The Expression of Myosin Genes in Developing Skeletal Muscle in the Mouse Embryo

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Abstract. Using in situ hybridization, we have investigated the temporal sequence of myosin gene expression in the developing skeletal muscle masses of mouse embryos. The probes used were isoform-specific, 35S-labeled antisense cRNAs to the known sarcomeric myosin heavy chain and myosin alkali light chain gene transcripts. Results showed that both cardiac and skeletal myosin heavy chain and myosin light chain mRNAs were first detected between 9 and 10 d post coitum (p.c.) in the myotomes of the most rostral somites. Myosin transcripts appeared in more caudal somites at later stages in a developmental gradient. The earliest myosin heavy chain transcripts detected code for the embryonic skeletal (MHCemb) and β-cardiac (MHCβ) isoforms. Perinatal myosin heavy chain (MHCpn) transcripts begin to accumulate at 10.5 d p.c., which is much earlier than previously reported. At this stage, MHCemb is the major MHC transcript. By 12.5 d p.c., MHCpn and MHCemb mRNAs are present to an equal extent, and by 15.5 d p.c. the MHCpn transcript is the major MHC mRNA detected. Cardiac MHCβ transcripts are always present as a minor component. In contrast, the cardiac MLC1A mRNA is initially more abundant than that encoding the skeletal MLC1F isoform. By 12.5 d p.c. the two MLC mRNAs are present at similar levels, and by 15.5 d p.c., MLC1F is the predominant MLC transcript detected. Transcripts for the ventricular/slow (MLC1V) and another fast skeletal myosin light chain (MLC3F) are not detected in skeletal muscle before 15 d p.c., which marks the beginning of the fetal stage of muscle development. This is the first stage at which we can detect differences in expression of myosin genes between developing muscle fibers. We conclude that, during the development of the myotome and body wall muscles, different myosin genes follow independent patterns of activation and accumulation. The data presented are the first detailed study of myosin gene expression at these early stages of skeletal muscle development.

THERE are seven known myosin heavy chain (MHC)[1] isoforms expressed in rodent striated muscle. Each is the product of a different gene in the MHC multigene family (Nguyen et al., 1982; Weydert et al., 1985). The isoforms encoded by these genes are expressed in cardiac muscle (MHCα and MHCβ), adult fast skeletal muscle fibers (MHCIIa and MHCIIb), adult slow muscle fibers (MHCβ), extraocular muscle fibers (MHCeo), and in developing skeletal muscle (MHCemb, MHCpn, and MHCβ) (reviewed in Weydert, 1988). The sequential expression of MHC genes from fetal (16 d post coitum [p.c.] in the rat [Narusawa et al., 1987] and 15 d p.c. in the mouse [Weydert et al., 1987]) to adult stages has been analyzed both at the protein level (Whalen et al., 1981; Rubinstein and Kelly, 1981; Lyons et al., 1983) and at the mRNA level (Nadal-Ginard et al., 1982; Weydert et al., 1983). In fetal muscles, MHCemb, MHCpn, and a low level of MHCβ have been detected. In the mouse, MHCpn transcripts become predominant in late fetal muscles and are replaced by adult isoforms after birth (Weydert et al., 1987).

Four myosin alkali light chain (MLC) genes are expressed in rodent skeletal muscle: MLC1F and MLC3F, encoded by the same gene and expressed in adult fast skeletal muscle, and MLC1V and MLC1A, cardiac isoforms which are also expressed in adult slow and fetal muscle, respectively (reviewed in Barton and Buckingham, 1985). At the earliest stage examined, 15 d p.c. in the mouse, MLC1F and MLC1A transcripts have been reported, while MLC3F mRNAs only begin to accumulate in late fetal muscle (Barton, et al., 1989).

Little is known about the expression of myosin genes at earlier stages of skeletal muscle development. During embryogenesis, all of the skeletal musculature in the body is formed from cells that originate in the somites. Somites con-

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1. *Abbreviations used in this paper*: MHC, myosin heavy chain; MLC, myosin light chain; UTR, untranslated region.

sist of three layers of cells: the dermatome, the myotome, and the sclerotome. The myotomes, which are the first skeletal muscles to form in the embryo, are initially mononucleated (Holtzer et al., 1957). They fuse to form multinucleated myotubes and give rise to the vertebral and intercostal muscles. All other muscles of the body and limbs are derived from premyogenic cells that migrate out from the ventrolateral edges of the dermamyotomes (Milaire, 1976; Jacob et al., 1979). Several laboratories have reported the presence of MHC proteins in the myotomes of somites based upon antibody studies. Jockusch et al. (1984) and Fürst et al. (1989) detected MHC in 9–10-d mouse somites using nonspecific myosin antibodies. Vivarelli et al. (1988), using specific polyclonal and monoclonal antibodies, determined that cells in 10-d p.c. mouse somites and in 13-d p.c. limb buds stained positively for slow/ventricular and embryonic (fast) MHCs. Similar results on MLC expression in early myogenic cells are not available.

To gain a more detailed understanding of myosin gene expression during the earliest stages of skeletal muscle formation in the mouse, we have used specific 35S-labeled cRNA probes to the 5′ and 3′ untranslated regions (UTRs) of cardiac and skeletal myosin gene transcripts for in situ hybridization. The results for muscle-specific actin gene expression between 7.5 and 14 d p.c. were reported by Sassoon et al. (1988). Here we present the results for MHC and MLC gene expression for the same developmental period. Our data show that the first MHC and MLC transcripts can be detected between 9 and 10 d p.c., and that the mRNA for each myosin isoform has a distinct pattern of accumulation during development. Our results also suggest that embryonic muscle masses share a common MHC and MLC phenotype. Differentiation into fast and slow fiber types was not detected before 15 d p.c.

Materials and Methods

Preparation and Prehybridization of Tissue Sections

The protocol that was used to fix and embed C3H and BALB/c mouse embryos and fetuses is described in detail in Sassoon et al. (1988). Ages and numbers of embryos examined are listed in Table I. Briefly, embryos were fixed in 4% paraformaldehyde in PBS dehydrated and infiltrated with paraffin. 5–7-μm thick serial sections were mounted on subbed slides (Gall and Pardue, 1971). One to three sections were mounted/slide, deparaffinized in xylene and rehydrated. The sections were digested with proteinase K, postfixed, treated with triethanolamine/acetic anhydride, washed, and dehydrated.

Probe Preparation

To distinguish between transcripts within the myosin multigene family, it is necessary to use probes derived from the 3′ or 5′ noncoding UTR of the mRNAs. Appropriate restriction fragments or oligonucleotides were subcloned into the vector, Bluescribe+ (Stratagene Cloning Systems, La Jolla, CA). Bluescribe+ was grown in *E. coli* TG1. The following probes were used. (*a*) 3′ UTR of mouse MLC1A (MLC1 embryonic) mRNA (Barton et al., 1988) 5′TTAATGAAACTCCAAGCTGGGGCTCTTTATTTCCAGGGAAGGTTGTGGGTCAGAGAAGCCATGTGAGTCCAATACTCCGTAACAGTAACAGCCGCTGTGGATCTCTTGCTTTCTCACGCAGGGCCAAGC3′. These sequence data are available from EMBL/GenBank/DDJB under accession numbers M20773, M19435, and J03932. (*b*) 5′ UTR of mouse MLC1F mRNA (Robert et al., 1984) 5′TCTCCTCCAGAAGAACCTGTCAGAGTGACACTTGGAAGAGCAGTGTGACTCGCTTGACCC3′. These sequence data are available from EMBL/GenBank/DDJB under accession number K02237. (*c*) 5′ UTR of mouse MLC3F mRNA (Robert et al., 1984) 5′CATGATGGAGTTCTAGGCTGCGAAACAGCAGTGGAGCTGGAGGATAAACTGAAGGCAGCTC3′. These sequence data are available from EMBL/GenBank/DDJB under accession number K02238. (*d*) 3′ UTR of the mouse MLC1V mRNA 5′GGAGTCCGAACCACTCCTTCCCGAAGCTCCAGACATGGTGTCAGCATCATGGTTGGGAGATGCTTGGCCTGCCCTGGGCTTCCTGA3′. (*e*) 5′ UTR of rat MHCembryonic mRNA (Strehler et al., 1986) 5′AGTGTTGGCTGAGTCACACCGGCAGGACAGCAGAGAGCAGCAGCAGCAGCTGTGGACCTATGGGACCTCT3′. These sequence data are available from EMBL/GenBank/DDJB under accession number X53489. (*f*) 3′ UTR of rat MHCslow/ventricular (MHCβ) mRNA (Mahdavi et al., 1982) 5′GGTCTCAGGGCTTCACAGGCATTCCTTAGGGTTGGGTAGCACATGATCTACTCTTCATTCAGGCCCTTG3′. These sequence data are available from EMBL/GenBank/DDJB under accession number K01463. (*g*) 3′ UTR of mouse MHCperinatal mRNA (Weydert et al., 1985) 5′TGATTTACCAATGCCTTGTAGTTTTTATTTAGTCAGCAAGTAGGAGAAAAGTAAACCCAGAGAGGCAAGAAGTGACCCAGCAAGCGACCCAAAGCAGCCCCTCCTGTGCATTTCCTTACAGCCCCTTGGG3′. These sequence data are available from EMBL/GenBank/DDJB under accession number M12289. (*h*) 3′ coding region of the rat MHCβ mRNA (Mahdavi et al., 1982) 5′TAGGTTCTTCTTGTCTTCCTCTGTCTGGTAGGTGAGCTCCTTGATGCGCCGCTCGCTCTTCCTCATGCCCTTCACCGACTCCGCATTGCGCTTCTGCTCAGCCTCCAGCTCATTCTCCAGCTC3′. These sequence data are available from EMBL/GenBank/DDJB under accession number J00751. (*i*) 5′ UTR of mouse cardiac α-actin mRNA (Sassoon et al., 1988) 5′CGTGCTAGGGGCGGCTGGATTCAGCTGGGCTGGCGCTGGTGGCAGGCACT3′. These sequence data are available from EMBL/GenBank/ DDJB under accession number X03767. (*j*) Myogenin 3′ coding and noncoding region (Wright et al., 1989). This cRNA corresponds to the terminal 700 nucleotides of the rat myogenin cDNA. The cRNA transcripts were synthesized according to manufacturer's conditions (Stratagene Cloning Systems) and labeled with 35S-UTP (>1,000 Ci/mmol; Amersham International, Amersham, UK). cRNA transcripts larger than 100 nucleotides were subjected to alkali hydrolysis to give a mean size of 70 bases for efficient hybridization.

Hybridization and Washing Procedures

The hybridization and posthybridization procedures were as described by Wilkinson et al. (1987). Sections were hybridized overnight at 52°C in 50% deionized formamide, 0.3 M NaCl, 20 mM Tris-HCl, pH 7.4, 5 mM EDTA, 10 mM $NaPO_4$, 10% dextran sulfate, 1× Denhardt's, 50 μg/ml total yeast RNA, and 50-75,000 cpm/μl 35S-labeled cRNA probe. The tissue was subjected to stringent washing at 56°C in 50% formamide, 2×SSC, 10 mM DTT and washed in PBS before treatment with 20 μg/ml RNAse A at 37°C for 30 min. After washes in 2× SSC and 0.1× SSC for 15 min at 37°C, the slides were dehydrated and dipped in Kodak NTB-2 nuclear track emulsion and exposed for 1 wk in light tight boxes with desiccant at 4°C. Photographic development was carried out in Kodak D-19. Slides were analyzed using both light- and dark field optics of a Zeiss Axiophot microscope.

Results

In the mouse, the first four somites form at 8 d p.c. (Rugh, 1968) in the cervical region of the embryo. Somite formation and maturation proceeds in a rostrocaudal gradient. Fig. 1 shows parallel parasagittal sections through the somites of an 8.5-d p.c. embryo. Using a coding region probe that hybridizes to all muscle-specific MHC gene transcripts, MHC mRNAs are not detected in developing myotomes at 8.5 d p.c. (Fig. 1 *C*). Similarly, no MLC transcript, including MLC1A mRNA, is detected in myotomal cells at this stage (Fig. 1 *D*). In the developing heart, MHC and MLC1A transcripts are present at high levels (Fig. 1, *C* and *D*). In contrast, transcripts of the myogenic differentiation factor, myogenin (Wright et al., 1989), are clearly detectable in the myotomes by in situ hybridization, but myogenin is not expressed in the heart (Fig. 1 *B*). We have previously reported the presence of α-actin transcripts in somites at this stage (Sassoon et al., 1988).

21 additional somites form between 8.5 and 9.5 d p.c. (Rugh, 1968). Between these two time points, MHC and

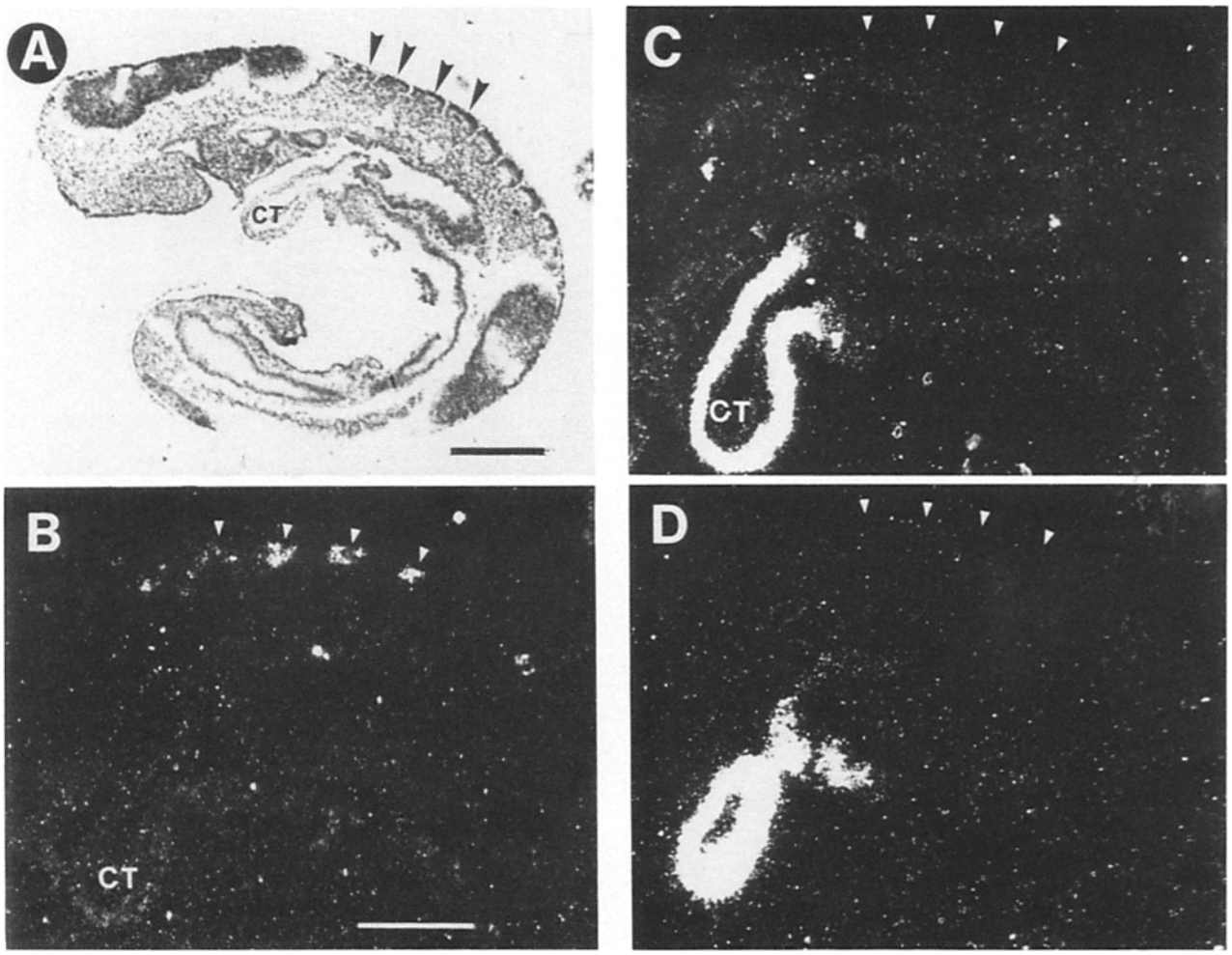

Figure 1. Myosin transcripts are not detectable in the first somites at 8.5 d p.c. (*A*) Phase-contrast micrograph of a parasagittal section of an 8.5-d p.c. mouse embryo showing several rostral somites (*arrowheads*) and the cardiac tube (*CT*). (*B*) Higher magnification dark field micrograph of the same section in *A*, which was hybridized to the myogenin probe. Strong hybridization signal is present over the myotomes of the somites, but there is no hybridization to the cardiac tube. (*C*) Dark field micrograph of a serial section to that in *B*, hybridized to a muscle-specific (slow/ventricular) MHC-coding region probe. (*D*) Parallel section to *B* and *C* hybridized with the MLC1A probe. There are high levels of MHC and MLC1A mRNAs in the myocardium of the cardiac tube, but myosin transcripts are not detectable in the myotomes at this early stage. Bars: (*A*) 300 μm; (*B–D*) 200 μm.

MLC transcripts can first be detected (Fig. 2). Cardiac and skeletal actin (Sassoon et al., 1988) and myosin isoforms are coexpressed at this early stage of skeletal muscle development in the mouse embryo. The first MHC mRNAs we detect at 9.5 d code for the ventricular/slow isoform, MHCβ (Fig. 2 *E*), which is coexpressed with those encoding the embryonic skeletal isoform, MHCemb (Fig. 2 *B*). Transcripts for the atrial MLC, MLC1A (Fig. 2 *C*) are the predominant MLC transcripts at this stage, but those for the fast skeletal isoform, MLC1F, are also detectable (Fig. 2 *F*).

At 10.5 d p.c., we begin to detect mRNA for the perinatal skeletal isoform, MHCpn, in the developing myotomes (Fig. 3 *C*) in addition to transcripts for MHCβ (Fig. 3 *B*) and MHCemb, which is the predominant MHC transcript in myotomes between 9.5 and 12.5 d p.c. (see Fig. 5). MLC1A (Fig. 3 *D*) is the major MLC transcript at 10.5 d p.c., and MLC1F mRNAs are also present (Fig. 3 *E*), but MLC1V (Fig. 3 *F*) transcripts are not detectable in myotomes at this stage. MLC1V is not expressed in developing skeletal muscle until 15.5 d p.c. (see Fig. 7 *F*).

The spatial distribution of muscle gene transcripts within the developing myotome of single somites is revealed in transverse sections of mouse embryos. Fig. 4 shows four consecutive 5-μm sections of a somite from a 10.5-d p.c. mouse embryo. The first (*A* and *B*) and last (*G* and *H*) sections were hybridized to the myogenin probe. The second in the series (*C* and *D*) was hybridized to the cardiac α-actin probe, and the third (*E* and *F*) with the MLC1A probe. α-Actin transcripts are detected in all areas of the myotome where myogenin mRNAs are present at a much higher level. However, MLC1A transcripts (Fig. 4, *E* and *F*) and other myosin mRNAs first appear in the central portion of the myotome and are detected in the dorsal and medial edges of the myotome at later stages. There is no detectable signal for any of these sequences in dermatomal cells.

As the myotomal premuscle masses develop in the growing embryo, the same myosin mRNAs are detected, but the levels of some transcripts change, as indicated by the intensities of autoradiographic signal. At 11.5 d p.c. (Figs. 5 and 6), MHCpn transcripts are increasing (Figs. 5 *D* and 6 *E*), and

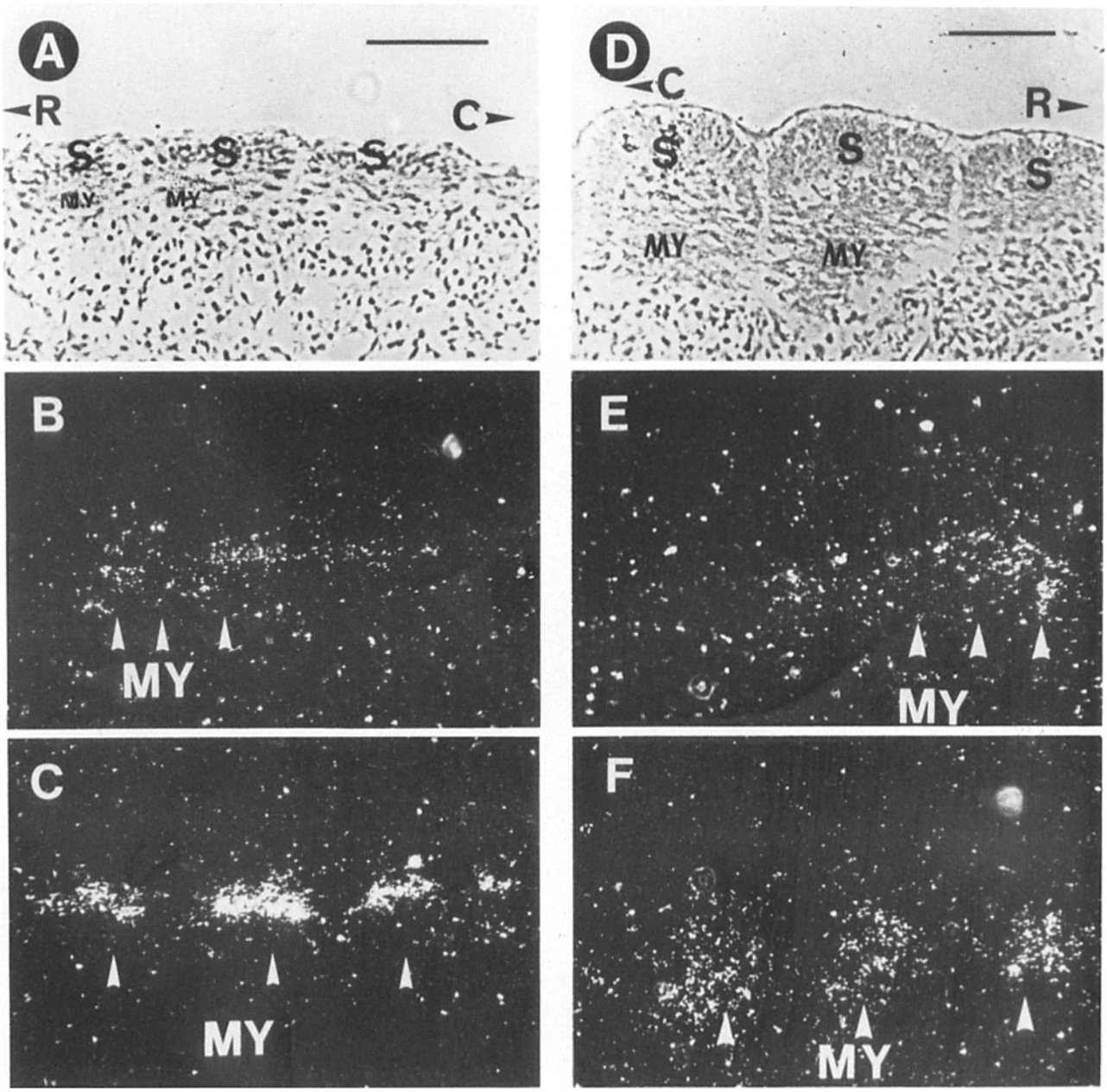

Figure 2. The first myosin gene transcripts are detected in somites by 9.5 d p.c. (*A* and *D*) Phase-contrast micrographs of rostral somites (*S*) in two different 9.5-d p.c. embryos. (*B*) Dark field micrograph of a parallel section to *A* and *C*, hybridized with the MHCemb probe. (*C*) Dark field micrograph of the same section as in *A*, hybridized with the MLC1A probe. (*E*) Parallel section to that in *D* hybridized to the MHCβ probe. (*F*) Same section as in *D* and parallel section to *E* hybridized to the MLC1F probe. MHCemb and MHCβ are expressed at low but detectable levels at 9.5 d. MLC1A is the predominant MLC mRNA at this stage of development. My, myotomes (arrows); *R*, rostral; *C*, caudal. Bars: (*A–C*)100 μm; (*D–F*)100 μm.

are present at a higher level than MHCβ transcripts (Fig. 6 *F*), but are still less abundant than those of MHCemb (Fig. 5 *C*). By 15.5 d p.c., MHCpn transcripts are the predominant MHC mRNAs expressed in skeletal muscle (Fig. 7 *C*). MLC1F mRNA levels increase in a similar fashion (Table I and Figs. 3 *E*, 6 *C*, and 7 *D*). At 15.5 d, MLC1F transcripts are present at a higher level than those of MLC1A in skeletal muscle fibers (Fig. 7, *D* and *E*). Transcripts encoding the second isoform typical of adult fast skeletal muscle, MLC3F, are not detectable before 15 d p.c.

Transcripts of the ventricular/slow MLC, MLC1V, begin to be detected at 15.5 d p.c. (Fig. 7, *F* and *F'*). This is the stage at which the mature pattern of muscle groups has formed (Kieny et al., 1986). Neuromuscular junctions are forming and secondary muscle fibers also begin to appear at this time (Ontell and Kozeka, 1984). Before 15.5 d p.c., skeletal muscle masses label uniformly with the different myosin probes. However, by 15.5 d p.c., this is no longer the case. MLC1V (Fig. 7 *F'*) transcripts are not uniformly distributed over the muscle mass. Grain distribution with the

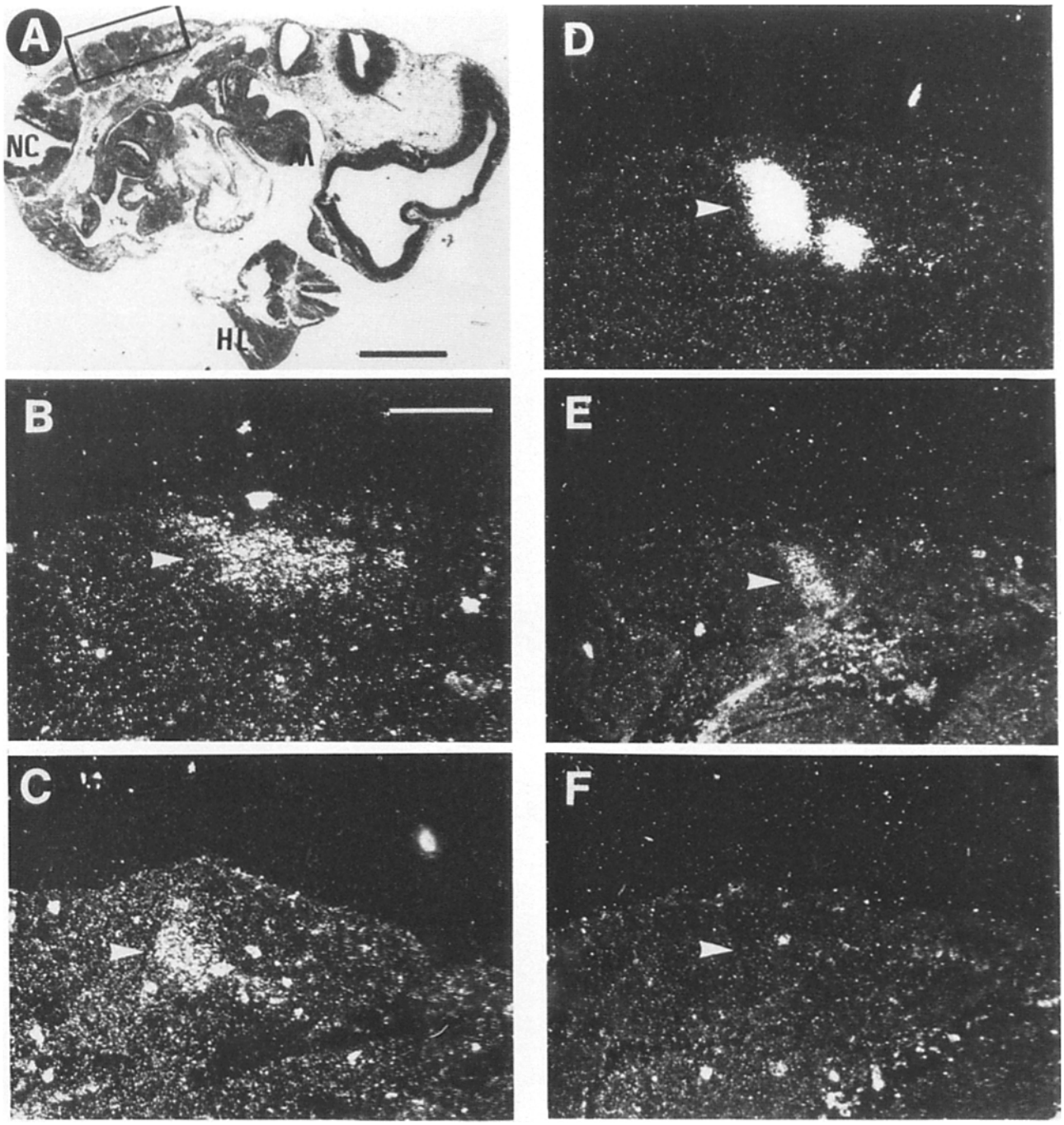

Figure 3. Cardiac and skeletal myosin genes are coexpressed in developing skeletal muscle. (*A*) Phase-contrast micrograph of a parasagittal section of a 10.5-d p.c. mouse embryo. Boxed area is magnified in (*B–F*). Dark field micrographs of somites in parallel sections which were hybridized with the (*B*) MHCβ probe; (*C*) MHCpn probe; (*D*) MLC1A probe; (*E*) MLC1F probe; and (*F*) MLC1V probe. Two cardiac myosin gene transcripts, MHCβ and MLC1A, are detected with the skeletal myosin gene transcripts, MHCpn and MLC1F, in somites (*arrowheads*) which are in this plane of section. In contrast, MLC1V, another cardiac myosin gene transcript, is not detected in myotomes at this stage (*F*). *NC*, neural canal; *HL*, hindlimb bud; *M*, mandible. Bars: (*A*) 620 μm; (*B–F*) 250 μm.

MLC1V probe differs from that for MHCpn (Fig. 7 *C'*). At this time, MHCβ transcripts begin to be restricted to the same regions where MLC1V is detected, whereas MLC1F mRNAs are more abundant in regions of muscles where MHCpn is expressed (data not shown). In general, the MLC1V and MHCβ probes hybridize most strongly to the central portions of muscle, whereas the MLC1F and MHCpn probes show strong hybridization signal over the peripheral portions of muscles.

At 16.5 d p.c., MLC1V transcripts are detected only in certain fibers of the body musculature. Fig. 8 *A* shows a transverse section of a gluteal muscle close to the pelvis. Parallel 5-μm sections were hybridized with the probes to MLC1A (Fig. 8 *B*), MLC1V (Fig. 8 *C*), and MHCβ (Fig. 8 *D*). The

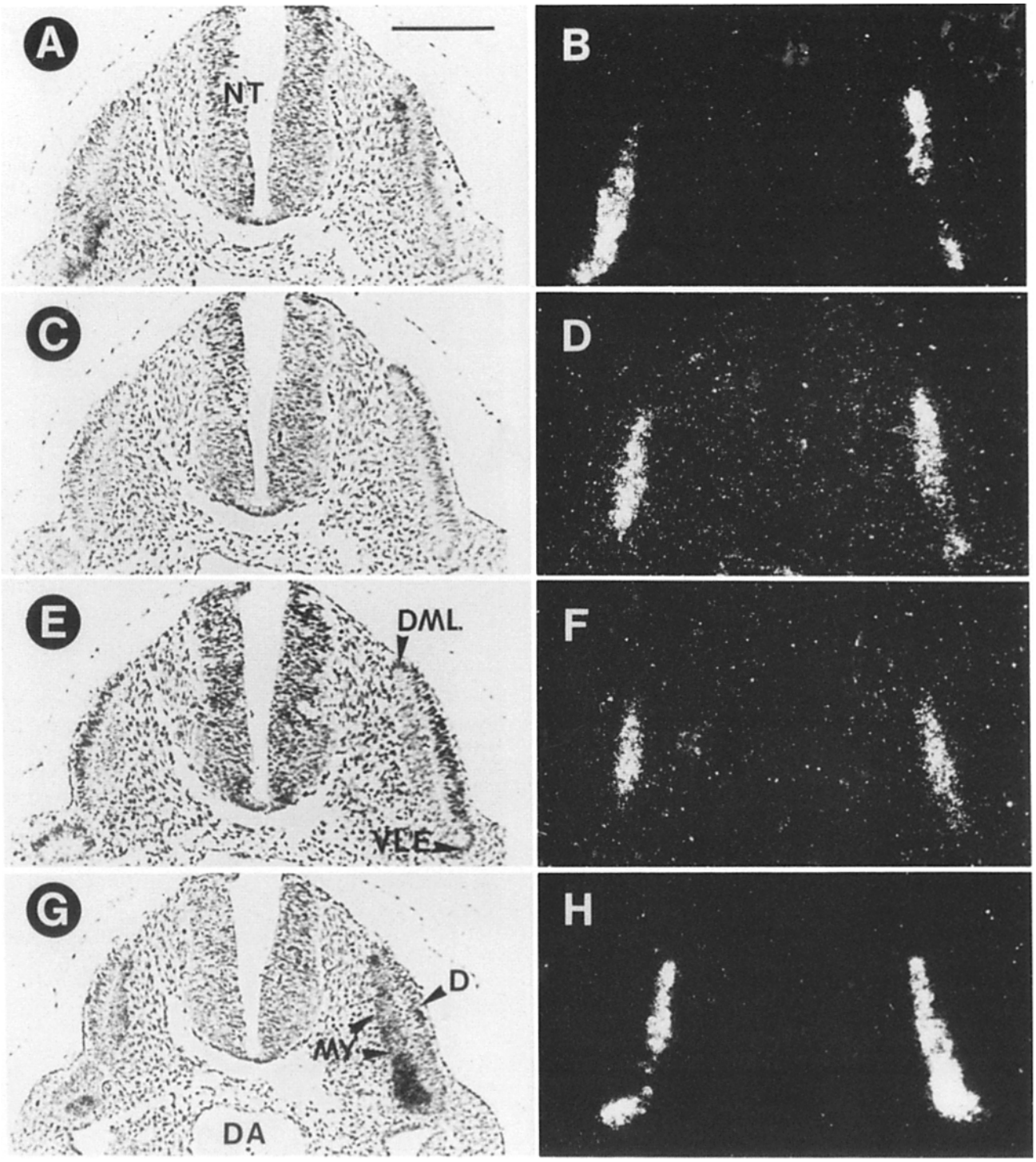

Figure 4. Muscle gene transcripts show similar patterns of distribution in developing myotomes. (*A*) Phase-contrast micrograph of a transverse section of a 10.5-d p.c. mouse embryo. (*B*) Dark field of the same section as in *A* hybridized with the myogenin probe. Consecutive sections were hybridized with (*C* and *D*) the cardiac α-actin probe; (*E* and *F*) the MLC1A probe; and (*G* and *H*) the myogenin probe. Comparison of *A–H* suggests that different muscle gene transcripts are all coexpressed in all myotomal cells, though at different levels, but these muscle mRNAs are not detectable in dermatomal (*D*) cells. Initially, myosin gene transcripts are expressed most abundantly in the middle region of myotomes (e.g., MLC1A; *E* and *F*). At a later stage, myosin mRNAs are expressed at high levels in the dorsal and medial edges of the myotomes. The sparse regions of silver grains in the middle portion of the right myotome in *B* and *D*, and the heavier region of grains at the ventral edge of the right myotome in *H* are the result of the angled plane of section through the myotome due to the curvature of the embryo. A second somite is in the lower left corner of *G* and *H*. *MY*, myotome; *DML*, dorsomedial lip; *VLE*, ventrolateral edge; *DA*, dorsal aorta; *NT*, neural tube. Bar, 150 μm.

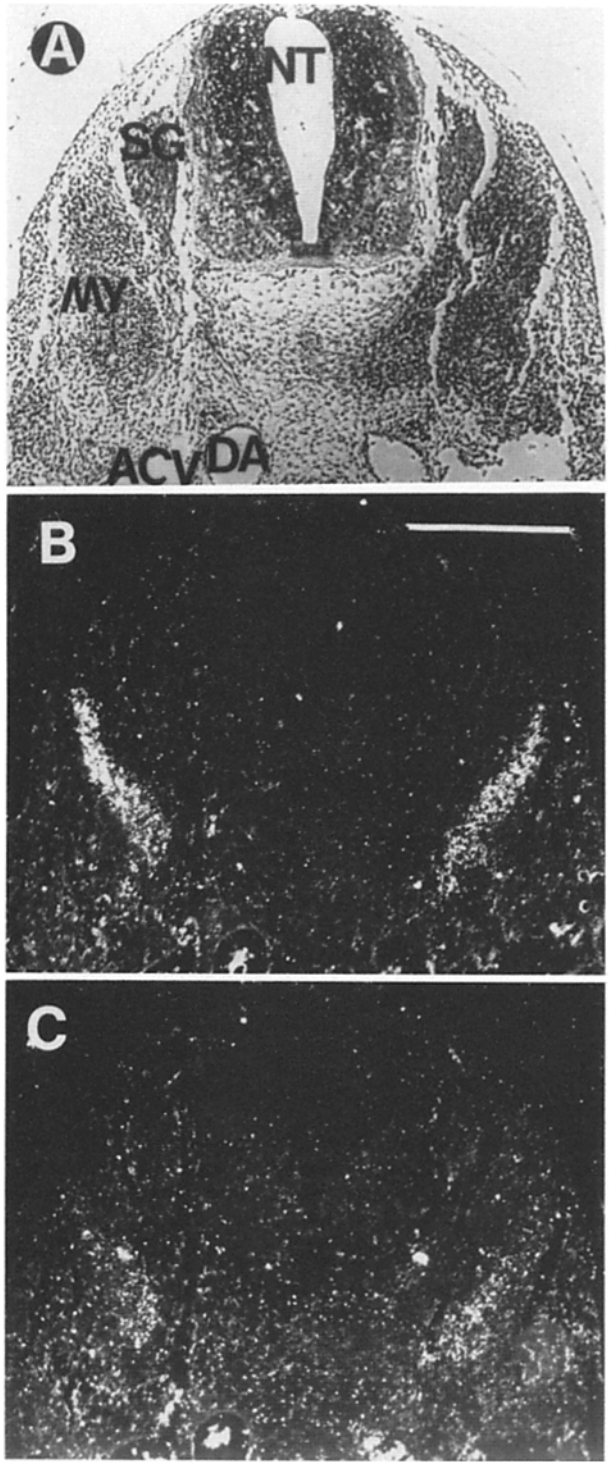

Figure 5. Transverse sections through myotomes of an 11.5-d p.c. mouse embryo show that MHCemb transcripts (*B*) are expressed at a higher level than MHCpn (*C*) at this early stage of skeletal muscle formation. (*A*) Phase-contrast micrograph of section serial to that in *B*. *NT*, neural tube; *SG*, spinal ganglion; *MY*, myotome; *ACV*, anterior cardinal vein; *DA*, dorsal aorta. Bar, 300 μm.

fibers which have high levels of MLC1V transcripts (Fig. 8 *C*) and MHCβ transcripts (Fig. 8 *D*), are probably primary muscle fibers which will form slow muscle fibers in the red portion of the gluteus medius muscle (Armstrong and Phelps, 1984) in the adult mouse. The fibers which do not express MLC1V and MHCβ mRNAs, but which do express MLC1A mRNAs (Fig. 8 *B*) will probably form the white portion of the gluteus medius muscle (Armstrong and Phelps, 1984).

Some myosin sequences are expressed both in cardiac and in skeletal muscle (Figs. 6, *B*, *D*, and *F* and 7, *E* and *F*). Developing skeletal muscles express a subset of myosin genes (MHCemb, MHCpn, and MLC1F) which are not detected in the heart (Figs. 6, *C* and *E* and 7, *A*, *C*, and *D*) at any of the stages we have examined. Cardiac myosin mRNAs are expressed at high levels in the cardiac tube at 8.5 d p.c. before any myosin mRNAs are detected in myotomes (Fig. 1, *C* and *D*), and it is evident that the pattern of expression of the common myosin sequences is regulated differently in cardiac versus skeletal muscle. Thus, in contrast to the pattern of expression in skeletal muscle, the two cardiac MLC genes, MLC1A and MLC1V, are coexpressed in embryonic cardiac muscle cells at all stages examined (Lyons, G., S. Schiaffino, D. Sassoon, and M. Buckingham, manuscript submitted for publication).

Table I summarizes the results for in situ hybridization with each of the myosin probes used and lists the number of embryos examined at each stage. We did not detect MLC3F transcripts until 15.5 d p.c., and we did not detect MHCIIa, MHCIIb, and MHCextraocular transcripts before birth (data not shown).

Discussion

Our results, taken together with the in situ hybridization results for α-actin transcripts (Sassoon et al., 1988) and for myogenin and MyoD1 transcripts (Sassoon et al., 1989), represent the first detailed analysis of muscle gene expression during skeletal muscle formation in the mouse embryo. It is important to place these results in the context of the morphological changes that occur during myotome formation.

Somitogenesis has not been as extensively studied in mouse embryos as in chick embryos. However, the sequence of developmental changes and their nature are the same in mice and birds despite large differences in cell number and cell size (Ede and El-Gadi, 1986). One model for myotome formation, suggests that the myotomal cells arise exclusively from the dorsomedial lip of the dermamyotome and migrate

Table I. Expression of Actin and Myosin Genes in Embryonic Mouse Skeletal Muscle

Days p.c.	8.5	9.5	10.5	11.5	12.5	14.5	15.5
α-cardiac actin	+	++	+++	+++	+++	+++	+++
α-skeletal actin	±	+	++	++	+++	+++	+++
MLC1A	−	+	+++	+++	+++	+++	+++
MLC1F	−	±	++	++	+++	+++	+++
MLC1V	−	−	−	−	−	−	+
MLC3F	−	−	−	−	−	−	+
MHCemb	−	±	++	+++	+++	+++	++
MHCpn	−	−	+	++	+++	+++	+++
MHCβ	−	±	+	+	+	+	+
No. embryos	3	6	7	7	4	3	6

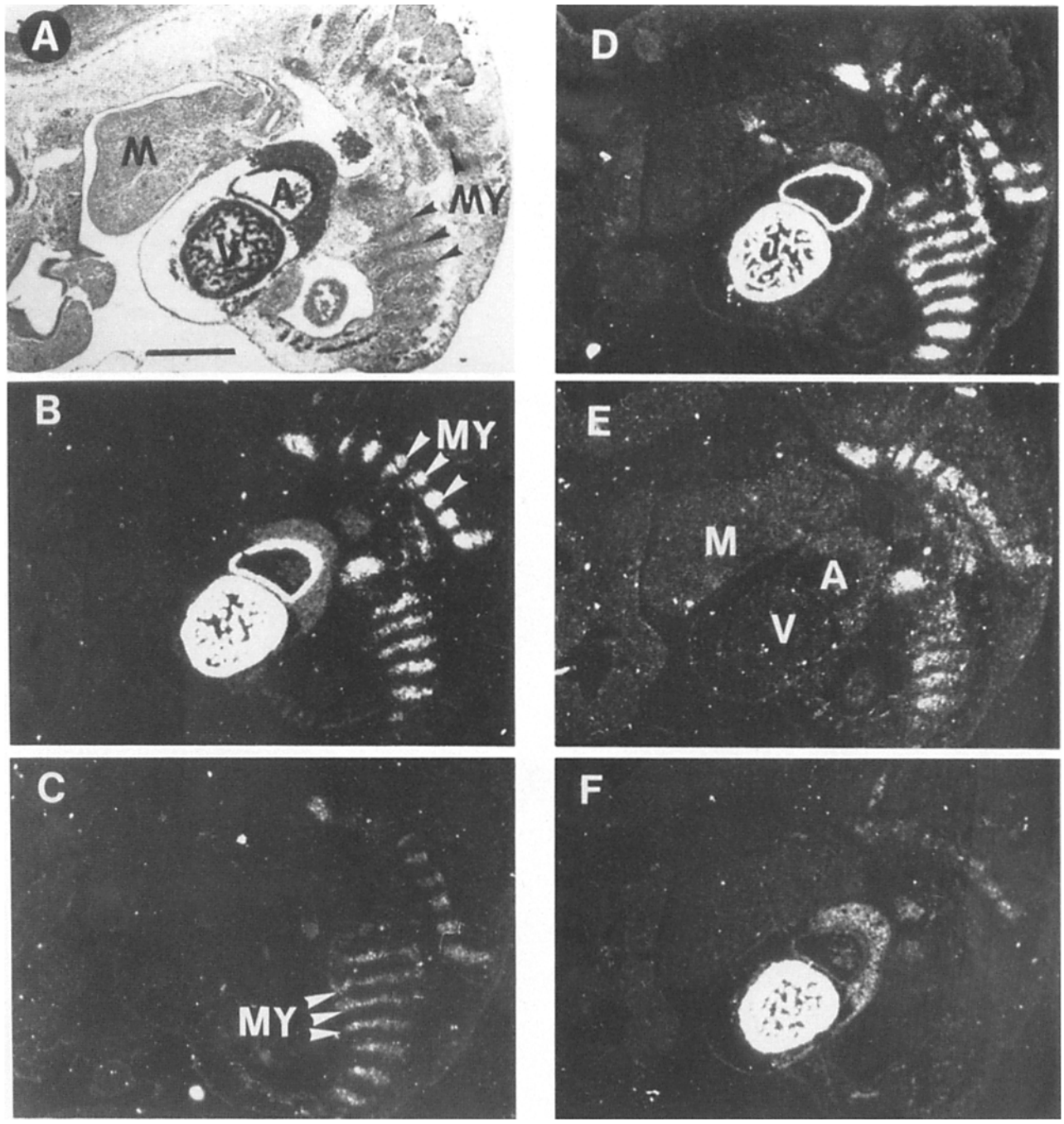

Figure 6. As the myotomes develop, the relative levels of myosin gene transcripts change in growing embryos. (*A*) Phase-contrast photomicrograph of an 11.5-d p.c. mouse embryo. (*B–F*) Dark field photomicrographs of parallel sections to *A*, which were hybridized with the (*B*) MLC1A probe; (*C*) MLC1F probe; (*D*) α-cardiac actin probe; (*E*) MHCpn probe; and (*F*) MHCβ probe. In comparison with 10.5 d (Fig. 3), the hybridization signals of the MHCpn and MLC1F probes over the maturing myotomes (*MY*), have increased, but the signals with the MHCβ and MLC1A probes have not changed. *M*, mandible; *V*, ventricle; *A*, atrium. Red blood cells around the atrium bind probe nonspecifically and appear refractile in dark field micrographs. Bar, 550 μm.

down the width of the dermatome (e.g., Ede and El-Gadi, 1986). An alternative model, based on studies of avian embryos, is that longitudinally oriented cells within the cranial edge of the dermamyotome give rise to the myotome. Beginning at the craniomedial corner of the dermamyotome, these cells elongate and migrate in a craniocaudal direction along the ventral surface of the dermatome until they extend along its length (Kaehn et al., 1988). As myogenic cells migrate in a craniocaudal direction from the cranial edge of the dermamyotome, they begin to accumulate myogenin and α-actin transcripts (Sassoon et al., 1989) and to synthesize muscle-specific proteins such as desmin (Kaehn et al., 1988) and titin (Fürst et al., 1989).

Myotomal cells are elongated, mononucleated cells that express MHC protein before fusion (Holtzer et al., 1957). Our results and those of Vivarelli and Cossu (1986), show

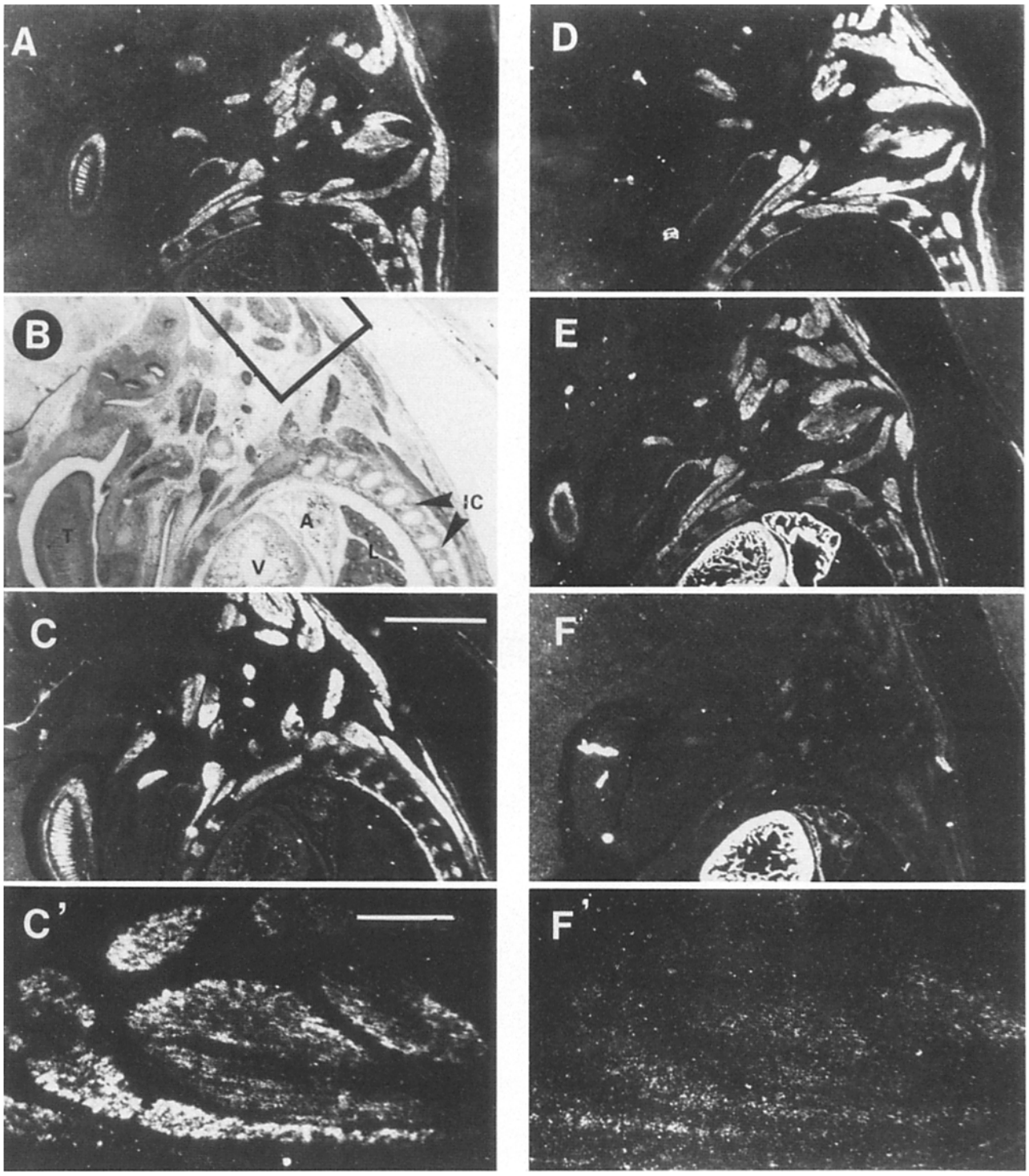

Figure 7. By 15.5 d p.c., the beginning of the fetal stage of muscle development, the mature pattern of muscle groups has formed. (*B*) Bright field micrograph of a parasagittal section of a 15.5-d mouse fetus. At this stage, MHCpn transcripts (*C*; same section as in *B*) are present at a higher level than those of MHCemb (*A*). MLC1F mRNAs (*D*) are now the predominant MLC transcripts compared with MLC1A (*E*) and MLC1V (*F*). This is the first stage at which MLC1V mRNAs are detectable in skeletal muscle. (*C'* and *F'*) Higher magnifications of neck and shoulder muscles corresponding to the area boxed in *B* (rotated ~120°). MLC1V transcripts (*F'*) are not uniformly distributed over the muscle masses; grain intensity differs from that for MHCpn (*C'*). (The apparent signal over the tongue in *F* is an artifact.) *A*, atrium; *V*, ventricle; *T*, tongue; *IC*, intercostal muscles; *L*, lung. Bars: (*A–F*) 1,000 μm; (*C'* and *F'*) 250 μm.

that MHCemb and MHCβ are the first MHCs expressed in the myotomes of mouse embryos. The recent paper of Sweeney et al. (1989) suggests that this is also true in the chick. We show that MLC1A and MLC1F, but not MLC1V, are the first MLC transcripts detected in developing myotomes. MLC3F transcripts are not detected in myotomes and

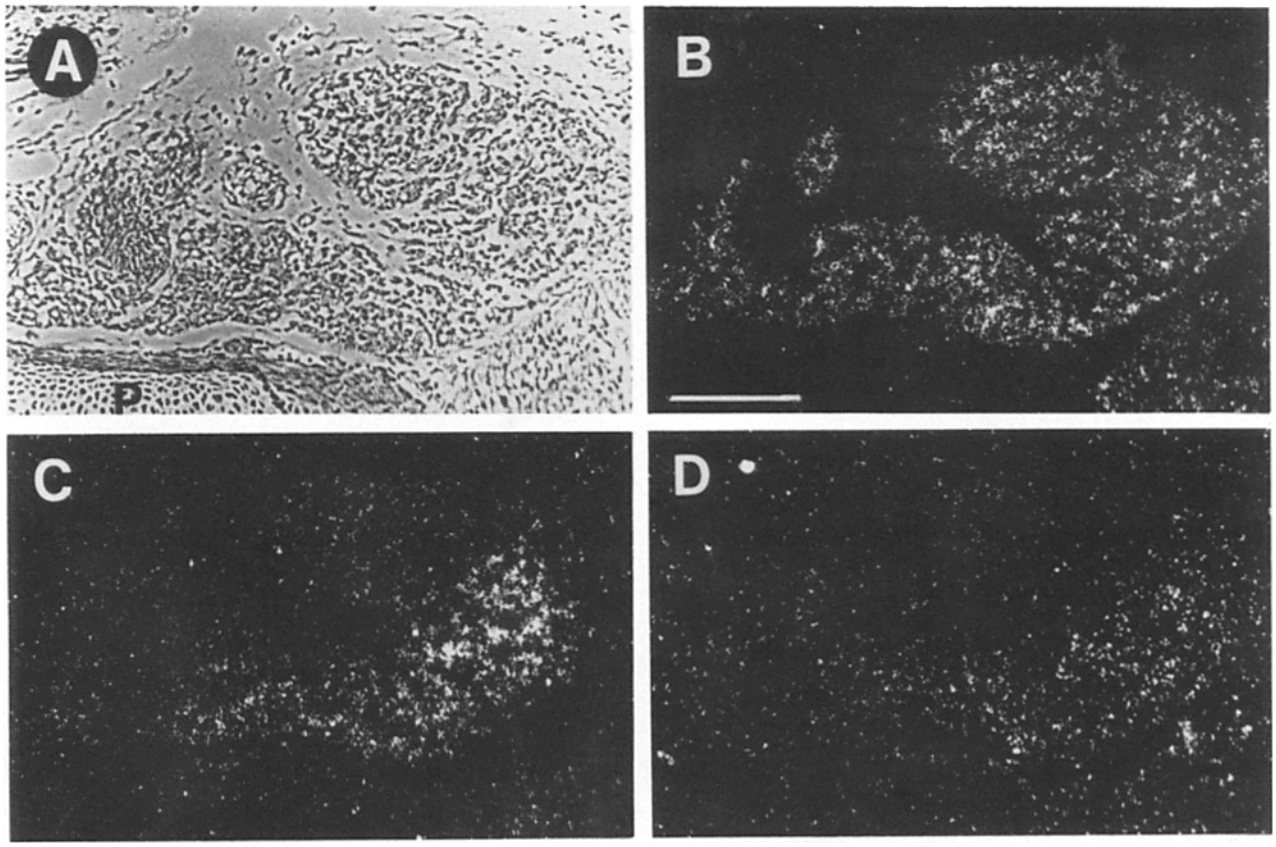

Figure 8. By 16.5 d p.c., MLC1V mRNAs are detected in a subpopulation of muscle fibers which will probably form slow fibers in the adult. (*A*) Phase-contrast micrograph of a transverse section of a gluteal muscle close to the pelvis (*P*). (*B*) Dark field micrograph of the same section as in *A* hybridized with the MLC1A probe. (*C*) Section parallel to *B* hybridized with the MLC1V probe. (*D*) Section parallel to *C* hybridized with the MHCβ probe. Both MLC1V mRNAs and MHCβ mRNAs are expressed in muscle fibers in the lower right portion of the muscle, which is probably the future red portion of the gluteus medius, but not in the fibers in the upper left portion of the muscle, which is probably the future white portion of the same muscle. In contrast, MLC1A is expressed in most of the muscle fibers. Bar, 300 μm.

only begin to be detected later in fetal mouse muscles (data not shown; Barton et al., 1989). The myosins and actins are assembled into functional myofibrils because myotomal myocytes have been observed to contain cross-striations and to contract (Holtzer et al., 1957). At a later stage, day 4 in the chick (Holtzer et al., 1957) and day 12–13 in the mouse (Vivarelli and Cossu, 1986), the mononucleated myotomal cells fuse to form multinucleated myotubes.

The timing of the detection of muscle gene transcripts and that of the corresponding protein appears to be very close. We did not perform antibody staining on our sections, but our observations are consistent with those of Vivarelli et al. (1988) and Fürst et al. (1989). There are, however, two examples in which mRNAs were detected in myogenic cells by in situ hybridization but the corresponding protein was not detected by antibody staining. Lawrence et al. (1989) found that single myogenic cells in vitro expressing cardiac actin mRNA did not express detectable levels of cardiac actin protein. We detect MHCpn transcripts beginning at 10.5 d p.c., but MHCpn protein is not detectable in 10-d somites or 13-d limb buds (Vivarelli et al., 1988). The first reported accumulation of the MHCpn protein in the rat is in fetal muscle, 16 d p.c. (Harris et al., 1989). Although the expression of most muscle genes is thought to be under transcriptional control, these observations and others (reviewed in Roy et al., 1984) suggest that there may be some posttranscriptional controls for some muscle genes during development (Cox, R., and M. Buckingham, manuscript submitted for publication).

Sweeney et al. (1989) propose that, in the chick, muscle cells in the embryo express a common MHC phenotype, which consists of MHCventricular and MHCfast. Our results also suggest that each of the embryonic myogenic cells expresses a common pattern of MHC gene transcripts. When MHC mRNAs are first detected between 9 and 10 d p.c., both MHCβ (ventricular) and MHCemb are present. We did not detect differential expression of either MHC isoform within the same muscle masses. At 10.5 d p.c., when MHCpn transcripts are also detectable, we do not find any myotomes expressing MHCemb and MHCβ that do not have MHCpn transcripts. Similarly, cardiac α-actin is expressed, although at a lower level, in all of the myotomal cells in which myogenin is expressed (Fig. 4). This is, however, within the limits of a comparison based on serial 5-μm sections, where only one type of mRNA is detected per section. The use of cold probes and/or appropriate antibodies on the same sections will, in future experiments, provide definitive evidence. It is only at later stages that fast and slow MHC isoform transcripts become segregated according to fiber type.

At 15 d p.c., we begin to detect differences in myosin gene

expression within muscles of the body and limbs. The most striking observation is the first expression of MLC1V in skeletal muscles (Fig. 7 *F*). MLC1V transcripts are detected mainly in the central portions of some skeletal muscles. In regions where MLC1V is expressed, MHCpn (Fig. 7 *C'*) and MLC1A (Fig. 8 *B*; Lyons et al., 1989) transcripts are often present at lower levels than elsewhere in the muscle mass. This stage defines the boundary between embryonic and fetal skeletal muscle development in that the muscle fiber groups now correspond in their spatial arrangement to those present in the adult (Kieny, et al., 1986). The onset of differential MLC gene expression in developing muscle occurs when secondary muscle fibers are forming and neuromuscular junctions are being established (Ontell and Kozeka, 1984). Our results suggest that MLC1A and MLC1V, in addition to the fast and slow MHCs, are good early markers for the differentiation of future fast and slow muscle fibers. At this stage, MLC1V (Fig. 8 *C*) and MHCβ transcripts (Fig. 8 *D*) as well as MHCβ protein begin to accumulate in a subset of fibers that will eventually become adult slow muscle fibers (Narusawa et al., 1987). However, as discussed, in contrast to MLC1V, MHCβ is present at a low level in all embryonic skeletal muscle masses before the onset of fiber differentiation. From 15 d p.c., MHCpn, MLC1F, and MLC1A expression begins to be restricted to secondary muscle fibers and to those primary muscle fibers that will become fast fibers in the adult.

The role of the myogenic differentiation factors, MyoD1 (Davis et al., 1987), myogenin (Wright et al., 1989), and myf-5 (Braun et al., 1989) in embryonic muscle development is not clear. Myogenin is a major transcript in the myotomes from their inception, whereas MyoD1 mRNA is not detected until 10.5 d p.c. (Sassoon et al., 1989), at a time when the α-actin and many of the myosin genes are already activated. If, as seems likely (Lassar et al., 1989), MyoD1 and the other myogenic sequences act as transcription factors for muscle structural genes, then both the combination of factors and their quantity must be finely tuned during development. There is no simple coordinated activation of a battery of actin and myosin genes in the embryo. Each gene examined has a different pattern of expression (Table I).

In addition to the potential role of the myogenic differentiation genes, other physiological factors influence the early stages of skeletal myogenesis. Of these, the neural tube is undoubtedly important. As the myotome forms and skeletal muscle gene transcripts and proteins begin to accumulate, processes from the neural tube have been observed to contact the myotome (Filogamo and Gabella, 1967). However, the role of the neural tube in the earliest stages of skeletal myogenesis is not well understood. Vivarelli and Cossu (1986) cultured 8.5-d and 10.5-d p.c. somitic cells in the presence and absence of the neural tube and assayed the number of cells that differentiated under these two conditions using a MHC antibody. They concluded that during early somitogenesis, 8.5 d, the presence of the neural tube is essential for the emergence of myosin-positive cells in culture, but by 10.5 d the number of myosin-positive cells appearing in culture is largely independent of the neural tube. Thus, the neural tube may be essential at least for the initiation of the myogenic program in somitic cells.

Understanding how such different regulatory influences are integrated at the molecular level to produce individual patterns of activation of different actin and myosin genes in the myotome is a major challenge which will require manipulation of the mouse embryo in vivo.

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