Mapping of *myogenin* Transcription During Embryogenesis Using Transgenes Linked to the *myogenin* Control Region

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Abstract. During vertebrate embryogenesis, the muscle-specific helix-loop-helix protein myogenin is expressed in muscle cell precursors in the developing somite myotome and limb bud before muscle fiber formation and is further upregulated during myogenesis. We show that *cis*-acting DNA sequences within the 5′ flanking region of the mouse *myogenin* gene are sufficient to direct appropriate temporal, spatial, and tissue-specific transcription of *myogenin* during mouse embryogenesis. *Myogenin-lacZ* transgenes trace the fate of embryonic cells that activate *myogenin* transcription and suggest that myogenic precursor cells that mi-grate from the somite myotome to the limb bud are committed to a myogenic fate in the absence of *myogenin* transcription. Activation of a *myogenin-lacZ* transgene can occur in limb bud explants in culture, indicating that signals required for activation of *myogenin* transcription are intrinsic to the limb bud and independent of other parts of the embryo. These results reveal multiple populations of myogenic precursor cells during development and suggest the existence of regulators other than myogenic helix-loop-helix proteins that maintain cells in the early limb bud in the myogenic lineage.

T HE formation of skeletal muscle during vertebrate development is associated with the withdrawal of proliferating myoblasts from the cell cycle, fusion to form multinucleate myotubes, and transcriptional activation of a large set of muscle-specific genes. While much has been learned about the mechanisms that regulate myogenesis in vitro, the intrinsic and extrinsic factors that influence muscle differentiation in vivo remain poorly understood.

Skeletal muscle in vertebrates is derived from the somites, which form by segmentation of the paraxial mesoderm along the neural tube (Chevallier et al., 1977; Christ et al., 1977). Somite formation in the mouse begins at ∼8 d post coitum (dpc)[1] and progresses caudally over a period of several days (Tam, 1981). As somites mature, they become compartmentalized, with the dorsal portions forming the dermamyotome and the ventral portions giving rise to the sclerotome. The first signs of muscle differentiation in the mouse are observed at 10.5 dpc within the myotome, which arises in the center of the somite through migration of cells from the dorsal medial portion of the dermamyotome. Limb muscles begin to form at ∼11.5 dpc of mouse development and are derived from myogenic progenitor cells that migrate out from the ventrolateral edge of the dermamyotome (Christ et al., 1978). Studies with chick-quail chimeras indicate that these migrating myogenic progenitors are committed to a skeletal muscle fate, but they do not express muscle markers until they reach the limb bud (Christ et al., 1978; Jacob et al., 1979).

The recent cloning of the MyoD family of myogenic regulators, which can activate muscle differentiation in tissue culture, has provided an opportunity to analyze the early events associated with establishment and maintenance of the skeletal muscle lineage in vivo. This family of regulatory factors, which includes MyoD, myogenin, myf5, and MRF4, activates muscle-specific transcription through binding to a DNA consensus sequence known as an E-box, which is found in the control regions of numerous muscle structural genes (for reviews, see Olson, 1990; Weintraub et al., 1991; Emerson, 1990). In addition to activating subordinate genes associated with terminal differentiation, members of the MyoD family participate in a complex regulatory circuit in which they auto- and cross-activate one anothers' expression (Thayer et al., 1989; Braun et al., 1989; Edmondson et al., 1991). Thus, positive feed-back interactions have been postulated to reinforce the expression of these regulatory factors and to provide stability to the myogenic phenotype.

Each member of the MyoD family exhibits a unique pattern of expression during development, suggesting that these genes respond to distinct developmental cues. The first member of the MyoD family to be expressed during mouse embryogenesis is myf5, which appears in the rostal somites at ∼8 dpc (Ott et al., 1991). Myogenin transcripts are detected in the somite myotome by day 8.5, followed two days

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1. *Abbreviations used in this paper:* CAT, chloramphenicol transferase; dpc, days post coitum; HLH, helix-loop-helix.

later by the appearance of transcripts for MyoD and other markers of terminal differentiation (Wright et al., 1989; Sassoon et al., 1989). MRF4 is expressed transiently in the somite myotome between days 9 and 12 and is then repressed until late stages of development (Hinterberger et al., 1991; Bober et al., 1991). In the developing limb bud, myf5 is initially detected between 10.5 and 11.0 dpc, with myogenin and MyoD appearing about a half-day later. Although the cells that migrate into the limb bud that eventually give rise to muscle are committed to a myogenic fate before day 10, these cells do not express transcripts for any member of the MyoD family (Sassoon et al., 1989), suggesting that other regulatory factors are responsible for maintaining them in the myogenic lineage. Whether one or more members of the MyoD family are expressed earlier in these cells and are subsequently extinguished during their migration from the myotome to the limb bud is unknown.

In contrast to myf5, MyoD, and MRF4, which are not expressed in many skeletal muscle cell lines, myogenin is expressed in all differentiated skeletal muscle lines (Wright et al., 1989; Edmondson et al., 1989; Braun et al., 1989), suggesting that the *myogenin* gene responds to a common myogenic regulatory pathway. Analysis of the mechanisms regulating *myogenin* transcription in muscle cells in vitro has revealed binding sites for myogenic helix-loop-helix (HLH) proteins and the muscle-specific enhancer factor MEF-2 within the *myogenin* promoter that mediate muscle-specific transcription and positive autoregulation (Edmondson et al., 1992).

To begin to define the mechanisms that regulate *myogenin* expression in vivo and to trace the embryonic fate of myogenic cells that activate *myogenin* transcription, we have created transgenic mice harboring reporter genes linked to the *myogenin* 5' flanking region. Here we show that DNA sequences immediately preceding the *myogenin* transcription initiation site are sufficient to direct appropriate temporal, spatial, and tissue-specific expression of a linked reporter gene during embryogenesis. *Myogenin-lacZ* transgenes, which trace the fate of cells that have activated *myogenin* transcription, show that myogenic progenitors that migrate from the somite into the developing limb bud arise from a population of cells in the myotome that are committed to a myogenic fate in the absence of *myogenin* transcription. Analysis of *myogenin* transcription in isolated limb bud explants, using *myogenin-lacZ* transgenes, indicates that the molecules required for activation of *myogenin* expression are intrinsic to the limb bud and are autonomous of other parts of the embryo.

Materials and Methods

Plasmids Construction

Myogenin 5' flanking sequences (Edmondson et al., 1992) were cloned into pCAT-basic (Promega Corp., Madison, WI) and pAUGLacZ (kindly provided by W. M. Perry, The University of Texas M. D. Anderson Cancer Center, Houston, TX). pMyo1565CAT was made by cloning an *Xba*I (−1565)-*Hae*III(+18) fragment into the pCAT-basic, which was then linearized by *Hind*III and *Bam*HI for microinjection. pMyo1565(-E)CAT contains the 5' flanking sequence from −1565 to −18 and was made by cloning an *Xba*I-*Ban*I fragment into the polylinker of pCAT-basic. For injection, a *Hae*II and *Bam*HI fragment of pMyo1565(-E)CAT was made. pMyo1565 (-E)LacZ was made by cloning of the *Xba*I-*Ban*I fragment described above into the polylinker of pAUGLacZ and linearized by *Hind*III and *Eco*RI di-

gestion for microinjection. pMyo1565LacZ was made by cloning the *Xba*I-*Hae*III fragment into the polylinker of the pAUGLacZ, and linearized by *Hind*III and *Eco*RI digestion. Additional details of the *myogenin* 5' flanking sequences in these constructions have been described (Edmondson et al., 1992). All plasmids to be injected were purified by double CsCl centrifugation, and the fragments isolated from SeaPlague (FMC Corp., Rockland, ME) agarose gel, phenol/chloroform cleaned, and passed through a NACS column (Life Technologies, Inc., Gaithersburg, MD).

Production and Analysis of Transgenic Mice

Hybrid inbred (C57BL6 × CBA)F1 mice (Jackson Laboratory, Bar Harbor, ME) were used as stud males, embryo donors, and mature females for breeding. Outbred ICR mice (Harlan Sprague Dawley, Indianapolis, IN) were used for vasectomized males and pseudopregnant females. The procedure for generating transgenic mice has been described (Hogan et al., 1986). Stable lines of transgenic mice were maintained by backcrossing to founder animals to nontransgenic (C57BL6 × CBAF1) hybrid mice. Transgene-positive animals were identified by PCR amplification of tail DNA (Hanley and Merlie, 1991) or by Southern blot analysis. Noon of the day vaginal plugs are detected is counted as 0.5 dpc with the diurnal cycle of dark from 7:00 PM to 5:00 AM. Embryonic development was also monitored according to Theiler (1972). Embryos were dissected out by removing all the coverings including the amnion, and fixed in 2% paraformaldehyde, 0.2% glutaraldehyde, in PBS for 30 to 90 min depending on size. The embryos were then washed twice in PBS for a total of 30 min. They were then stained with 1 mg/ml X-gal, 2 mM $MgCl_2$, 5 mM $K_3Fe(CN)_6$, and 5 mM $K_4Fe(CN)_6$ in PBS at room temperature for 12–16 h, after which time they were cleared in PBS for more than 10 h and then they were further fixed and stored in 4% formaldehyde (Sanes et al., 1991). Whole-mount embryos were dehydrated, cleared, and embedded in paraffin. Sections 5 μM thick were dewaxed and counterstained with hematoxylin and eosin. Different organs and tissues were isolated from 3-wk old tail-positive mice for chloramphenicol acetyltransferase (CAT) assay as described (Donoghue et al., 1991).

CAT activity in extracts of transgenic tissues was measured using 0.1–100 μg of extract protein, as described (Donoghue et al., 1991). All values from the CAT assay were normalized to 10 μg of cellular extract. Assays were linear with respect to time and protein concentration. Activities were determined by separating substrate and product by thin-layer chromatography and counting radioactivity in the different species.

Results

The Myogenin 5' Flanking Region Directs Muscle-specific Expression of a Linked CAT Gene in Transgenic Mice

To begin to define the DNA sequences that regulate *myogenin* transcription in vivo, we generated transgenic mice harboring the transgene pMyo1565CAT, which contains the *CAT* reporter gene linked to a DNA fragment extending from +18 to −1565 relative to the *myogenin* transcription initiation site (Fig. 1 *a*). Analysis of nine independent lines of transgenic mice revealed three lines that expressed the transgene in the F1 generation. In all three lines, high levels of CAT expression were observed in skeletal muscle, with no detectable expression in any other tissues examined, including cardiac and smooth muscle (Fig. 1 *b*). The level of CAT expression in skeletal muscle from different transgenic lines varied severalfold, but was in all cases at least 3,000-fold higher in skeletal muscle than in nonmuscle tissues. These results are consistent with previous studies which showed that DNA sequences immediately upstream of the *myogenin* gene were sufficient for muscle-specific transcription in C2 muscle cells and chick primary myotubes (Edmondson et al., 1992; Salminen et al., 1991).

An E-box that binds myogenic HLH proteins with high-affinity is located between the TATA element and the transcription start site of the *myogenin* gene (Edmondson et al.,
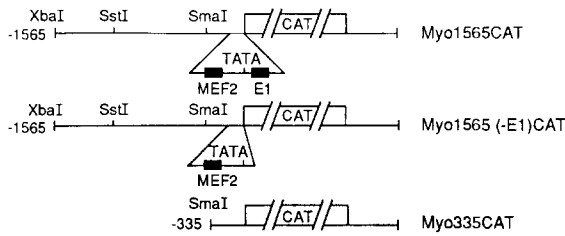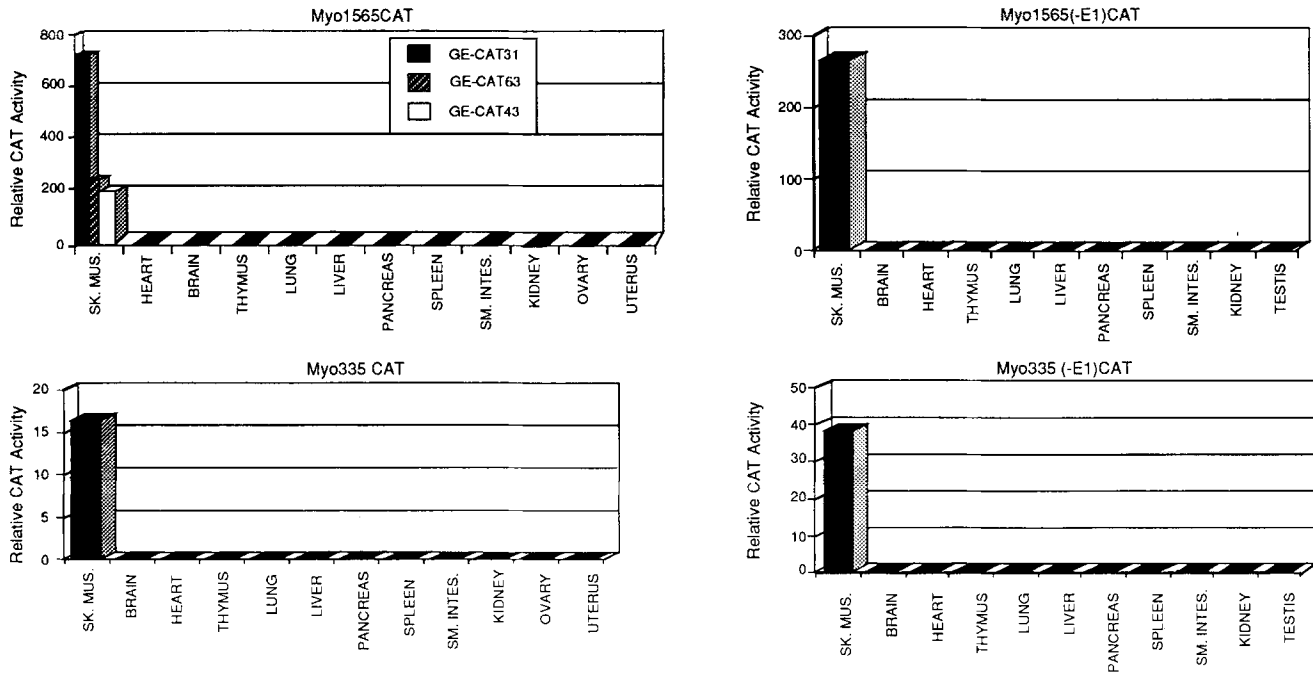
Figure 1. Muscle-specific expression of myogenin-CAT transgenes. (A) Expression vectors for creation of transgenic mice. Restriction fragments encompassing the myogenin promoter and extending to the XbaI and SmaI restriction sites at −1565 and −335, respectively, were inserted immediately upstream of CAT or lacZ. The proximal promoter contains a MEF-2 site and an E-box (E-1) surrounding the TATA box. Constructions lacking E-1 are indicated (-E1). Details of the constructions are described in the text. (B) CAT activity was determined in extracts of adult mouse tissues from transgenic lines GECAT-31, GECAT-63, and GECAT-43, harboring Myo1565CAT and transgenic line GCAT-28, harboring Myo1565(-E1)CAT. (C) CAT activity was determined in extracts of adult mouse tissues from transgenic line G2CAT-22, harboring Myo335CAT, and transgenic line G2CAT-53, harboring Myo335(-E1)CAT. Values are normalized to the percent conversion of substrate to product by 10 μg of protein from the indicated tissue extract. Assays with skeletal muscle extracts were linear in the range of 1-100 μg of protein and were diluted sufficiently to allow enzyme activity to be determined quantitatively. Skeletal muscle was obtained from the hind limb, and it was the only tissue that showed activity above background.
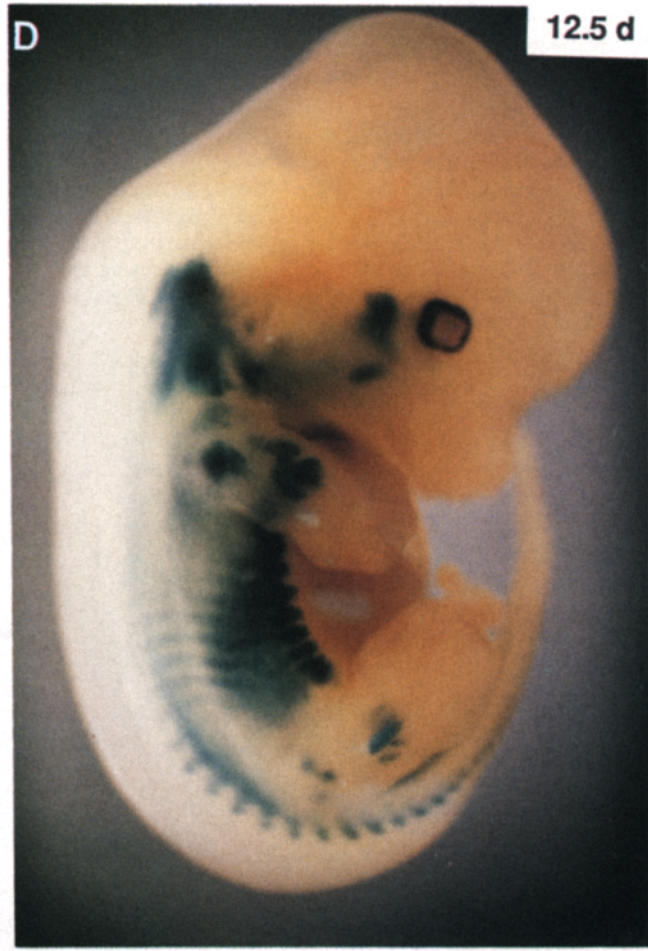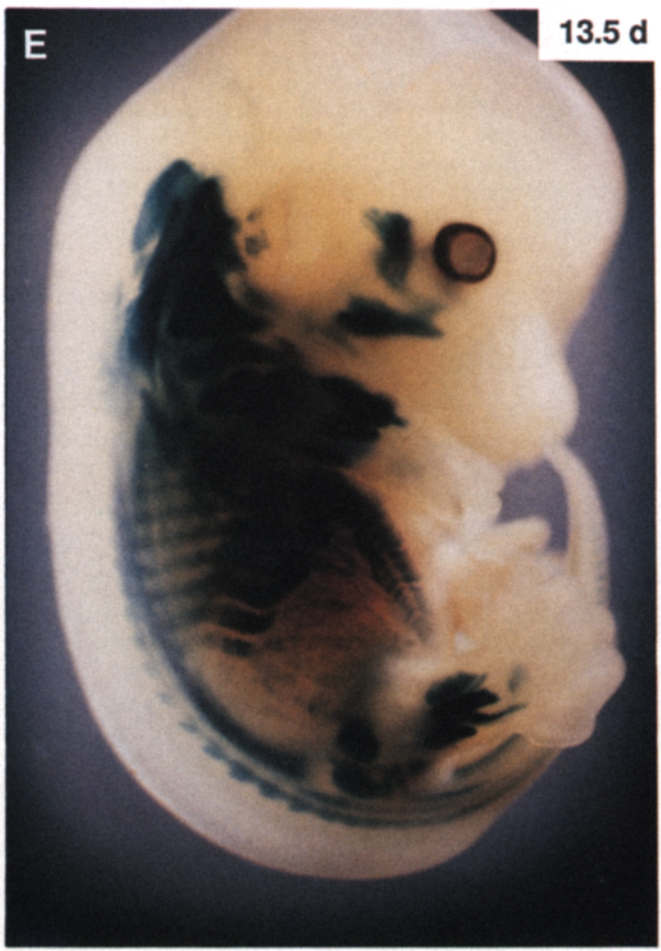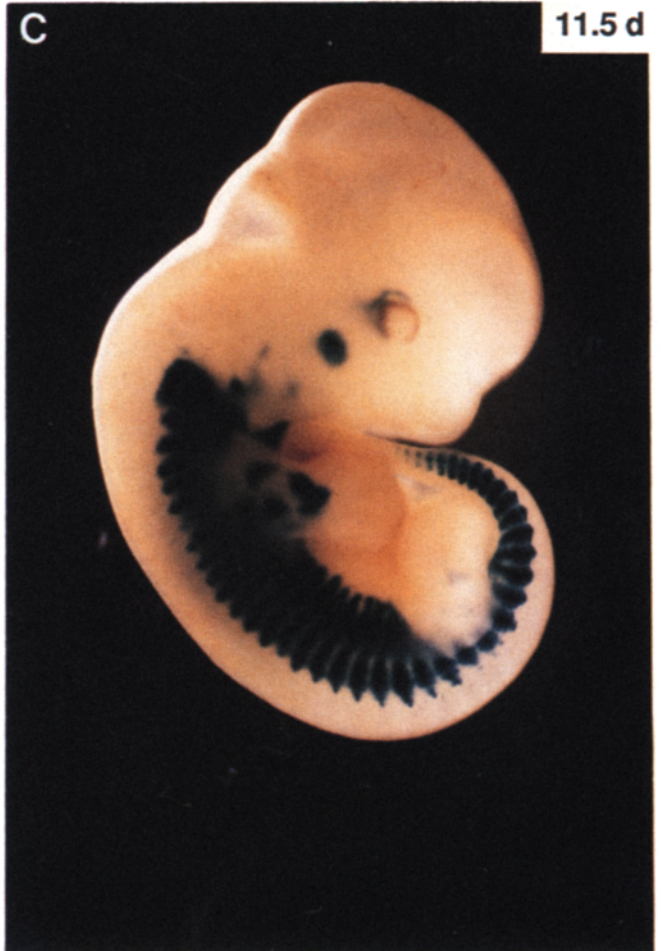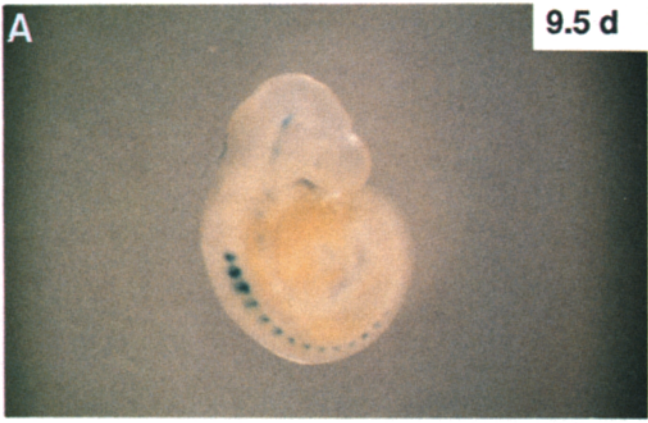
1992). In cultured muscle cells, this E-box, referred to as E-1, has less than a twofold effect on the level of muscle-specific transcription and does not influence the site of transcription initiation (Edmondson et al., 1992). To determine whether E-1 contributed to myogenin transcription in vivo, we generated lines of mice using a derivative of pMyo1565-CAT, in which E-1 was deleted (Fig. 1 b). Like the parental transgene, this construction, designated pMyo1565(-E1)CAT, directed high-level expression of CAT in skeletal muscle (Fig. 1 b). There was no apparent difference in the overall level of CAT expression in hind limb muscles from mice harboring Myo1565CAT and Myo1565(-E1)CAT, suggesting that E-1 is nonessential for myogenin transcription in adult skeletal muscle.

We also examined the expression of a CAT transgene linked to the 335 bp of DNA preceding the myogenin tran-

scription initiation site with and without E-1 (Fig. 1 a). As with the larger 5' flanking region, CAT expression directed by the 335-bp promoter was strictly muscle-specific and was not dependent on E-box E-1 (Fig. 1 c). The level of CAT expression in these transgenic lines, however, was significantly less than that of the lines derived with Myo1565CAT. These results suggest that elements required for muscle-specific expression are contained within the 335-bp promoter, but that elements required for maximal expression may lie between −335 and −1565.

A lacZ Reporter Gene Linked to the Myogenin 5'
Flanking Region Recapitulates the Temporal, Spatial,
and Tissue-specific Pattern of Expression of the
Endogenous Myogenin Gene During Embryogenesis

To analyze the temporal and spatial pattern of myogenin tran-

scription at the single cell level, we generated stable lines of transgenic mice harboring a *lacZ* reporter linked to the region from +18 to −1565 bp of the *myogenin* 5′ flanking region (Myo1565lacZ) (Fig. 1 *a*). Whole-mount staining for lacZ activity revealed high levels of expression of this transgene by 9.5 dpc in the rostral-most somites (Fig. 2 *a*), which represent the initial site of myogenin mRNA accumulation detected by in situ hybridization (Wright et al., 1989; Sassoon et al., 1989). By day 10.5, strong expression of the transgene was detected within more than 30 somites (Fig. 2 *b*). This rostral-to-caudal progression of transgene expression parallels the pattern of somite maturation and agrees with the time course for expression of the endogenous gene.

Transverse sections through the rostral somites of 10.5-d embryos showed high levels of transgene expression in the central region of the somite, corresponding to the myotome (Fig. 3, *a* and *b*) and allowed mapping of individual cells that activated *myogenin* transcription. The boundary of transgene expression in the somite was well defined and did not extend beyond the myotomal region. The compartmentalization of transgene expression was also evident in sections through the more caudal somites, in which the dermatome, myotome and sclerotome were clearly delineated (Fig. 3 *c*).

LacZ staining became detectable within mononucleate myogenic cells in the limb buds as well as in the visceral arches at day 11.5 (Fig. 2 *c*), at about the same time as the endogenous gene becomes activated in these structures (Sassoon et al., 1989). Expression of lacZ was observed in the forelimbs about a half day before the hindlimbs; this is consistent with the derivation of limb muscles from the somites, which show a rostral-to-caudal pattern of maturation. High-level expression of lacZ was subsequently observed in newly formed muscle fibers in the trunk, limbs, and face and served as a marker for the sequential formation of skeletal muscles throughout the developing embryo (Fig. 2). We detected little or no ectopic expression of lacZ in nonmuscle cell types at any stage of development in mice harboring Myo1565-lacZ, with the exception of one line which showed low level expression of lacZ in specific regions of the brain (Fig. 2, *a* and *b*). Identical expression in the somites, limb buds and skeletal muscle fibers was observed in transgenic mice that were sacrificed in the F0 generation.

Migrating Myogenic Progenitors Do Not Express Myogenin

It is well established that skeletal muscle in the limbs arises from myogenic progenitor cells that migrate out from the ventrolateral aspect of the somite myotome (Christ et al., 1978). Although these migrating cells are committed to a myogenic fate, they do not express transcripts for the myogenic HLH proteins at detectable levels (Sassoon et al., 1989), which suggests that they are derived from a unique population of somitic cells or that these cells extinguish transcription of these genes during migration. To distinguish between these possibilities, we analyzed transgenic embryos

harboring Myo1565lacZ between days 9.5 and 11.5 of development for the presence of lacZ-positive cells in the zone of migration between the somite myotome and limb bud. LacZ-positive cells were easily detectable in the somite myotomes at day 10.5 (Figs. 2 *b* and 4, *a* and *b*), but there was no lacZ staining in the limb buds at that time. By day 11.5, regions of lacZ-positive cells were observed throughout the limb bud (Figs. 2 *c* and 4 *c*). LacZ-positive cells were never observed within the zone between the somites and the limb bud where myogenic precursors are known to migrate before day 11.5. Because the lacZ protein has a relatively long half-life and was expressed at high levels in cells within the somite myotome by day 9.5, the enzyme should have marked these myogenic precursors if the *myogenin* gene was indeed transcribed in these cells before their migration. Indeed, lacZ-positive cells appear to migrate from the rostral somites to the visceral arches at 10.5–11.5 dpc. The failure to detect lacZ staining in cells that migrate into the limb bud suggests that these committed myogenic cells are derived from a population of cells in the somite myotome that never expressed myogenin.

Myogenin-lacZ Transgenes Become Activated in Limb Bud Explants

To determine whether endogenous regulators of *myogenin* transcription could activate *myogenin-lacZ* transgenes in the limb bud in the absence of other parts of the embryo, we isolated forelimb buds from 10.5 d transgenic embryos harboring Myo1565lacZ and placed them in culture. There was no detectable lacZ expression within the limb buds at the time they were isolated. However, after 4 d in culture, expression of lacZ was readily detectable in the distal region of the limb bud (Fig. 5). These results confirm that myogenic precursors that are destined to form muscle are present in the limb by day 10.5, but these cells do not activate *myogenin* transcription until at least 1 d later.

Discussion

Myogenin transcription is activated when skeletal muscle cells are induced to differentiate by exposure to growth factor-deficient medium, preceding by several hours the expression of genes associated with terminal differentiation (Wright et al., 1989; Edmondson and Olson, 1989). To begin to define the mechanisms regulating *myogenin* transcription during embryogenesis, we have analyzed the expression of reporter genes linked to the *myogenin* control region in transgenic mice. Our results show that the *cis*-acting sequences required for appropriate temporal, spatial, and tissue-specific transcription of *myogenin* lie within 1,565 bp 5′ of the gene. When linked to reporter genes such as *lacZ*, these sequences allow mapping at the single cell level of cells in the developing embryo that activate *myogenin* transcription, and they suggest the existence of distinct types of myogenic precursors in the somite myotome and limb buds.

Figure 2. Developmental regulation of myogenin-lacZ transgenes. Transgenic embryos harboring Myo1565lacZ were stained for lacZ activity. (*A*) 9.5 dpc; (*B*) 10.5 dpc; (*C*) 11.5 dpc; (*D*) 12.5 dpc; (*E*) 13.5 dpc. Embryos in *D* and *E* harbor Myo1565(-E1)lacZ, which shows the same pattern of expression as the transgene with E1 at these developmental stages. Transverse sections through the embryos in *B* and *C* are shown in Figs. 3 and 4.
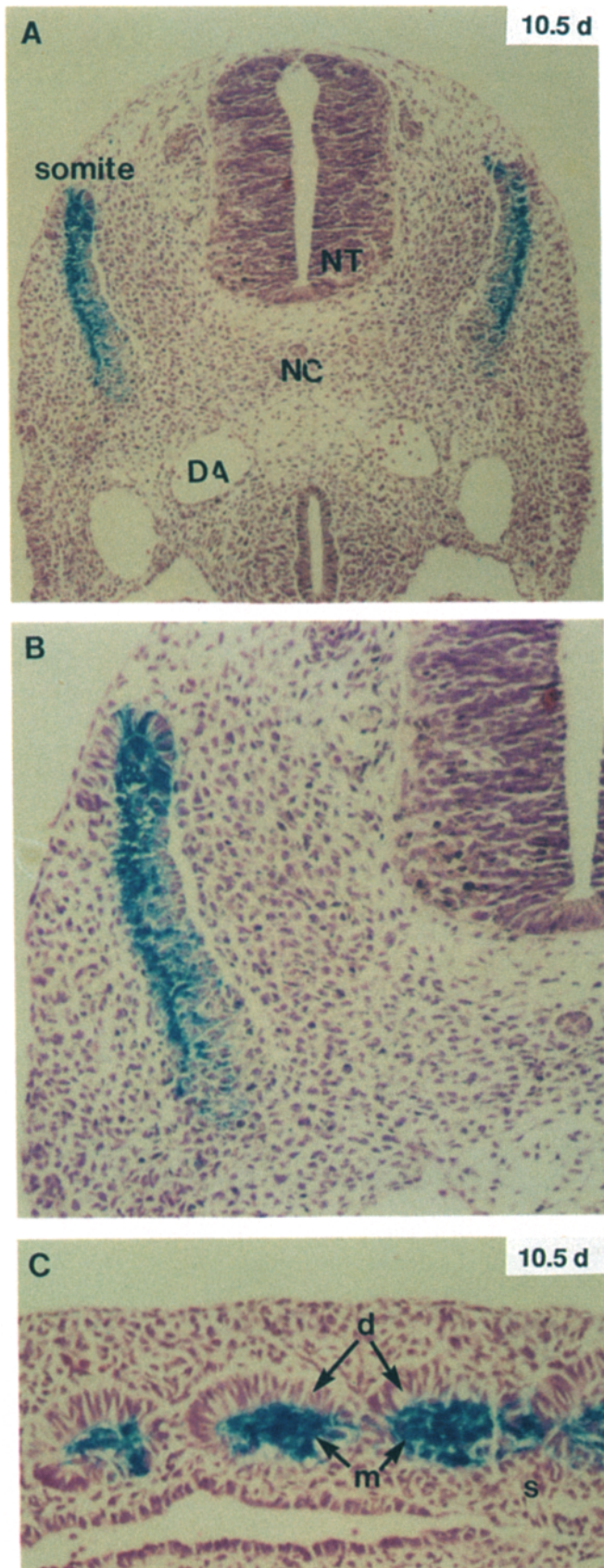
Migrating Myogenic Precursors Do Not Activate Myogenin Transcription Until They Reach the Limb Bud

Myogenin expression is first detected in the somite myotome at 8.5 dpc, a half day later than myf5 and 2 d earlier than MyoD (Wright et al., 1989; Sassoon et al., 1989; Bober et al., 1991; Ott et al., 1991). Skeletal muscle in the limbs is derived from the somites. However, transcripts encoding myogenic HLH proteins are not detected in the developing limb bud until day 11, despite the fact that cells within this region are committed to a myogenic fate before that time. At least two explanations could account for the lack of expression of myogenic regulatory factors in committed myogenic cells within the limb bud. These cells could be derived from a population of cells within the somite myotome that fails to express myogenic HLH proteins prior to migration to the limb bud and are committed to a myogenic fate by regulatory proteins that are as yet unidentified. Alternatively, myogenic cells could extinguish expression of the myogenic regulators during migration, but previous expression of these factors may have committed them to a myogenic fate. Because of the short half-lives of myogenin and MyoD mRNAs and proteins (Thayer et al., 1989; Edmondson et al., 1991), these gene products would be expected to disappear rapidly from migrating precursors if transcription of these genes was repressed when cells exit the somite myotome and enter the limb bud.

Using the *myogenin* control region linked to *lacZ*, we show that the lacZ protein, which exhibits a long half life in vivo, is expressed at high levels in the somite myotome before day 9.5, but is not detectable in the limb buds until day 11.5. The absence of lacZ expression in myogenic precursor cells that migrate into the limb bud well before day 11.5 indicates that *myogenin* transcription has not been previously activated in these cells and suggests that cells that contribute to skeletal muscle of the limbs are derived from a unique population of cells in the somite, distinct from that which activates *myogenin* transcription in the myotome.

We also analyzed *myogenin* transcription in limb bud explants from transgenic mice harboring a *myogenin-lacZ* transgene. There was no detectable *myogenin* transcription in limb buds until day 11.5, but if limb bud explants from 10.5 d embryos were incubated in vitro for several days, the *myogenin-lacZ* transgene became activated. These results confirm that cells destined to become muscle are present in the limb at least 1 d before the activation of *myogenin* transcription. These results also suggest that the mechanism that specifies myogenin expression is already in place in the limb by day 10.5 and requires no further contribution from other parts of the embryo.

If cells committed to a myogenic fate are present in the early limb bud, but these cells do not express members of the MyoD family, how might they be maintained in the lineage? We favor the notion that there may be additional regulators that participate in myogenic lineage commitment, which could be operating in these cells. Indeed, considerable

Figure 3. Myogenin-lacZ expression within the somite myotome. Transgenic embryos harboring Myo1565lacZ were analyzed for lacZ expression in sections through the somites. (*A*) Transverse section through the rostral somites of a 10.5 d transgenic embryo. (*B*) Higher magnification of *A*. (*C*) Transverse section through the caudal somites of a 10.5 d transgenic embryo. *NT*, neural tube; *DA*, dorsal aorta; NC, notochord; *m*, myotome; *d*, dermatome; *s*, sclerotome.
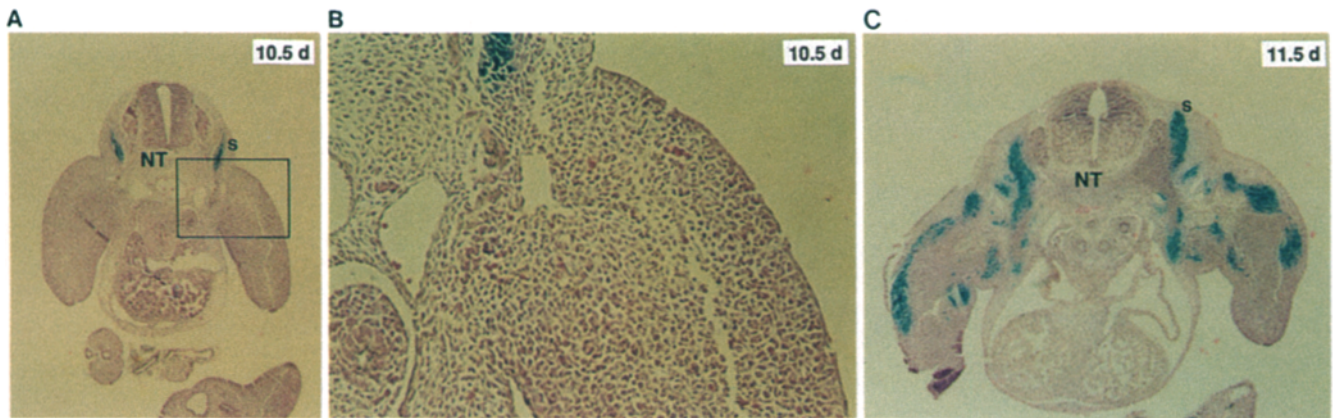
Figure 4. Expression of myogenin-lacZ transgene in the somites and limb buds of 10.5 and 11.5 d embryos. (*A*) Transverse section through the level of the forelimb bud of a 10.5 d transgenic embryo harboring Myo1565lacZ from Fig. 2 *B*. (*B*) High magnification of the region in the box (*A*). (*C*) Transverse section through the level of the forelimb bud of an 11.5 d transgenic embryo from Fig. 2 *C*. NT, neural tube.

evidence is accumulating to suggest the existence of regulatory factors that can control myogenic lineage commitment in the absence of myogenic HLH proteins. *Caenorhabditus elegans* mutants, deficient for *MyoD*, for example, are able to express skeletal muscle-specific genes (Chen et al., 1992). Similarly, expression of activated Ras and Fos or incorporation of the thymidine analog 5-bromo-2′deoxyuridine into myoblast DNA extinguishes expression of myogenic HLH proteins, but cells under these conditions remain committed

Figure 5. Expression of myogenin-lacZ transgenes in limb bud explants. Forelimb buds were dissected from 10.5 d transgenic embryos harboring Myo1565lacZ and were placed in culture in DME containing 10% FBS and 3% chick embryo extract. There was no detectable expression of lacZ in the limb buds at the time they were isolated. 4 d after incubation in culture, limb buds were stained for lacZ. High-level expression of lacZ is detected in the distal region of the limb bud. (*p, d, a*) Proximal, distal, and anterior regions of the limb, respectively.

to a myogenic fate (Lassar et al., 1989; Tapscott et al., 1989). Mutational analysis of the *myogenin* promoter in transgenic mice should permit the identification of *cis*-acting sequences, and eventually *trans*-acting factors, responsible for initial activation of *myogenin* transcription in the limb bud. Such regulatory factors would be likely candidates for regulators of myogenic lineage commitment.

Analysis of the *myogenin* promoter in muscle cells in culture has revealed that muscle-specificity and positive autoregulation are dependent on a binding site for the muscle-specific enhancer factor MEF-2 in the *myogenin* promoter (Edmondson et al., 1992). The temporal and spatial regulation of MEF-2 expression during embryogenesis has not yet been defined, but it is possible that MEF-2 may contribute to *myogenin* transcription in the somite myotome and limb bud.

Myogenin-lacZ Transgenes Reveal Multiple Myogenic Cell Types in the Somite Myotome and Limb Buds

The existence of two distinct populations of myogenic progenitors that give rise to muscles of the trunk and limbs is consistent with recent studies of Ordahl and LeDouarin (1991). By somite grafting experiments in chick-quail chimeras, they showed that cells in the lateral portion of the somite contribute to muscles of the limbs whereas cells in the medial region of the somite contribute to the axial muscles of the trunk. The fate of cells in these two regions are plastic and can be altered when transplanted to the opposite region. Our results indicate that transcription of *myogenin* in the myotome may be an initial marker of the axial muscle precursors, but not the limb muscle precursors.

The control region for *MyoD* was also analyzed recently in transgenic mice (Goldhammer et al., 1992). In contrast to *myogenin*, in which the regulatory region lies within the proximal promoter, the *MyoD* control region is located ∼15 kb upstream of the gene (Goldhammer et al., 1992). Expression of *MyoD-lacZ* transgenes has been reported for a single time point during development, and the regulatory elements have been mapped only to a 4-kb region, so it remains to be determined whether this region is necessary and sufficient for the full pattern of *MyoD* transcription throughout embryogenesis.
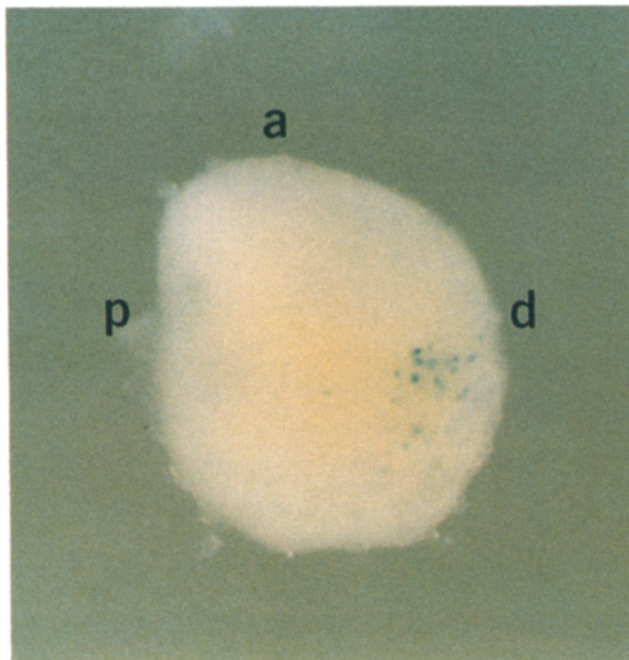
The muscle-specificity of and autoregulatory interactions among members of the MyoD family of myogenic regulatory genes suggest that commonalities may exist in the mechanisms regulating these genes. However, each member of this regulatory gene family also exhibits distinct aspects of regulation which are likely to arise from unique regulatory mechanisms. Further analysis of the control regions that govern expression in somites and limb buds of these different genes should permit the details of the myogenic regulatory circuit to be defined.

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