo is a practical model for investigating the developmental potentialities of mouse embryo somites because it is easily accessible to experimentation during the entire embryonic period, whereas the mammalian embryo, because of its implantation in the uterus, is especially difficult to study during the active phases of early organogenesis.
In ovo mouse somite graft as a model of developmental study

In our approach, we replaced somites in chick embryo unilaterally with somites from mouse embryo. Due to differences in the staining properties of chick and mouse nuclei after use of the Feulgen-Rossenbeck method (pink for chick nuclei versus deep purple for mouse nuclei), the subsequent fate of donor somitic cells could be determined histologically from host cells. Although the distinction was less acute than in the quail/chick system and sometimes difficult to assess at a single cell level, groups of at least three mouse cells could be recognized in the chick environment. Through use of the transgenic lacZ line, the myogenic cells in the grafted tissue were specifically identified.

Fig. 9. Expression of transgene desmin in a chick embryo examined 2 days after implantation of the brachial somites of a 9 dpc transgenic mouse at the 18-20 somite level. (a) The graft site is identified by X-gal blue staining. (b) A transverse section shows evidence of transgene activity in grafted myotomes (×240). Transverse section of 8-day-old chick host embryo implanted at the 18-somite stage, at brachial level, with 9 dpc brachial transgenic mouse somites. (c) In a muscle located in the proximal region of the right limb bud, myotubes derived from the graft exhibit blue nuclei and have accumulated myosin (×240). (d) High magnification of the framed area in c. In graft-derived myotubes, myosin appears in well-organized striated structures (×360).
Fig. 10. (a) Examination of a 4-day-old chick embryo implanted at the 19 somite stage, at brachial level, with posterior-most somites of a 9 dpc transgenic mouse embryo. The graft site was clearly evidenced by Xgal reaction. (b) Examination of a 4.5-day-old chick embryo grafted at the 18-somite stage, at brachial level, with 10 dpc mouse dermomyotomes. Grafted cells (arrows) participated in chick somitogenesis (×240). (c) High-power view of framed area in b (×360). Feulgen-Rossenbeck staining. (d) Transverse section of a 10-day-old chick embryo implanted at the 18 somite stage, at brachial level, with 10 dpc transgenic mouse dermomyotomes. An intercostal muscle was composed of lacZ-positive cells derived from the graft, host ribs (r) (×240). (e) In vitro culture of 9 dpc desmin lacZ transgenic mouse somites. When the posterior-most somites were explanted, transgenic activation occurred after 2 days in vitro differentiated myotubes (×960).
We demonstrated that the developmental potentialities of the different components of the grafted somites (i.e., muscle, dermis and vertebrae) can be expressed in the chick. Orthotopic transplantation of 9 dpc somitic cells resulted in the participation of mouse cells in host dermis, skeleton and muscle at the graft site, whereas previous experiments had totally failed to achieve this. Kieny et al. (1987), who grafted either mouse somitic mesoderm or limb bud presumacular mass into chick hosts, found that mouse cells were unable to participate in any skeletal musculature of the chick host. These authors concluded that mammalian myogenic cells are unable to differentiate in an avian matrical environment and interact with avian cells. Our results demonstrate that mouse somitic cells are not only capable of differentiation in the chick host but that a few of them can also migrate into the wing bud. The mouse graft-derived cells penetrate into some appendicular muscles related to the graft level. However, whether the migratory pathway or the destination of the migratory mouse cells is determined remains to be studied. In the chicken, cell migration from the lateral edge of the somites to the wing takes place from the 22-somite stage (Chevallier, 1978). It appears that mouse cells can acquire migratory properties when grafted in appropriate locations and use the same migratory pathways as do host somitic cells in reaching the host limb.

Although it is widely assumed that limb muscle precursors in mammals arise from migratory somite cells (Milaire, 1976), such migration has not been experimentally demonstrated (Beddington and Martin, 1989). The latter authors in the mouse and Lee and Sze (1993) in the rat previously addressed the question of whether mammalian somites can participate in the development of the limb bud. They labeled somites adjacent to the prospective region of the forelimb and cultured experimental embryos. Even though mouse embryos could be maintained for only a few days in culture, the authors observed that labeled somitic cells were detached from the ventrolateral edge of the dermomyotome at about the 22-somite stage in order to invade the proximal territories of the forelimb. Beddington and Martin (1989) reported that somite-derived cells in the limb bud developed mainly into blood vessel endothelium, but also described a few somitic cells in the limb, which were tentatively considered to be myoblasts. Lee and Sze (1993) argued that labeled cells in the forelimb of injected rat embryos were myoblastic precursors and suggested that brachial musculature arose from somites, whereas cartilage and bone were formed by limb somatopleure. These observations strengthened the opinion that, in mammals as in avians, the limb is formed by cells migrating from somitic myotomes. However, one disadvantage of the mass culture technique used to study cell migration process in mammals is its limited duration. After 3 days, the core of embryo organs becomes necrotic and thus uninformative, whereas our in ovo graft technique enabled us to study the fate of implanted mouse cells throughout the embryonic period in chick hosts. Avian embryonic environment is able to promote the fate of the mammalian cells.

**Transgenic desmin nls lacZ mouse used in ovo for dynamic monitoring of the fate of early myogenic cells**

Particular attention has been given to the myogenic behavior of grafted mouse cells through use of the transgenic mouse model, and it has been determined that desmin transcription is activated when skeletal muscle precursor cells begin to differentiate. Li et al. (1993) analyzed the expression of reporter genes linked to the desmin control region in transgenic mice, a region requiring 280 bp for appropriate transcription. When linked to reporter gene lacZ, the transgene allowed the mapping of cells that activate desmin transcription in embryos and thus the tracing of myogenic cells in living embryos.

In our mouse-chick graft system, the behavior of grafted myogenic cells proved of particular interest since the expression of desmin lacZ transgene could be useful as a specific marker of muscle cells. When anterior-most somites were implanted into the chick host, the implantation site was clearly identified after X-gal staining, and histological analysis evidenced mouse myotubes with blue nuclei. In our experiments involving in ovo grafting of mouse somites negative for β-galactosidase staining, transgene activation also occurred. Such activation was also evidenced in cultures. Taken together, these results suggest that the mechanisms specifying desmin transgene expression were already in place and that somitic cells were specified early for myogenesis. lacZ protein was expressed at a high level in myotomes at graft time, whereas transcripts encoding desmin were not yet detected. Migrating myogenic precursors did not activate desmin transcription until they reached the limb bud, and lacZ gene products become detectable in the host wing from day 5 postsurgery. Our results, together with those of Cheng et al. (1992), Buckingham and Tajbakhsh (1993) and Li et al. (1993), suggest that migrating myogenic precursors do not activate transcription before settling up into the limbs. However, recent studies demonstrating that migratory cells possess active gene transcription has led to the identification of Pax gene products (Gruss and Walther, 1992). Bober et al. (1994) showed that Pax3 transcripts in mouse embryo are present in migratory cells that constitute the prospective limb myoblasts. Pax3 actually represents the only molecular marker for these cells in chick (Williams and Ordahl, 1994) as in mouse (Bober et al., 1994) embryos. Pax3 expression domains in mouse embryo limb buds are similar to those seen for avian embryos and, more importantly, are absent in the limbs of Splotch mouse embryos (Franz, 1993; Franz et al., 1993; Goulding et al., 1994). The use of Pax3 as a marker for migratory limb muscle precursors and of mouse-chick transplantation could constitute an important step in the understanding of mammalian muscle development.

Replacement of the presumptive myogenic areas of the somites adjacent to the prospective wing level with portions of 10 dpc somitic dermomyotomes resulted in the participation of the grafted cells in chick axial muscles and dermis. Implanted cells were also examined in peripheral muscles such as intercostal and triceps muscles composed of cells migrating out from brachial somites. As in birds (Mauger and Kieny, 1980; Auda-Boucher and Fontaine-Perus, 1994), it appeared that myogenic cells had migratory capacities beyond the normal migration phase after being grafted into appropriate locations.

It may be concluded that the disadvantages in handling mammalian embryos can be overcome by a combination of procedures. In ovo transplantation provides a suitable environment for the development of mammalian cells, and the information supplied by this environment is capable of promoting mouse cell differentiation. The replacement of chick somitic cells with their transgenic mouse counterparts should help specify the interrelationships between the development of...
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