Development 117, 947-959 (1993)
Printed in Great Britain © The Company of Biologists Limited 1993



# Desmin sequence elements regulating skeletal muscle-specific expression in transgenic mice

**Z. Li[1], P. Marchand[2], J. Humbert[1], C. Babinet[2] and D. Paulin[1,*]**

[1]Biologie Moléculaire de la Différenciation, Université Paris 7 et Institut Pasteur SCME 25, rue du Dr Roux, 75724 Paris Cedex 15, France
[2]Génétique des mammifères, Institut Pasteur 25, rue du Dr Roux, 75724 Paris Cedex 15, France

*Author for correspondence

## SUMMARY

**During the development of the mouse embryo, desmin is one of the first muscle proteins detected in both the heart and the somites. The expression of the desmin gene differs from most other muscle genes, since it is initiated in replicating myoblasts and accumulates as the muscle differentiates. We have characterized a muscle-specific enhancer which directs the expression of desmin in vitro in the myoblasts and myotubes of C2 cells but not in non-myogenic cells. We report here on the generation and characterization of transgenic mice bearing a transgene in which the 1 kb DNA 5′ regulatory sequence of the desmin gene is linked to a reporter gene coding for *Escherichia coli* β-*galactosidase (Des1-nlacZ).* The enhancer activity of the desmin promoter is very strong and the reporter gene expression is easily detected in tissue sections. We have demonstrated that the regulatory elements present in the transgene *Des1-nlacZ* are sufficient to direct muscle-specific and developmentally regulated expression of nlacZ in skeletal muscles. Endogenous desmin expression and transgene activity were found to be correlated during the development of skeletal muscles. The transgene was expressed in the committed mononucleate myoblasts as well as in the myotubes. In addition, we have shown that the desmin-derived sequences direct a highly selective expression of nlacZ in cells that leave the somites and invade the limb bud, indicating that the cells that migrate from the somites are already predetermined for myogenesis. In contrast, smooth and cardiac muscle cells were β-*galactosidase* negative both during embryonic and foetal development. Interestingly, the transgene was found to be expressed in the conduction system of the heart, which exhibits many features characteristic of skeletal muscles.**

Key words: conduction system, desmin gene, β-galactosidase, heart, Krox, MEF2-MyoD1, myotome, neuroectoderm, skeletal enhancer, transgenic mice

## INTRODUCTION

Desmin is a muscle cytoskeletal protein whose gene belongs to the family of intermediate filament proteins (Lazarides and Hubbard, 1976; Small and Sobieszek, 1977; Geisler and Weber, 1982). Desmin is derived from a fully characterized single copy gene (Li et al., 1989; Quax et al., 1985), which has been mapped to band q35 to the long arm of human chromosome 2 (Viegas-Péquignot et al., 1989) and to band C3 for mouse to chromosome 1 (Li et al., 1990).

Desmin is one of the first muscle-specific proteins to be detected in the mammalian embryo since it is expressed before titin, skeletal muscle actin, myosin heavy chains and nebulin (Hill et al., 1986; Fürst et al., 1989; Babai et al., 1990). During the development of the mouse embryo, desmin was first detected at 8.25 d.p.c. in the ectoderm where it was transiently coexpressed with keratin and vimentin (Schaart al., 1989). At 8.5 d.p.c., desmin was found in the heart rudiment and remained present with increasing intensity in the myocardial cells during later cardiogenesis (Schaart et al., 1989). From 9 d.p.c. onwards, desmin could also be detected in the myotomes (Mayo et al., 1992). Levels of desmin expression in skeletal and cardiac muscles remained high throughout embryogenesis and in early postnatal life.

More than 20 genes are known to be coordinately induced during skeletal myogenic differentiation (Caravatti et al., 1982; Gunning et al., 1987). It was suggested that the regulation of desmin gene expression is distinct from that of myofibrillar proteins such as myosin heavy chain and titin. The expression of the desmin gene differs from most of these genes, which share the property of being repressed in proliferating undifferentiated myoblasts and of being concomitantly expressed with myoblasts fusion. In contrast, desmin expression is initiated in replicating myoblasts and accumulates to a high level as muscle cells differentiate (Pieper et al., 1987; Kaufman and Foster, 1988; Li and Paulin, 1991). This is illustrated by the mouse C2.7

myogenic cell line where desmin is expressed at a low level in myoblasts. To date, all the muscle-specific enhancers, such as the rat and mouse muscle creatine kinase enhancer (Horlick and Benfield, 1989; Jaynes et al., 1988; Sternberg et al., 1988), rat myosin light chain (MLC1/3) enhancer (Donoghue et al., 1988), quail troponin I enhancer (Yutzey et al., 1989), chicken and mouse AchR δ-subunit enhancer (Baldwin and Burden, 1989; Wang et al., 1990), and chicken AchR α-subunit enhancer (Wang et al., 1988) function in differentiated cells but not in their precursors.

The results of our previous studies have indicated that the regulatory apparatus of the human desmin gene is complex. A positive regulatory element that is important for high level expression is located in the region between −973 and −693 bp relative to the start site of transcription and shows properties of a muscle-specific enhancer. Downstream this enhancer, a negative region located between nt −693 and −228 was shown to cause the decrease by about 3-fold of chloramphenicol acetyl transferase (CAT) expression in myogenic cells. Following the negative region, another positive region between nt −228 and +75 has been found. This positive region, containing a classical TATA box and transcriptional initiation sites, was defined as the desmin promoter and can confer low level muscle-specific expression on a CAT gene (Li and Paulin, 1991).

An interesting feature is that the desmin regulatory region contains many sequences sharing extensive homologies with the sequences within the enhancer of other muscle-specific genes, such as two consensus sequences recognized by MyoD1, myogenin, myf5 or MRF4, which are muscle-specific trans-acting factors (Buskin and Hauschka, 1989; Brennan and Olson, 1990; Lassar et al., 1989), and one consensus sequence recognized by MEF2, a muscle *trans*-acting factor described by Gossett et al. (1989).

Thus, even though cardiac, skeletal and smooth muscles express desmin, it was of considerable interest whether the desmin expression is regulated by identical or different *cis*-elements in these three compartments. However, the use of in vitro models to address this question has inherent limitations. Therefore, in order to determine if the 1 kb genomic DNA characterized in vitro contains sufficient information for appropriate regulation when subjected to a normal developmental environment, we decided to use the transgenic methodology, which permits analysis of both the temporal and the spatial regulation of the transgene. As an initial step in such a study, we report here on the generation and characterization of transgenic mice bearing a transgene in which the previously well-characterized 1 kb 5′ regulatory sequences (Li and Paulin, 1991) of the desmin gene were linked to a reporter gene. Because the histochemical stain for β-galactosidase provides a sensitive means for studying the patterns of expression at a cellular level, we have used as reporter gene the *Escherichia coli LacZ* including a nuclear localisation sequence which targets it to the nucleus (Kalderon et al., 1984; Sanes et al., 1991).

We found that endogenous desmin and transgene activities coincide in skeletal muscle. However, no expression of transgene was observed either in cardiac or in smooth muscles. Therefore, these results demonstrate that the 1 kb DNA regulatory sequence is competent to promote specific expression of desmin in skeletal muscles and suggest that other sequences are necessary for cardiac and smooth muscle expression. Our study provides evidence for distinct cardiac, smooth and skeletal muscle gene programs.

## MATERIALS AND METHODS

### Construction of *HuDes1-nlacZ* expression vectors

A 2.3 kb *Pst*I-*Acc*II fragment of the human desmin promoter, which ends at nt −2225 and +75 relative to the transcription start site, was isolated from the clone pHuDes16 (Li and Paulin, 1991). This fragment was first inserted upstream of the CAT gene into the promoterless pBLCAT3 to obtain the p2.3HuDesCAT expression vector, as previously described (Li and Paulin, 1991). The CAT gene was removed by appropriate digestion in the polylinker and replaced by the *lacZ* gene-derived from the pCH110 by *Hin*dIII-*Bam*HI digestion. An oligonucleotide: AGGTTAC-CATGGAGCCAAAAAAGAAGAGAAAGGTAGAAGACCC-CGTAC, containing both a nuclear localization signal, which targets *Escherichia coli* β-galactosidase to the nucleus, and the Kozak sequence to enhance the efficiency of translation (Kozak, 1987), was inserted to give the *Des2.3-nlacZ* expression vector. The plasmids: *pHuDes0.3-nlacZ; pHuDes0.7-nlacZ; pHuDes1-nlacZ.* were obtained from *Des2.3-nlacZ* by digestion respectively with *Pst*I, *Hin*dIII or *Kpn*I.

### DNA transfer to cell lines

For transient transfections, supercoiled plasmid DNA was purified on CsCl gradients, then introduced into C2.7 or 3T3 cells using the calcium phosphate method as described previously (Li and Paulin, 1991). The CAT activity was measured 48 hours after transfection for myoblasts and fibroblasts. For myotubes, the same protocol was used except that cells were incubated with 2% horse serum 48 hours after transfection and harvested 48 hours later. The CAT activity is expressed as picomoles of acetylated chloramphenicol per minute. Plasmids including various lengths of 5′ regulatory region linked to the *nlacZ* gene were transfected into C2.7 myoblasts, which fuse in culture to form multinucleate myotubes, and into 3T3 fibroblasts. Blue staining for β-galactosidase was assayed 48 hours and 96 hours later.

### Production of transgenic mice

*Bsm*I fragment containing the 1.2 kb regulatory sequences of human desmin gene (nt −1150 to +75) fused to the *nlacZ* gene was excised from *Des2.2-nlacZ.* The fragments were isolated by electrophoresis in an agarose gel, then purified further by the Geneclean kit (Bio1O1, La Jolla, CA) and finally resuspended in 10 mM Tris-HCI pH 7.5, 0.25 mM EDTA. DNA solutions (3 μg/ml) were microinjected into the pronuclei of fertilized oocyctes as described by Brinster et al. (1985). The fertilized eggs were obtained from superovulated (C57/SJL/J) $F_1$ hybrid females mated to identical males. Eggs that survived microinjections were implanted into the oviducts of pseudopregnant (C57bl/6×CBA) $F_1$ foster females. Transgenic mice were identified by a polymerase chain reaction (PCR) and Southern blot using tail DNA. Transgenic founders and subsequent progeny generations were bred by backcrossing to C57BL6/J×CBA $F_1$ hybrids or C57BL/6. Thus, all experiments were performed with mice hemizygous for the transgene. Seven founders carried the transgene. Two lines were established from two founders $F_1$, which express the transgene in all transgenic animals of the progeny. As a control, two lines were established from two founders $F_1$ which do not express the transgene in any animals of the progeny.

### Histochemical staining for β-galactosidase

Mice were killed by cervical dislocation and dissected in PBS (150 mM NaCl, 10 mM sodium phosphate, pH 7.3). Whole embryos, tails or individual muscles were fixed for 15 minutes to 1 hour in 2% paraformaldehyde plus 0.2% glutaraldehyde in PBS, washed thoroughly in PBS, and stained for β-galactosidase as described by Sanes et al. (1986). The staining solution contained 2 mM 5-bromo-4-chloro-3-indolyl-β-D-galactoside (X-gal; United States Biochemicals, Cleveland Ohio), 5 mM potassium ferrocyanide, 5 mM potassium ferricyanide, and 2 mM $MgCl_2$ in PBS. Staining was for 16-20 hours at 30°C. Embryos or tissues were then rinsed several times in PBS. Some specimens were embedded in resin (Leitz) or paraffin. Sections (2-7 μm thick) were cut on a microtome. When necessary, sections were further stained with antibodies. Photographs were taken using a Leitz stereomicroscope.

### Desmin immunodetection

For indirect immunostaining of desmin, 5 μm thick tissue sections were incubated with rabbit polyclonal antibody to desmin (Bio Makor code 1085). Then, they were incubated with peroxidase-conjugated anti-rabbit IgG (Dako) diluted 1/40 with PBS for 30 minutes at room temperature and washed in PBS. All readings were performed on a Leitz microscope. Controls were made using peroxidase-conjugated anti-rabbit IgG alone.

## RESULTS

### (1) Muscle-specific expression of desmin gene in vitro

In a previous attempt to identify the *cis*-acting DNA sequences involved in the regulation of the desmin gene, a series of constructs have been made by fusing different lengths of human desmin 5′-flanking fragments to the bacterial chloramphenicol acetyl transferase (CAT) gene (Li and Paulin, 1991).

These constructs were introduced into mouse cells, either myogenic (C2.7 cells) or non-myogenic cells (3T3 fibroblasts). Comparison of the transient expression of CAT in myogenic and non-myogenic cells (see Table 1) demonstrated that the sequence of 228 base pairs upstream of the transcription initiation site is sufficient to confer low level muscle-specific expression of the desmin gene, even in myoblasts. High level expression of the gene depends on a 280 bp muscle-specific enhancer located between −693 and −973 bp upstream of the transcription initiation site (Li and Paulin, 1991). This enhancer includes three consensus Krox elements and an adjacent Sp1 and M box as well as MyoD1- and MEF2-binding sites (see Fig. 1, upper part).

**Table 1. Expression of Des-CAT recombinants in myogenic and non myogenic cells**

| (a) Recombinants | (b) Myoblasts | (c) Myotubes | (d) Fibroblasts |
|---|---|---|---|
| −227 | 23 | 41 | 1.8 |
| −693 | 8 | 12 | 1 |
| −973 | 75 | 300 | 1 |
| −1700 | 70 | 280 | 1 |
| −2300 | 70 | 280 | 1 |

(a) All constructions share the same 3′ end at nt +75 relative to the transcription start site. Numbers represent the nucleotide lengths of the inserts from the transcription start site. The constructions were transfected into C2.7 or 3T3 cells. The CAT activity was measured 48 hours after transfection for myoblasts (b) and fibroblasts (d). For myotubes (c) the same protocol was used except that cells were incubated with 2% horse serum 48 hours after transfection and harvested 48 hours later. The CAT activity is expressed as picomoles of acetylated chloramphenicol/minute.

Because the aim of this study was to examine gene expression in vivo, DNA sequences identical to those described above were fused to a modified *lacZ* gene; β-galactosidase was used rather than CAT because its product can be detected histochemically on whole-mount preparations (Sanes et al., 1986) and at the single cell level. In addition, for a better visualization of the reporter gene expression, the β-galactosidase was targeted on the nucleus using a short sequence that encodes a nuclear localizing signal. (Kalderon et al., 1984; Dingwall and Laskey, 1986). After transfection of the different constructs (five plasmids including −2300, −1700, −973, −693, −227 bp upstream of the initiation site of the desmin gene, respectively), β-galactosidase was assayed using the X-gal reaction either in myoblasts or in myotubes using the C2.7 cells or in 3T3 fibroblasts. Levels of expression were estimated by visual inspection (Fig. 1). Results essentially similar to those of the CAT experiments were obtained. No expression was detected in fibroblasts and a lower expression was found in myoblasts compared to myotubes. It is evident that the constructs that do not contain the 280 bp enhancer (*Kpn*I-*Hin*dIII) could not direct high level expression in myogenic cells. As shown in Fig.1D β-galactosidase accumulated preferentially in myotube nuclei when using a construct containing the *nlacZ* gene under the control of the specific enhancer.

### (2) Establishment of transgenic mouse lines

Having established that the *Des1-nlacZ* construct, which contains a 1000 bp fragment of the desmin promoter including the desmin enhancer (Li and Paulin, 1991), directs specific expression in C2.7 cells, we asked whether they act identically in vivo and promote temporal and spatial expression of the reporter gene. Transgenic mice were therefore generated by microinjection of the *Des1-nlacZ* hybrid gene into fertilized mouse eggs: seven transgenic mice were obtained: M-*Des1-nlacZ* number I to VII. The PCR and Southern blot analysis of DNA from the tails showed that the seven transgenic founders had incorporated multiple copies of the gene (data not shown). None of the transgenic mice showed any abnormalities. Four of them transmitted the *Des1-nlacZ* gene to their offspring. The progeny and their founders were tested for β-galactosidase expression. As described in Materials and methods, the staining for β-galactosidase was performed on tail sections from newborn mice (see lower part of the Fig. 2F). Two founders were found to express nlacz in the tail of the newborn. Expression was stably inherited in all transgenic offspring (not shown). These two independent lines expressing the transgene (M-Des1-nlacZ I and M-Des1-nlacZ V) were used for our studies. Controls were performed using non-expressing transgenic lines and non-transgenic mice.

The fusion gene seems to have no harmful effect on the development of mice. During this study, we have produced and examined a large number (at least 200) of transgenic

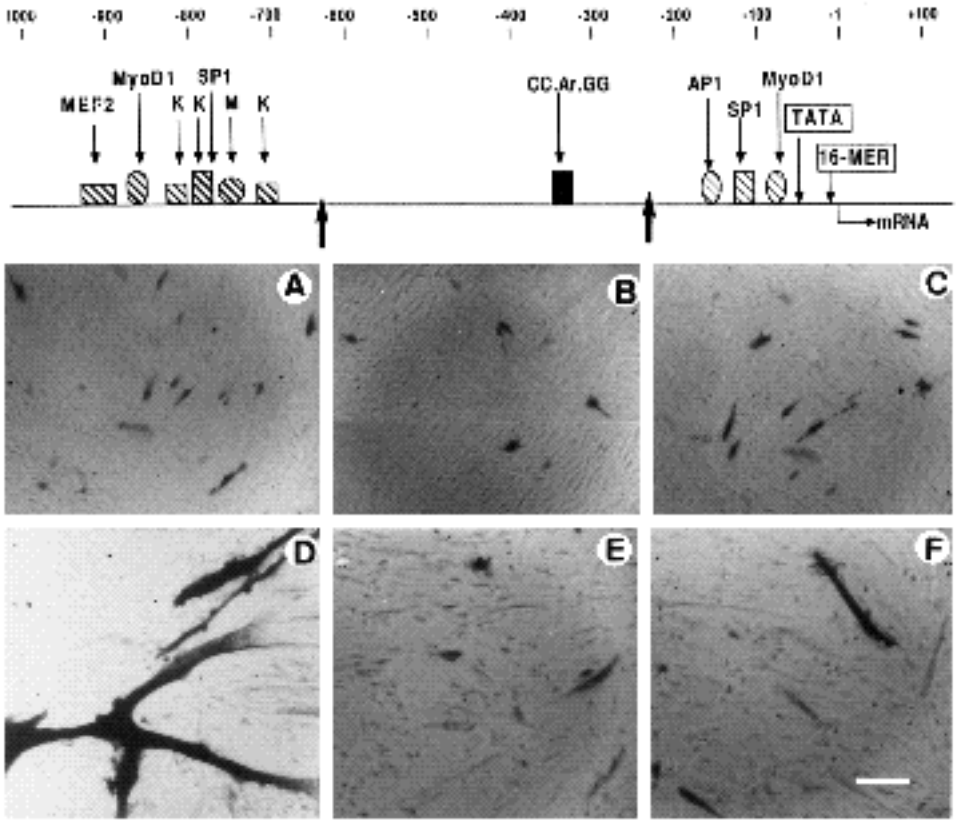


**Fig. 1.** Muscle-specific expression of *Des1-nlacZ* recombinant in cultured cells. (Top) Location of regulatory sequences in the desmin transgene. Summary of the characterized nuclear factors that interact with the desmin enhancer sequences. These include MyoD and myogenin, MEF2, a muscle-specific factor that interacts with other muscle-specific regulatory sequences, and three consensus Krox (K) binding sites and an adjacent Mb box (M), which bind uncharacterized nuclear factors. (Bottom) Expression of *Des1-nlacZ* recombinants. Fusion genes were introduced into C2.7 cells and 3T3 fibroblasts. The β-galactosidase staining was performed 48 hours and 96 hours after transfection. Transient expression of similar CAT constructs were performed in parallel (see Table 1). (A-C) Myoblasts; (D-F) myotubes. No expression was detected in fibroblasts. The small recombinant, which corresponds to the basal promoter (+), ends to −227 bp. In the second recombinant, the negative sequence (−) is added and ends at −693 bp. The recombinant *Des1-nlacZ*, which displayed the high expression, ends at −973 bp and exhibited the 280 bp muscle-specific enhancer quoted (+++). Note high expression of β-galactosidase in myotubes bearing the *Des1-nlacZ* recombinant (see D). The scale bar indicates 200 μm.

mice carrying the *Des1-nlacZ* gene. As yet no abnormality has been observed in either foetuses or adults.

### (3) Developmental expression of *Des1-nlacZ* transgene

Embryos and foetuses at 7 to 15 days of gestation were stained in toto. Then 5 μm sections of paraffin or resin embedded samples were produced according to Kaufman (1992). Analysis of these sections confirmed that the staining seen in toto corresponded to the nuclear location of the reporter enzyme. For each stage of development (based on the staging system of Theiler), about 30 embryos or foetuses were tested (day 8 to 15). The staining pattern was very reproducible and identical results were regularly found.

During developement of the embryo, transgene activity was first detected at 8 d.p.c. in some particular neuroectoderm region (see below), and in the conduction system of the heart rudiment. From 9 d.p.c. the transgene expression was found in the myotomes of the cervical and thoracic somites. By day 11, blue-stained clusters of cells were also detected in cephalic tissues. By day 15, muscles were β-galactosidase-positive in the head as well as in the trunk and limbs. The intensity of staining remained high throughout embryogenesis. At birth, staining of nuclei was found in all skeletal muscles from body, head and limbs. However none of the smooth muscles that were examined was stained at any time during development. All together transgene expression was found to be restricted to skeletal tissues.

A detailed description of the transgene expression illustrated in Figs 3 to 7 follows.

#### From 9 days both the transgene and the endogenous desmin were found to be expressed in the somites

Myotomes arise from somites that form in the cervical region at about 7.5 days. At this stage, no desmin RNA can be detected by in situ hybridization and no protein by immunofluorescence, respectively. Expression of the *nlacZ* was found in the somites of embryos starting at 9 days of gestation (Fig. 2) and thereafter. At day 9, the most anterior somites were intensely stained while the posterior ones

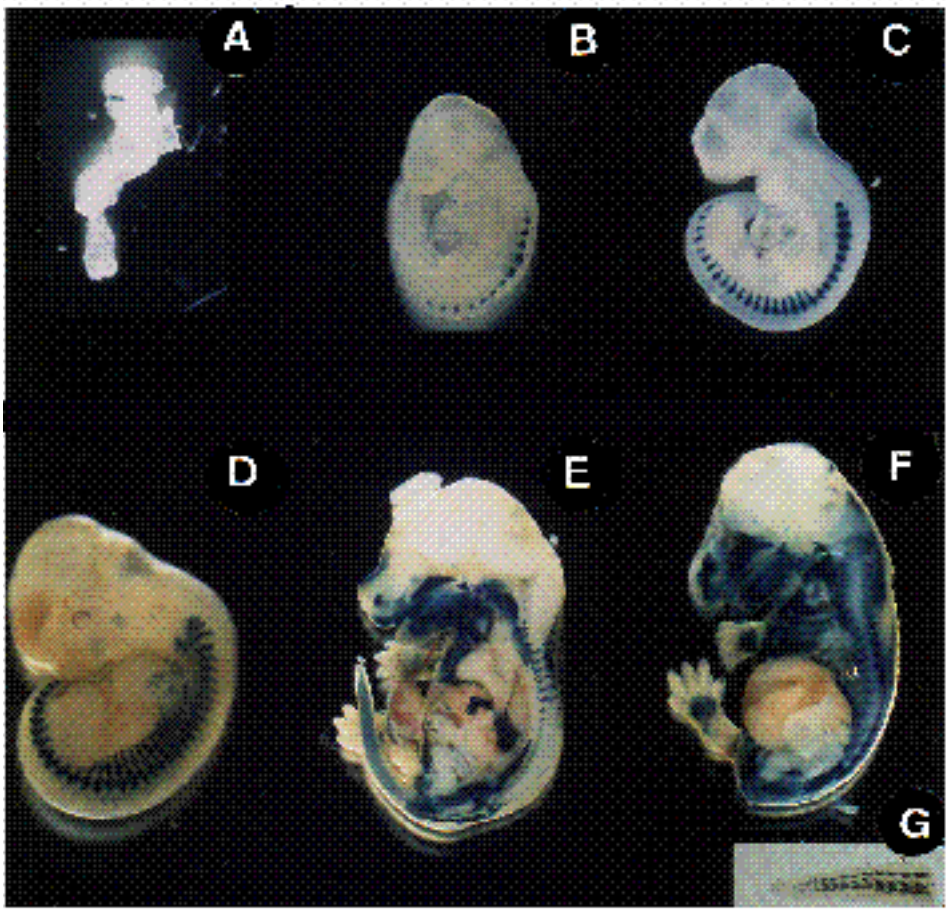


**Fig. 2.** Photograph of mouse embryos from 9 to 15 days of gestation (lateral view). The 8-, 9-, 10-, 11-, 13- and 15-day-old embryos (A-F) were stained for β-galactosidase. Expression of *nlacZ* was found in the somites of transgenic embryos starting at 9 days of gestation; the most anterior somites were intensely stained while the posterior ones were unstained, which is in keeping with the rostrocaudal of somite differentiation gradients. Note the positive reaction in muscular skeletal system and negative reaction in smooth and cardiac muscle for 15-day transgenic embryos. The *Des1-nlacZ* transgene is expressed in the 3rd rhombomere in day 8 embryos and continues to be expressed until day 14 in the neuroepithelium at the level of the pontine flexure forming the floor of the 4th ventricle. The expression of the nlacZ could be detected in some scattered cells of the heart in the 8-day embryo, which appeared to belong to the conduction system judging from their anatomical localization. This expression of the transgene in the conduction system was detected throughout the foetal development. All the non-transgenic animals were negative for X-gal reaction. Tail of a newborn is shown in lower insert (G).

were unstained, which corresponds to the rostrocaudal somite differentiation gradients. In somites, the staining was restricted to the myotome. We compared the X-gal staining to the in situ hybridization signal obtained with a desmin cDNA probe on sections of a 9-day embryo, and found a similar pattern of expression at the level of somites for both the desmin RNA and protein (data not shown) as well as the *nlacZ* transgene. By 10 days, all somites were stained for the β-galactosidase as was the case for endogenous desmin RNA as evidenced by in situ hybridization (data not shown).

By 11 days, myotomes appear with longitudinally directed myoblasts. All was intensively stained for β-galactosidase activity. In 11-day embryos β-galactosidase was also detected in the mandibular arches, ocular muscles and in developing limb buds. The photographs reported in Fig. 3 demonstrate that these cells could be easily detected throughout the forelimb bud.

### Muscle fibers of the trunk

By 13 days, myoblasts show active proliferation and somite-derived muscle fibrils can be found by 14.5 days. By 15 days, skeletal muscles become contractile and they display cross striations. At this stage in the transgenic embryos (Fig. 2), the skeletal muscle fibers of the body and limbs, which arise from a common somitic progenitor pool, the myotome, expressed *nlacZ* transgene and exhibited blue nuclei. Fig. 4 illustrates the cellular resolution of the β-galactosidase signal, which allows the unambiguous identification of labelled nuclei.

### Cephalic skeletal muscles

Studies on chick embryos have shown that the prechordal mesoderm gives rise to three of the ocular muscles (rectus ventralis and medialis, and oblicus ventralis). These three as well as the remaining ocular muscles (Couly et al., 1992), (rectus dorsalis, oblicus dorsalis and rectus lateralis) all arise from the median paraxial mesoderm. In sections of the 11-day embryos, blue-stained cells were easily detected in the ocular and mandibular region. All the corresponding muscles in transgenic embryos displayed blue-stained fibers. The occipital somites (2-5) of avian and mammalian embryos have been shown to be the source of the myogenic lineage cells that give rise to the intrinsic musculature of the tongue (see in Mayo et al., 1992). Lingual muscles contained both unstained and stained fibers at day 13, appeared to be fully stained at birth.

### At 8 days transgene expression is detected in the neural tube

At 8.5 days, the anterior neuropore begins to close and the optic sulcus appears, then the three primary brain vesicules and the cranial flexure form (Rugh, 1968). At this stage, in the transgenic embryo, the neural fold and neural groove are intensely stained at the level of the 3rd hindbrain rhombomere, a localization that was confirmed on sections (Fig.

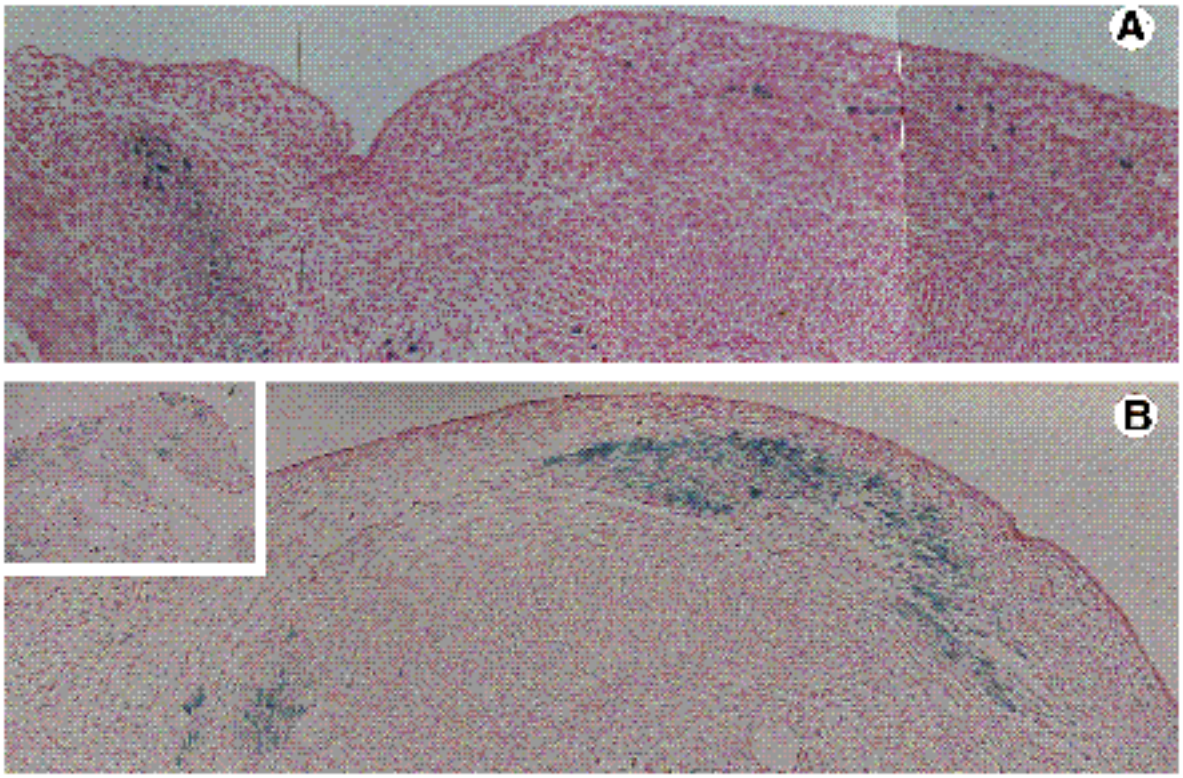


**Fig. 3.** Photograph of somites and limbs from 11- and 12-day embryos. (A) Photograph of forelimb bud at 11 days when the first desmin-positive cells have left somites and migrated to the limb. Desmin transgene expression is detected by blue staining prior to the time that the myoblasts begin to fuse to form the primary fibers. (B) Forelimb bud at 12 day. Muscle fibrils are intensively stained. The insert shows a complete section of the corresponding 12-day embryo.

5A,C). At day 9.5 the elongated myelencephalon displayed groups of blue cells in the wall of neural tube (Fig. 5B,D). At day 11, a triangular patch of blue-stained cells appeared at the pontine flexure, at the time when the cephalic flexure was formed as indicated by a ventral bulge in the floor of the myelencephalon. This β-galactosidase expression was found until day 14 with the same location in the pontine flexure and then disappeared. However, no expression of endogeneous desmin was observed, either by immunoreaction or by in situ hybridization on neuroectoderm sections of 8- to 12-day-old embryos (data not shown).

### The transgene is not expressed in the cardiocytes of the heart

As early as 5.5 to 6 days of development, paired mesocardial primordia appear and cardiac myoblasts can be identified by 7.5 days (Rugh, 1968). By day 8, a tubular heart is formed where myocardial contractions are beginning. The tubular portion of the heart elongates and bends into an S-shape structure. The atrial and bulbe ventricular regions are clearly recognizable with the latter contracting. In the 9-day embryo (13 to 20 somites), the heart is well developed and functional with four chambers each lined with endocardium, consisting of myocardium and surrounded by a pericardium.

Endogenous desmin was first detected by in situ hybridization and by peroxidase immunostaining in the tubular heart of an 8-day embryo. In contrast, the transgene was not expressed in these cells. However, the expression of the *nlacZ* could be detected in some scattered cells of the 8-day embryo, which appeared to belong to the conduction system judging from their anatomical localization. This expression of the transgene in the conduction system was detected throughout the foetal development and in newborn mice (Fig. 6).

### (4) Endogenous desmin expression and transgene activity in skeletal muscles

The pattern of transgene expression was compared with the endogenous expression of desmin synthesized by the mouse embryo after detection with a specific antibody directed against desmin. Fig. 7 illustrates desmin expression in 13-day embryos, which appears as a brown staining. Endogenous desmin was present in the skeletal muscles of the head, trunk and limbs and in cardiac muscle as well (see Table 2). Correlation between endogenous and transgenic desmin expression was observed in skeletal muscles (diaphragm, neck and vertebral muscles, in axial and limb musculature as well as in the cephalic muscles). This is illustrated by the clear correspondence between brown staining of the endogenous desmin and blue nuclei within the same muscle fibers. However, the X-Gal staining was not present at this time in all muscle fibers. These results could not be explained by an artefactual loss of X-gal staining due to the subsequent treatments because in other muscles, such as the diaphragm, all of nuclei were stained blue (Fig. 4). The

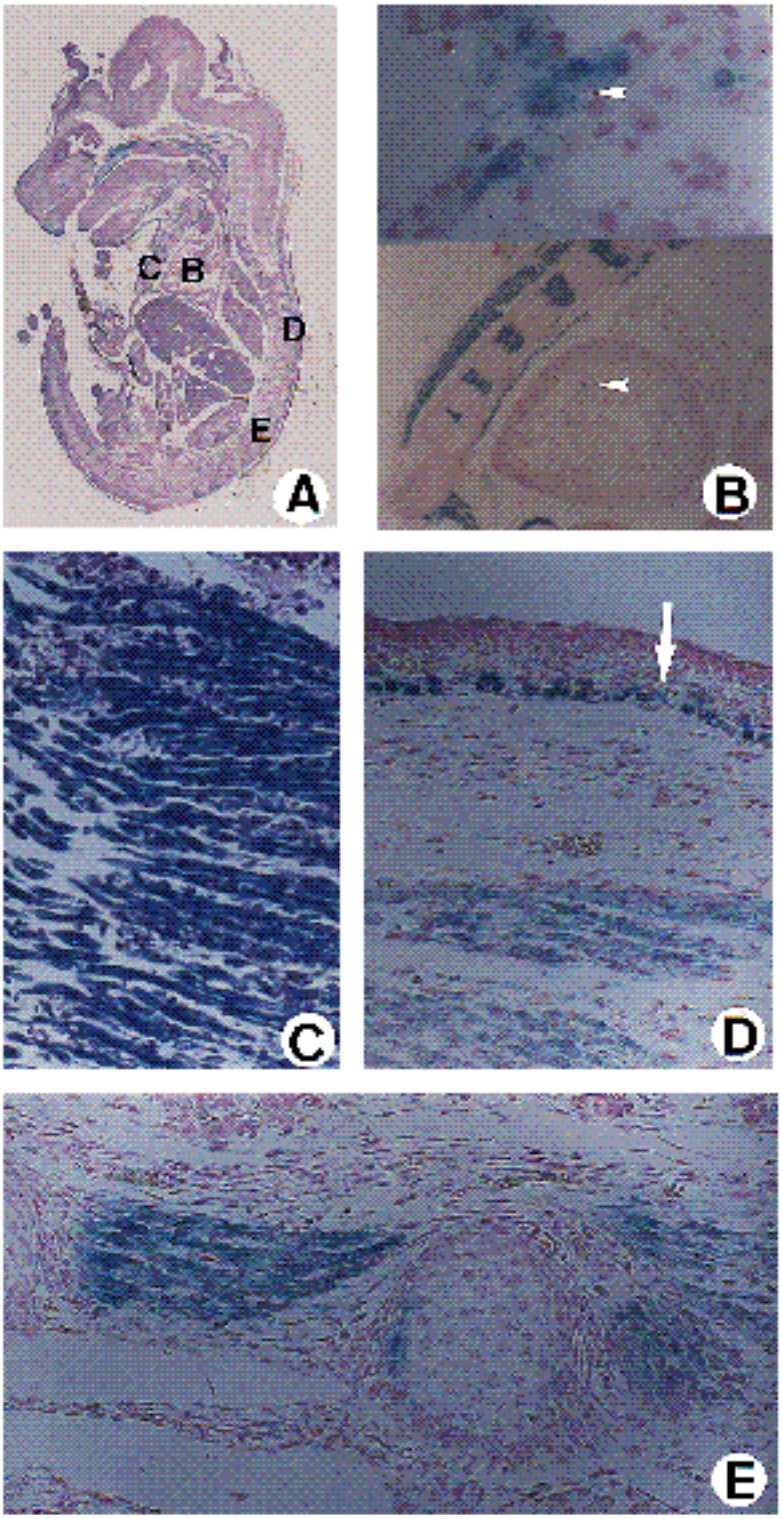


**Fig. 4.** Transgene expression in skeletal muscles and precursors. After fixation, 14-day embryos were submitted to X-gal reaction, embedded in paraffin and 5 μm sections cut and stained with hematoxylin. (A) Mid-sagittal section of a whole embryo: activity of the desmin transgene is visualized in blue. Letters indicate the enlarged regions. (B) Heart. The arrow points out the positive reaction corresponding to the cells of the conduction system (upper part, higher magnification). (C) Diaphragm, (D) subcutaneous and (E) vertebral muscles exhibit blue nuclei.

intensity of expression of *nlacZ* varied between different muscles. These variations were more readily documented because staining extended along entire muscle fibers. Internal intercostals and sternomastoid muscles contained large regions in which nearly all muscle fibers were stained whereas for example, the tibialis anterior was composed of a mixture of stained and unstained fibers.

## DISCUSSION

The results presented have shown that the desmin transgene containing the skeletal muscle enhancer was expressed in all the skeletal muscles during development of the transgenic mice but never in either the smooth or the cardiac muscles. In addition, the transgene was expressed in the

committed mononucleate myogenic cells (as shown by their expression in the cells of the limb bud) as well as in the myotubes/myofibers.

Endogenous desmin expression and transgene activity were found to be correlated in the skeletal muscles. The desmin transgene began to be expressed within the loose somitic mesoderm and remained active throughout the conversion from myoblast to myotubes and during the maturation of muscle fibers of the trunk and limbs. The desmin transgene was also active in a derivative of the prechordal and para-axial mesoderm that gave rise to the cephalic muscles. In contrast, it is remarkable that cardiac and smooth

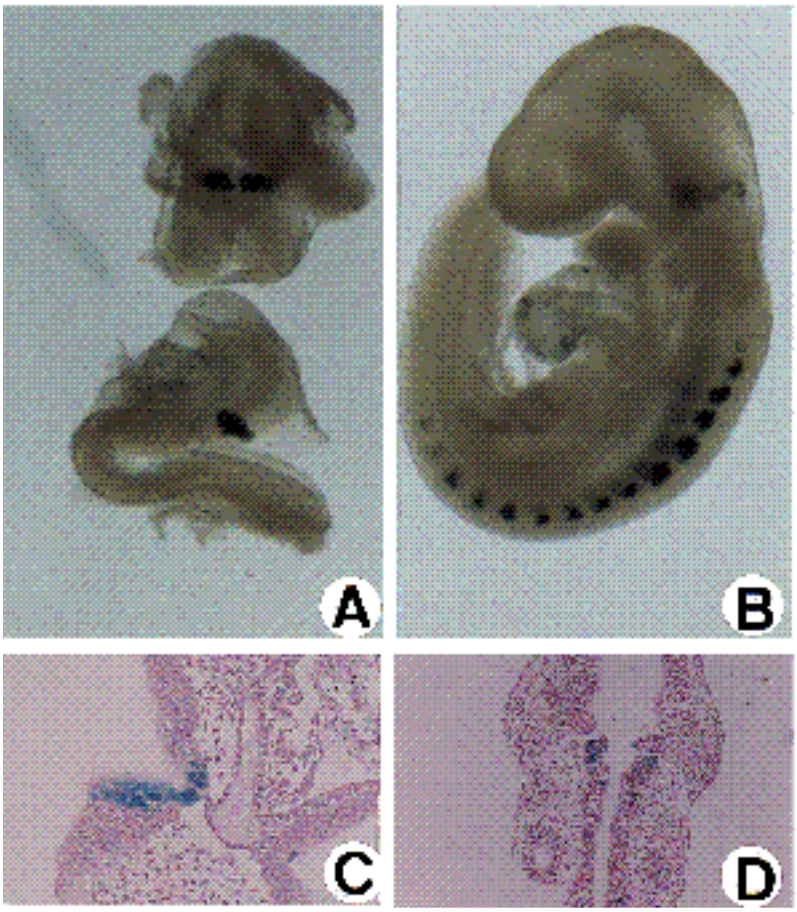


**Fig. 5.** Transitory expression of transgene in neural tube. (A, C) 8-day embryo, (B, D) 9.5-day embryo. (A, B) Embryos are stained in toto. (C) Transverse section of neural groove of 8-day, 10-somite embryo at the level of the future neuromeres. Neural fold and neural groove are stained. The section shows evidence of cranial and cervical flexure by proximity of brain and cord. (D) Sagittal section to dorsal side of the 9.5-day embryo at the otic vesicles level. The elongated myelencephalon is well identified. Groups of blue cells are distributed along the neuromeres.

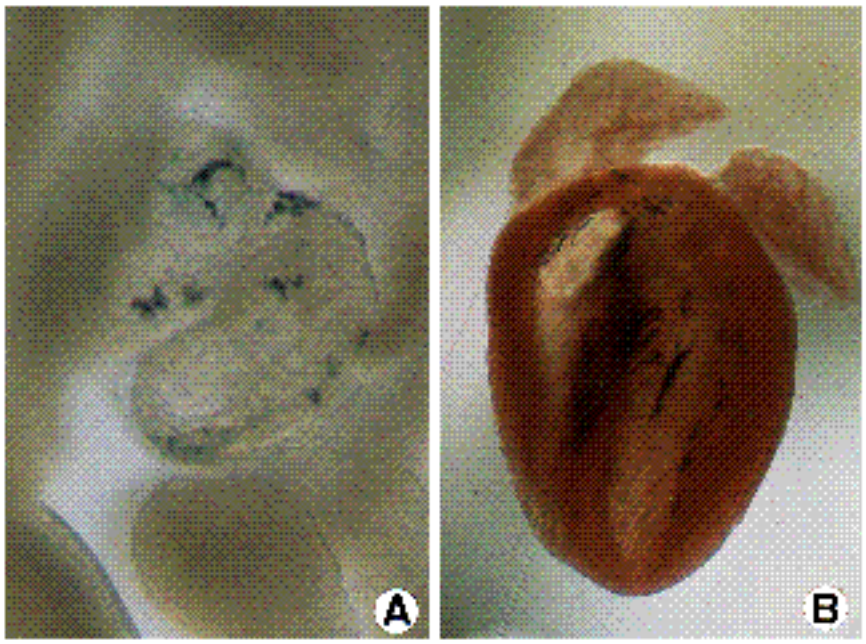


**Fig. 6.** Expression of desmin transgene in the heart. Heart of 8-day (A) or 18-day (B) transgenic embryos stained for β-galactosidase expression. In the transgenic embryo at day 8, when a tubular heart with the beginning of myocardial contraction takes place, some cells appear positive for the X-gal reaction. These cells were identified as belonging to the conduction system (Viragh and Challice, 1977). During all the embryonic development and in newborn, these cells appear reactive to Xgal, demonstrating the transgene activity. No other cells of the cardiac muscle show transgenic expression whereas the endogenous desmin gene is active at days 8.

muscles, which are both formed from splanchnic mesoderm, were uniformly β-galactosidase negative.

### The 1 kb desmin promoter-*nlacZ* fusion gene is functional both in vitro and in vivo

The 1 kb genomic fragment fused to the *nlacZ* gene used here was demonstrated to direct the cell-type-specific and differentiation-dependent expression of desmin in myogenic C2.7 cells. Furthermore, it was shown to act as a regulatory element in the skeletal muscles during the development of transgenic mice.

In myogenic C2.7 cells, high level tissue- and stage-specific expression of the desmin gene depends upon a 280 bp muscle-specific enhancer located between −693 and −973 bp upstream of the transcription initiation site. Important functional elements within this fragment, able to bind myogenic factors belonging to the myogenic helix-loop-helix superfamily, have been mapped (Li and Paulin, 1991). Each one of the four muscle regulatory factors (MyoD1, myogenin, myf5, and MRF4) has the ability to bind to the 14 bp sequence 5′TTGGCAGCTGTTGC3′ present in the desmin transgene. It has been shown by in situ hybridiza-

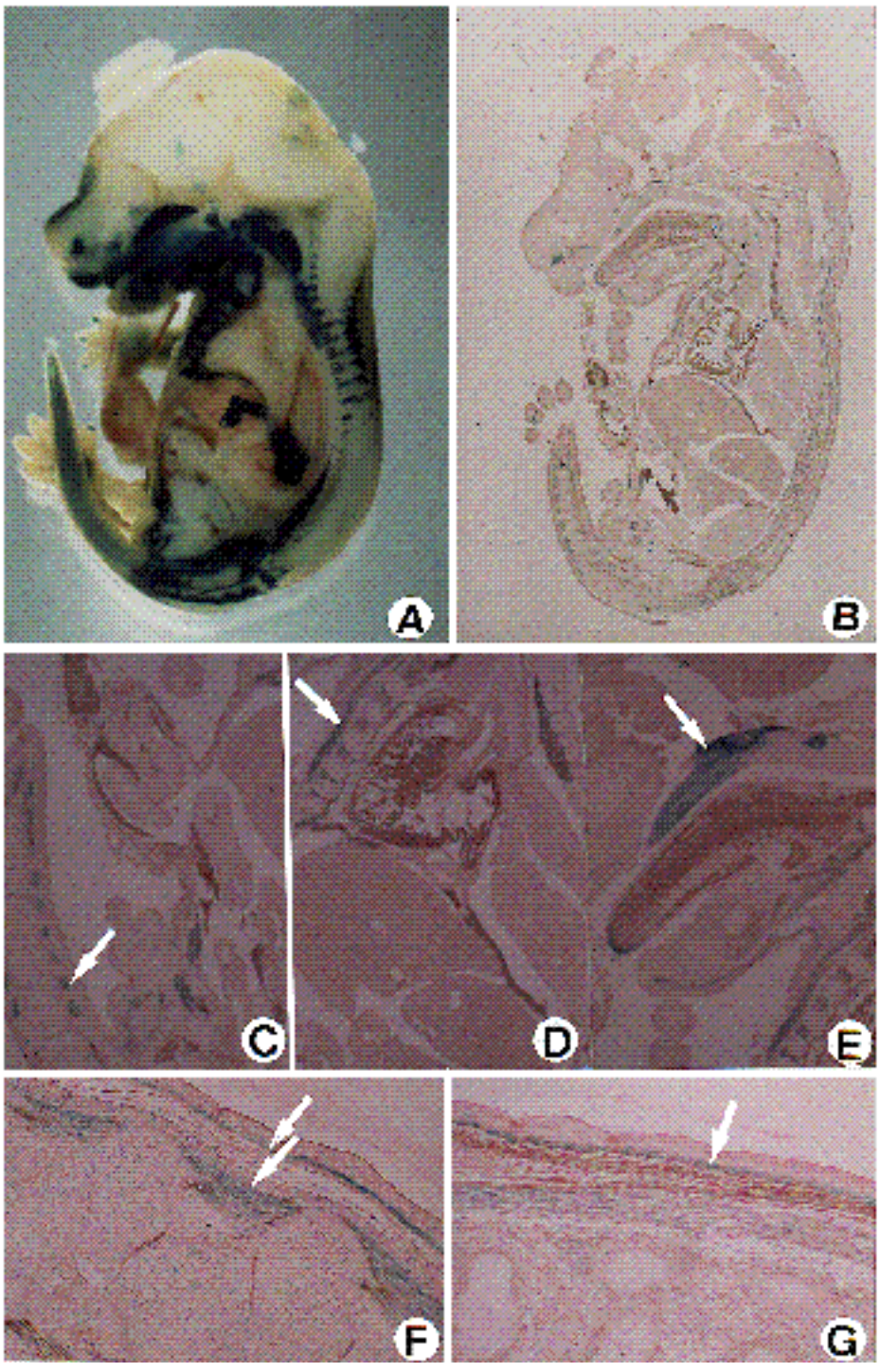


**Fig. 7.** Endogenous desmin expression in a 13-day embryo. Transgenic embryo, was stained with X-gal (A) and embedded in paraffin, and sagittal sections were made and treated for immunodetection of desmin revealed by peroxidase-conjugated antibodies (brown colour) (B). Note the absence of expression of the transgene in cardiac (D) and in smooth muscles: kidney, intestine, genital eminence, bladder and vessels (C). See Table 2 for all correspondence of endogenous and transgenic desmin expression in skeletal muscles. White arrows point out the coexpression in diaphragm (D); vertebral muscles (F,G); tongue and palate (E).

**Table 2. First time of desmin detection in mouse embryo of endogene and transgene activities**

| | (a) Endogene desmin | (b) Transgene Des1-nlacZ |
|---|---|---|
| Neuroectoderm | | |
| 3rd rhombomere | ND | 8 |
| Somites | | |
| 1-14 | 9 | 9 |
| 15-24 | 9.5 | 9.5 |
| 25-64 | 10 | 10 |
| Trunk | | |
| Diaphragm | 11 | 10.5 |
| Intercostal | 11 | 10.5 |
| Limb buds | | |
| Fore limb | ND | 11 |
| Hind limb | ND | 11.5 |
| Cephalic muscle | | |
| Mandibular | 11 | 11 |
| Lingual | 11 | 11 |
| Tongue | 11 | 11 |
| Palate | 11 | 11 |
| Heart | | |
| Atria | 8 | NE |
| Ventricle | 8 | NE |
| Conduction system | 8 | 8 |

(a) Desmin was characterized by immunostaining: this work and Babai et al., 1990; Hill et al., 1986; Mayo et al., 1992; Shaart et al., 1989.
(b) Desmine transgene *Des1-nlacZ* expression was detected by histochemical staining: this work.
(ND) not detected by immunocytochemistry.
(NE) never expressed.

tion that Myf-5 transcripts could be detected in the first somites from 8-day-old embryos, thus prior to myogenin in the dermomyotome and before the formation of the myotome (see Table 3).

Transgene expression was first detected as early as day 9 in the myotomal compartment of the somites and was progressively visible in more posterior structures in a rostrocaudal differentiation gradient (cf Fig. 2). The desmin protein expression coincides with the detection of myogenin RNA transcripts; however, the corresponding myogenin protein could only be detected after 10.5 days of development (Cusella De Anjelis et al., 1992). At this stage, no MyoD1 transcripts were found (Sassoon et al., 1989). By day 10, all somites were stained and, by day 10.5, the myotome of the somites differentiated into segmented blocks of myotomal muscle and blue staining for β-galactosidase was observed throughout the myotomal compartment. At this stage, MyoD1 began to be detected in the somites and in the muscle myotomes. Taken together these results and the relatively late appearance of MyoD1 indicates that myogenesis in the myotomal muscle of the embryo most probably begins in the absence of MyoD1.

The possibility that unknown regulations would control the initial expression of desmin could be considered. For instance, Pax 7, the murine paired-box gene (Jostes et al., 1991) was also detected at day 9 in the somitic mesoderm at the same time as the myogenin transcripts with a similar pattern of expression. Later, the three regulatory factors MyoD1, myogenin and Pax 7 exhibit the same pattern of expression in the intercostal muscles. However, it remains to be shown if Pax 7 can also act as a muscle-specific regulatory factor.

## Desmin is a marker of cells having migrated in limb buds

In several systems, it has been demonstrated that the myoblasts that invade the developing limb bud originate from the dermomyotome. (Christ et al., 1977; Jacob et al., 1978, 1979; Tam et al., 1987; Chevallier et al., 1977). In the mouse, a significant proportion of the mesodermal cells that will eventually contribute to the limb musculature are presumably present in the limb bud at 9 days. (Milaire, 1976). The lack of detectable myogenin and MyoD1 transcripts before 11.5 days in the limb bud indicates a difference between the precursor cells for myotomal muscles (see above) and precursor for musculature of the limb as suggested by Ordahl and Le Douarin (1992).

A striking demonstration of the additional information that can be obtained using a histochemically detectable reporter is the finding that the *Des1-nlacZ* transgene is expressed in cells that have migrated into the limb buds of 11-day-old embryos suggesting that these cells are already determined for myogenesis even though they do not express MyoD1 and myogenin. These cells appear as a cluster near the myotome and become aligned along the limb. Desmin transgene expression is detected by blue staining prior to the time that the myoblasts begin to fuse to form the primary fibers. The fact that the transgene was expressed in the commited mononucleate myogenic cells as well as in the myotubes is consistent with the organization of the desmin enhancer, which includes two regions; one part

**Table 3. Expression of desmin and myogenic regulatory factor genes in embryonic mouse somites and skeletal muscles of the trunk**

| Days p.c. | 8 | 9 | 9.5 | 10.5 | 11.5 | 12.5 | 14.5 | 15.5 |
|---|---|---|---|---|---|---|---|---|
| Desmin[a] | | + | ++ | ++ | +++ | +++ | +++ | +++ |
| Myf-5[b] | + | + | ++ | ++ | ++ | + | +/− | − |
| Myf-6MRF4[b] | | + | + | + | − | − | − | + |
| Myogenin[b] | − | *(++) | *(+++) | +++ | +++ | +++ | +++ | +++ |
| MyoD1[b] | − | − | − | ++ | +++ | +++ | +++ | +++ |

(a) Desmin was characterized by immunostaining: this work and Babai et al., 1990; Hill et al., 1986; Schaart et al., 1989; Mayo et al., 1992.
(b) Expression of regulatory myogenic factors was monitored by in situ hybridization or/and immunocytochemistry. Results come from Cussella-De-Anjelis et al., 1992; Montarras et al., 1991; Bober et al., 1991; Hannon et al., 1992; Sassoon et al., 1989.
*Myogenin protein could only be detected 10.5 d.p.c. while the corresponding messenger was clearly present at 9.5 d.p.c.

would be active in myotube through the MyoD1 binding site and the second one in the myoblasts, through the Krox elements (see drawing at the top of the Fig. 1).

### Transitory expression in the neural tube

The *Des1-lacZ* transgene is expressed in the 3rd rhombomere in 8-day-old embryos and continues to be expressed until day 14 in the neuroepithelium at the level of the pontine flexure forming the floor of the 4th ventricle.

According to Wilkinson et al. (1989), Krox-20 RNA transcripts coding for a transactivation factor are also expressed in the same zone in 8- to 13-day-old embryos. In this context it is intriguing to note that the genomic fragment of the transgene contains three consensus sequences able to bind the Krox-20 and Krox-24 proteins (unpublished data). Thus, one can hypothesize that the expression of the desmin transgene is induced by Krox acting as a muscle regulatory factor, even though we did not find endogeneous desmin expression in vivo. The fact that we find an activity of the desmin fusion gene in the neuroepithelium of our mice might be indicative of myogenic potential of mammalian neuroectoderm, which has previously been recognized in relation to skeletal muscle differentiation in vitro and in vivo (Lennon et al., 1979).

### Expression in the conduction system of the heart

Although the endogenous desmin was detected by in situ hybridization and by immunostaining in the cardiac muscle of an 8-day-old embryo (Schaart et al., 1989), at this stage the cardiac cells were not positive for staining.

However, the expression of the *nlacZ* was detected in particular cells that form the conduction system. The cardiac conduction tissue is a specialized cell system designed to conduct the electrical impulses for the sequential contraction of the cardiac muscle. This conduction system of the heart exhibits special morphological features and differs from the contractile myocardium in several respects, including cell form and size intercellular contacts, and the absence of T tubules (Thornell and Eriksson, 1981). The conduction cells contain myofibrillar material with different histochemical, biochemical and morphological properties compared with the contractile myocardium. For example, in the conduction system cell, sarcomeric myosin heavy chains are expressed (Gorza et al., 1986). It has been reported that these muscle fibers express a neural crest cell surface marker recognized by the monoclonal antibody NHK1, suggesting that they originate from a population of neural-crest-derived cells that have migrated from the branchial arches (Gorza et al., 1987). Furthermore, studies in the chick embryo have shown that neural-crest-derived cells (Le Douarin, 1984) from the branchial arches 3, 4 and 6, migrate into the heart and give rise to mesenchymal derivatives (Phillips et al., 1987). These results taken together thus raise the possibility that ectoderm-derived cells express desmin during embryogenesis. This possibility is further strengthened by our intriguing observation that the desmin transgene is expressed in the neuroectoderm.

Although the endogeneous desmin is expressed at high levels in cardiac muscle, the *Des1-nlacZ* gene is not expressed in the transgenic embryos, suggesting that distinct regulatory programs may have evolved for cardiac and skeletal muscle-specific expression of the desmin gene. In this event, the sequences controlling desmin gene expression in cardiac muscle should be located outside of the 1 kb region either in the gene itself or in the flanking regions.

In other transgenic lines, carrying fusion genes between different portions of the 5′ or 3′ regulatory regions of muscle-specific gene and reporter genes, expression of the reporter genes has been studied in cardiac and skeletal muscles. The enhancer of the rat myosin light chain 1/3 (MLC11/3) gene linked to the CAT reporter gene is known to be sufficient to induce a strong skeletal muscle-specific expression. Activation of the CAT gene reflected the onset of endogeneous MLC1 transcription at the same fetal stage (Rosenthal et al., 1989). In another system, a myotube-regulated expression was observed in mouse embryos carrying 842 bp of the 5′ upstream sequences of the chicken AchR alpha-subunit or 3500 bp of the mouse AchR epsilon-subunit genes linked to the *lacZ* gene. Thus 842 bp conferred a preferential synaptic expression within myofibres in vivo (Klarsfeld et al., 1991; Sanes et al., 1991).

A skeletal and cardiac expression is induced in mice carrying 3300 bp of the 5′ upstream sequences of the myosin creatine kinase (MCK) gene linked to the CAT gene. Progressive 5′-deletions from the nucleotide −3300 resulted in a preferential decrease in expression in cardiac tissue relative to that in skeletal muscle (Johnson et al., 1989). Transgenic mice harboring a luciferase reporter gene under the control of either a 2100 bp or a 250 bp of the myosin light chain (MLC-2) promoter fragment demonstrated high levels of luciferase activity in cardiac muscle and only background levels in slow skeletal muscle (Lee et al., 1992).

Thus, the results obtained with our transgenic mice provide evidence that the 1 kb *cis*-regulatory sequences confer specific developmental control for skeletal muscles and reveal distinct programs for cardiac and skeletal muscle-specific expression of the single desmin gene.

We thank Professor H. Condamine, Dr P. Duprey and P. Vicart for helpful discussions. We are indebted to Dr G. Buttler Browne, Dr P. Cochard and Dr S. Tajjbakhsh for critical revision of the work. We should like thank Dr P. Gounon for having followed the progress of this work with interest. We thank P. Ave for performing the sections of embryos and H. Cage for preparing the manuscript. This work is supported by grants from the Association Française contre les Myopathies (AFM 92/886), the Ministere de la Recherche et de la Technologie (MRT 91C0943), the Université Paris 7 and the Institut National de la Santé et de la Recherche Médicale (INSERM 920810).

## REFERENCES

**Babai, F., Musevi-Aghdam, J., Schurch, W., Royal, A. and Gabbiani, G.** (1990). Coexpression of α-sarcomeric actin, α-smooth muscle actin and desmin during myogenesis in rat and mouse embryos I. Skeletal muscle. *Differentiation* **44,** 132-142.

**Baldwin, T. J. and Burden, S. J.** (1989). Muscle-specific gene expression controlled by a regulatory element lacking a MyoD1-binding site. *Nature* **341,** 716-720.

**Bober, E., Lyons, G. E., Braun, T., Cossu, G., Buckingham, M. and Arnold, H. H.** (1991). The muscle regulatory gene, myf-6 has a biphasic pattern of expression during early mouse development. *J. Cell Biol.* **133,** 1255-1265.

**Brennan, T. J. and Olson, E. N.** (1990). Myogenin resides in the nucleus

and acquires high affinity for a conserved enhancer element on heterodimerization. *Genes Dev.* **4,** 582-595.

**Brinster, R. L., Chen, H. Y., Trumbauer, M. E., Yagle, M. K. and Palmiter, R. D.** (1985). Factors affecting the efficiency of introducing foreign DNA into mice by microinjecting eggs. *Proc. Natl. Acad. Sci. USA* **82,** 4438-4442.

**Buskin, J. N. and Hauschka, S. D.** (1989). Identification of a myocyte nuclear factor that binds to the muscle-specific enhancer of the mouse muscle creatine kinase gene. *Mol. Cell. Biol.* **9,** 2627-2640.

**Caravatti, M., Minty, A., Robert, B., Montarras, D., Weydert, A., Cohen, A., Daubas, P. and Buckingham, M.** (1982). Regulation of muscle gene expression. The accumulation of messenger RNAs coding for muscle-specific proteins during myogenesis in a mouse cell line. *J. Mol. Biol.* **160,** 59-76.

**Chevallier, A., Kieny, M. and Mauger, A.** (1977). Limb-somite relationship: origin of the limb musculature. *J. Embryol. Exp. Morph.* **41,** 245-258.

**Christ, B., Jacob, H. J. and Jacob, M.** (1977). Experimental analysis of the origin of the wing musculature in avian embryos. *Anat. Embryol.* **150,** 171-186.

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

**Cusella-De-Anjelis, M. G., Lyons, G., Sonnino, C., De Angelis, L., Vivarelli, E., Farmer, K., Wright, W. E., Molinaro, M., Bouché, M., Buckingham, M. and Cossu, G.** (1992). MyoD, myogenin independent differenciation of primordial myoblasts in mouse somites. *J. Cell. Biol.* **116,** 1243-1255.

**Dingwall, C. and Laskey, R. A.** (1986). Protein import into the cell nucleus. *Ann. Rev. Cell. Biol.* **2,** 367-390.

**Donoghue, M., Ernst, H., Wentworth, B., Nadal-Ginard, B. and Rosenthal, N.** (1988). A muscle-specific enhancer is located at the 3′ end of the myosin light-chain 1/3 gene locus. *Genes Dev.* **2,** 1779-1790.

**Fürst, D. O., Osborn, M. and Weber, K.** (1989). Myogenesis in the mouse embryo: differential onset of expression of myogenic proteins and the involvement of titin in myofibril assembly. *J. Cell. Biol.* **109,** 517-527.

**Geisler, N. and Weber, K.** (1982). The amino acid sequence of chiken muscle desmin provides a common structural model for intermediate filament proteins. *EMBO J.* **1,** 1649-1656.

**Gorza, L., Sartore, S., Thornell, L. E. and Schiaffino, S.** (1986). Myosin type and fiber types in cardiac muscle. III. Nodal conduction tissue. *J. Cell Biol.* **102,** 1758-1766.

**Gorza, L., Schiaffino, S. and Vitadello, M.** (1987). Heart conduction system: a neural crest derivative? *Brain Res.* **457,** 360-366.

**Gossett, L. A., Kelvin, D. J., Sternberg, E. A. and Olson, E. N.** (1989). A new myocyte-specific enhancer-binding factor that recognizes a conserved element associated with multiple muscle-specific genes. *Mol. Cell. Biol.* **9,** 5022-5033.

**Gunning, P., Hardeman, E., Wade, R., Ponte, P., Bains, W., Blau, H. M. and Kedes, L.** (1987). Differential patterns of transcript accumulation during human myogenesis. *Mol. Cell. Biol.* **7,** 4100-4114.

**Hannon, K., Smith II, C. K., Bales, K. R. and Santerre, R. F.** (1992). Temporal quantitative analysis of myogenic regulatory and growth factor gene expression in the developing mouse embryo. *Dev. Biol.* **151,** 137-144.

**Hill, C. S., Duran, S., Lin, Z., Weber, K. and Holtzer, H.** (1986). Titin and myosin, but not desmin, are linked during myofibrillogenesis in postmitotic mononucleated myoblasts. *J. Cell. Biol.* **103,** 2185-2196.

**Horlick, R. A. and Benfield, P. A.** (1989). The upstream muscle-specific enhancer of the rat muscle creatine kinase gene is composed of multiple elements. *Mol. Cell. Biol.* **9,** 2396-2413.

**Jacob, M., Christ, B. and Jacob, J. H.** (1978). On the migration of myogenic stem cells into the prospective wing region of chick embryos. *J. Anat. Embryol.* **153,** 179-193.

**Jacob, M., Christ, B. and Jacob, H. J.** (1979). The migration of myogenic cells from the somites into the leg region of avian embryos. *Anat. Embryol.* **157,** 291-309.

**Jaynes, J. B., Johnson, J. E., Buskin, J. N., Gartside, C. L. and Hauschka, S. D.** (1988). The muscle creatine kinase gene is regulated by multiple upstream elements, including a muscle-specific enhancer. *Mol. Cell. Biol.* **8,** 62-70.

**Johnson, J. E., Wold, B. J. and Hauschka, S. D.** (1989). Muscle creatine kinase sequence elements regulating skeletal and cardiac muscle expression in transgenic mice. *Mol. Cell. Biol.* **9,** 3393-3399.

**Jostes, B., Walther, C. and Gruss, P.** (1991). The murine paired box gene, Pax7, is expressed specifically during the development of the nervous and muscular system. *Mech. Dev.* **33,** 27-38.

**Kalderon, D., Roberts, B. L., William.D. R. and Smith, A. E.** (1984). A short amino acid sequence able to specify nuclear location. *Cell.* **39,** 499-509.

**Kaufman, S. J. and Foster, R. F.** (1988). Replicating myoblasts express a muscle-specific phenotype. *Proc. Natl. Acad. Sci. USA* **85,** 9606-9610.

**Kaufman, M. H.** (1992). *The Atlas of Mouse Development.* London: Academic Press Limited.

**Klasfeld, A., Bessereau, J. L., Salmon, A. M., Triller, A., Babinet, C. and Changeux, J. P.** (1991). An acetylcholine receptor α-subunit promoter confering preferential synaptic expression in muscle of transgenic mice. *EMBO J.* **10,** 625-632.

**Kozak, M.** (1987). An analysis of 5′-noncoding sequences from 699 vertebrate messenger RNAs. *Nucl. Acids Res.* **15,** 8125-8131.

**Lassar, A. B., Buskin, J. N., Lockshon, D., Davis, L., Apone, S., Hauschka, D. and Weintraub, H.** (1989). MyoD is a sequence-specific DNA binding protein requiring a region of myc homology to bind to the muscle creatine kinase enhancer. *Cell* **58,** 823-831.

**Lazarides, E. and Hubbard, B. D.** (1976). Immunological characterization of the subunit of the 100 A filaments from muscle cells. *Proc. Natl. Acad. Sci.* USA **73,** 4344-4348.

**Le Douarin, N. M.** (1984). Cell migrations in embryos. *Cell* **38,** 353-360.

**Lee, K. J., Ross, R. S., Rockman, H. A., Harris, A., O'Brien, T., Van Bilsen, M., Shubeita, H. E., Kandolf, R., Brem, G., Price, J., Evans, S. M., Zhu, H., Franz, W. M. and Chien, K. R.** (1992). Myosin light chain-2 luciferase transgenic mice reveal distinct regulatory programs for cardiac and skeletal muscle-specific expression of a single contractile protein gene. *J. Biol. Chem.* **267,** 15875-15885.

**Lennon, V. A., Peterson, S. and Shubert, D.** (1979). Neurectoderm markers retained in phenotypical skeletal muscle cells arising from a glial cell line. *Nature* **281,** 586-588.

**Li, Z., Lilienbaum, A., Butler-Browne, G. and Paulin, D.** (1989). Human desmin coding gene : complete nucleotide sequence, characterization and regulation of expression during myogenesis and development. *Gene* **78,** 243-254.

**Li, Z., Mattei, M. G., Mattei, J. F. and Paulin, D.** (1990). Assignment of the mouse desmin gene to chromosome 1 band C3. *Genet. Res. Camb.* **55,** 101-105.

**Li, Z. and Paulin, D.** (1991). High level desmin expression depends on a muscle-specific enhancer. *J. Biol. Chem.* **266,** 6562-6570.

**Mayo, M., Brinbas, M., Santos, V., Shum, L. and Slavkin, H. C.** (1992). Desmin expression during early mouse tongue morphogenesis. *J. Dev. Biol.* **36,** 255-263.

**Milaire, J.** (1976). Contribution cellulaire des somites à la genèse des bourgeons de membres postérieurs chez la souris. *Arch. Biol.* **87,** 315-343.

**Montarras, D., Chelly, J., Bober, E., Arnold, H., Ott, M. O., Gros, F. and Pinset, C.** (1991). Developmental patterns in the expression of myf5, MyoD1, myogenin and MRF4 during myogenesis. *The New Biologist* **3,** 592-600.

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

**Phillips, M. T., Kirby, M. L. and Forbes, G.** (1987). Analysis of cranial neural crest distribution in the developing heart using quail-chick chimeras,. *Circ. Res.* **60,** 27-30.

**Pieper, F. R., Slobbe, R. L., Ramaekers, F. C. S., Cuypers, H. T. and Bloemendal, H.** (1987). Upstream regions of the hamster desmin and vimentin genes regulate expression during in vitro myogenesis. *EMBO J.* **6,** 3611-3618.

**Quax, W., Broek, L. V. D., Egberts, W. V., Ramaekers, F. and Bloemendal, H.** (1985). Characterization of the hamster desmin gene: expression and formation of desmin filaments in nonmuscle cells after gene transfer. *Cell* **43,** 327-338.

**Rosenthal, N., Kornhauser, J. M., Donoghue, M., Rosen, K. M. and Merlie, J. P.** (1989). Myosin light chain enhancer activates muscle-specific, developmentally regulated gene expression in transgenic mice. *Proc. Natl. Acad. Sci.USA* **86,** 7780-7784.

**Rugh, R.** (1968). *The Mouse its Reproduction and Development.* Oxford University Press: Oxford Science Publications.

**Sanes, J. R., Rubinstein, J. L. and Nicolas, J. F.** (1986). Use of a recombinant retrovirus to study post-implantation cell lineage in mouse embryos. *EMBO. J.* **5,** 3133-3142.

**Sanes, J., Johnson, Y., Kotzbauer, P., Mudd, J., Hanley, T., Martinou, J. C. and Merlie, J.** (1991). Selective expression of an acetylcholine receptor-lacZ transgene in synaptic nuclei of adult muscle fibers. *Development* **113,** 1181-1191.

**Sassoon, D., Lyons, G., Wright, W. E., Lin, V., Lassar, A., Weintraub, H. and Buckingham, M.** (1989). Expression of two myogenic regulatory factors myogenin and MyoD1 during mouse embryogenesis. *Nature* **341,** 303-307.

**Schaart, G., Viebahn, C., Langmann, W. and Ramaekers, F.** (1989). Desmin and titin expression in early postimplantation mouse embryos. *Development* **107,** 585-596.

**Small, J. V. and Sobieszek, A.** (1977). Studies on the function and composition of the 10-nm (100-A) filaments of vertebrate smooth muscle. *J. Cell Sci.* **23,** 243-268.

**Sternberg, E., Spizz, G., Perry, W. M., Vizard, D., Weil, T. and Olson, E. N.** (1988). Identification of upstream and intragenic regulatory elements that confer cell-type-restricted and differentiation-specific expression on the muscle creatine kinase gene. *Mol. Cell. Biol.* **8,** 2896-2909.

**Tam, P. P. L. and Beddington, R. S.** (1987). The formation of mesodermal tissues in the mouse embryo during gastrulation and early organogenesis. *Development* **99,** 109-126.

**Thornell, L. E. and Eriksson, A.** (1981). Filament system in the Purkinje fibers of the heart. *Am. J. Physiol.* **241,** 291-305.

**Viegas-Péquignot, E., Li, Z., Dutrillaux, B., Apiou, F. and Paulin, D.** (1989). Assignment of human desmin gene to band 2q35 by nonradioactive in situ hybridization. *Human Genetics* **83,** 33-36.

**Viragh, Sz. and Challice, C. E.** (1977). The development of the conduction system in the mouse embryo heart. *Dev. Biol.* **56,** 397-411.

**Wang, Y., H.P., X., Wang, X. M., Ballivet, M. and Schmidt, J.** (1988). A cell type-specific enhancer drives expression of the chick muscle acetylcholine receptor -subunit gene. *Neuron* **1,** 527-534.

**Wang, X. M., Tsay, H. J. and Schmidt, J.** (1990). Expression of the acetylcholine receptor δ-subunit gene in differentiating chick muscle cells is activated by an element that contains two 16 bp copies of a segment of the α-subunit enhancer. *EMBOJ.* **9,** 783-790.

**Wilkinson, D. G., Bhatt, S., Chavrier, P., Bravo, R. and Charnay, P.** (1989). Segment-specific expression of a Zinc-finger gene in the developing nervous system of the mouse. *Nature* **337,** 461-463.

**Yutzey, K. E., Kline, R. L. and Konieczny, S. F.** (1989). An internal regulatory element controls troponin I gene expression. *Mol. Cell. Biol.* **9,** 1397-1405.

(Accepted 17 December 1992)