

# Developmental Regulation of Myosin Gene Expression in Mouse Cardiac Muscle

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**Abstract.** Expression of the two isoforms of cardiac myosin heavy chain (MHC), MHC $\alpha$  and MHC $\beta$ , in mammals is regulated postnatally by a variety of stimuli, including serum hormone levels. Less is known about the factors that regulate myosin gene expression in rapidly growing cardiac muscle in embryos. Using isoform-specific 35S-labeled cRNA probes corresponding to the two MHC genes and the two myosin alkali light chain (MLC) genes expressed in cardiac muscle, we have investigated the temporal and spatial pattern of expression of these different genes in the developing mouse heart by *in situ* hybridization. Between 7.5 and 8 d post coitum (p.c.), the newly formed cardiac tube begins to express MHC $\alpha$ , MHC $\beta$ , MLC1 atrial (MLC1A), and MLC1 ventricular (MLC1V) gene transcripts at high levels throughout the myocardium. As a distinct ventricular chamber forms between 8 and 9 d p.c., MHC $\beta$  mRNAs begin to be restricted to ventric-

ular myocytes. This process is complete by 10.5 d p.c. During this time, MHC $\alpha$  mRNA levels decrease in ventricular muscle cells but continue to be expressed at high levels in atrial muscle cells. MHC $\alpha$  transcripts continue to decrease in ventricular myocytes until 16 d p.c., when they are detectable at low levels, but then increase, and finally replace MHC $\beta$  mRNAs in ventricular muscle by 7 d after birth. Like MHC $\beta$ , MLC1V transcripts become restricted to ventricular myocytes, but at a slower rate. MLC1V mRNAs continue to be detected at low levels in atrial cells until 15.5 d p.c. MLC1A mRNA levels gradually decrease but are still detectable in ventricular cells until a few days after birth. This dynamic pattern of changes in the myosin phenotype in the prenatal mouse heart suggests that there are different regulatory mechanisms for cell-specific expression of myosin isoforms during cardiac development.

CARDIAC muscle is known to contain at least two different isoforms of myosin heavy chain (MHC)<sup>1</sup> and two myosin alkali light chains (MLC), which are encoded by distinct genes (Mahdavi et al., 1982; Barton et al., 1985, 1988). The genes encoding the two MHC proteins, called MHC $\alpha$  and MHC $\beta$  (Hoh et al., 1978), are organized in tandem (Mahdavi et al., 1984) and are developmentally and hormonally regulated (Lompré et al., 1984). These genes are also regulated differentially in response to cardiac hypertrophy due to hemodynamic overload (Izumo et al., 1987; reviewed in Nadal-Ginard and Mahdavi, 1989). The MLC genes, MLC1A and MLC1V, are coexpressed and developmentally regulated in cardiac and skeletal muscle (Barton and Buckingham, 1985; Lyons et al., 1990b). These

MLC genes are regulated differentially during cardiac hypertrophy in humans (Cummins, 1982; Kurabayashi et al., 1988) but not in rodents (Rovner et al., 1990), and do not appear to be hormonally regulated (Schwartz et al., 1982). In the adult rodent heart, MLC1V is present in the ventricles and MLC1A in the atria, while MHC $\alpha$  is the major isoform in both compartments.

Most of the reports on myosin expression in embryonic cardiac muscle have involved antibody studies of the chick heart. Zhang et al. (1986) and Sweeney et al. (1987) report that at Hamburger-Hamilton stage 10 (1.25 d in ovo), only the adult ventricular MHC is detectable. Approximately 1 d later, stage 12–15, atrial MHC starts to be expressed in embryonic chick hearts. There is one report of an antibody study of MHCs in the embryonic rat heart. De Groot et al. (1989) show that MHC $\alpha$  and MHC $\beta$  are coexpressed in tubular rat hearts (embryonic day 10–11). Biochemical studies of developing mammalian hearts have shown that MHC $\beta$  is the predominant MHC in late fetal ventricles (Lompré et al., 1981; Schwartz et al., 1982). However, there are variations in the developmental patterns of MHC protein

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

expression in the ventricles of different mammalian species (Lompré et al., 1981). Variations in MHC proteins in atria and ventricles appear to be related to animal size (Syrový, 1985). There are a few studies of the MLC protein content of fetal cardiac muscle; these have shown that MLC1A is expressed in fetal ventricles (Whalen and Sell, 1980; Cummins and Lambert, 1986).

We are interested in the regulation of myosin gene expression in the developing mammalian heart, particularly at early stages in the embryo. To characterize the normal pattern of expression of MHC and MLC genes, we have used isoform-specific cRNA probes to MHC $\alpha$ , MHC $\beta$ , MLC1A, and MLC1V mRNAs for *in situ* hybridization of the mouse embryonic and fetal heart. We show that, after an initial high level of coexpression in the myocardium of the cardiac tube at 8 d post coitum (p.c.), each of these transcripts has a different pattern of accumulation suggesting that each is regulated in a different cell-specific and stage-specific manner in developing atrial and ventricular myocytes.

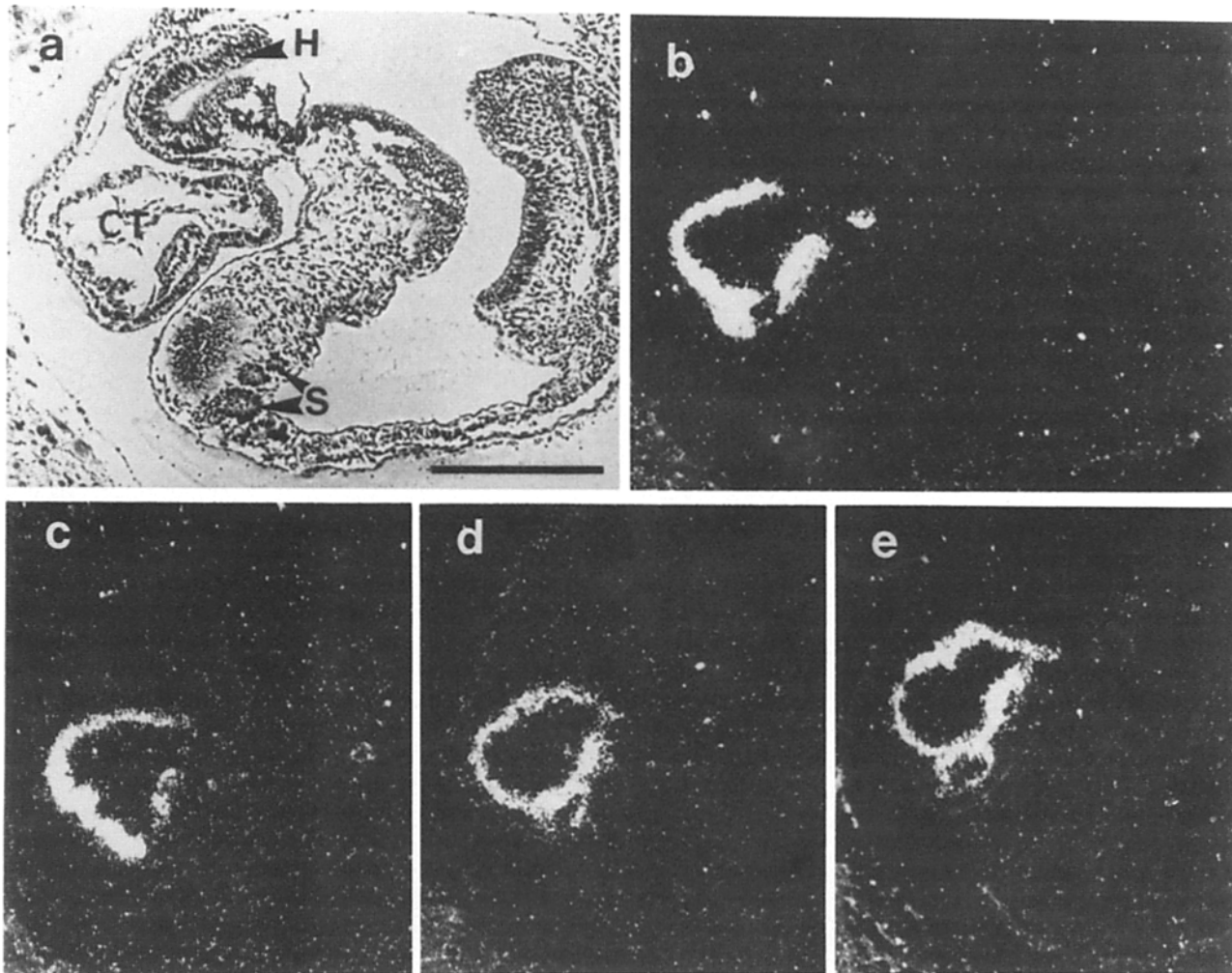
## Materials and Methods

### Preparation and Prehybridization of Tissue Sections

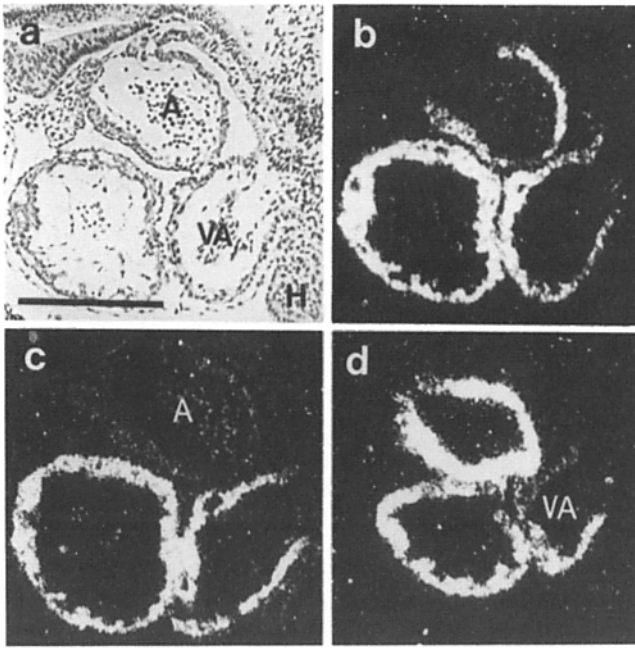
The protocol that was used to fix and embed C3H and BALB/c mouse embryos, fetuses, and postnatal hearts is described in detail in Sassoon et al. (1988). Ages and numbers of embryos and fetuses examined are listed in Table I. Briefly, embryos were fixed in 4% paraformaldehyde in PBS, dehydrated, and infiltrated with paraffin. 5–7- $\mu$ m-thick serial sections were mounted on subbed slides (Gall and Pardue, 1971). One to three sections were mounted per slide, deparaffinized in xylene, and rehydrated. The sections were digested with proteinase K, postfixated 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 untranslated regions (UTR) of the mRNAs. Appropriate restriction fragments or oligonucleotides were subcloned into the vector, Bluescribe+ (Stratagene Cloning Systems, La Jolla, CA) and grown in *E. coli* TGI. The following probes were used. (1) 3' UTR of mouse MLC1Atrial mRNA (Barton et al., 1988) 5'TTAATGAAACTCCAAGCTGGGGCTCTTTATTTCCAGGGAAGGTT-

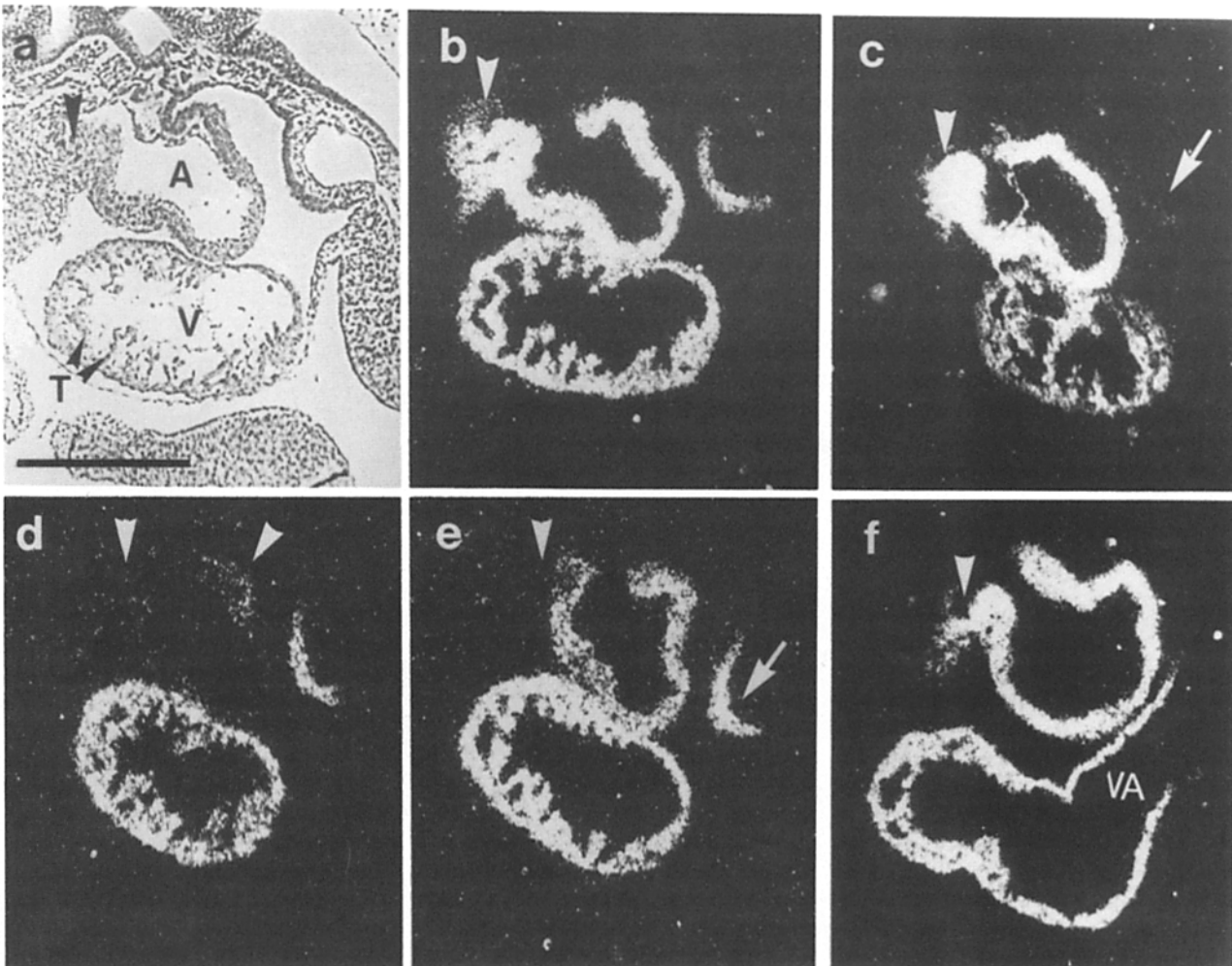


**Figure 1.** Myosin gene expression in the atrioventricular junction of the newly formed cardiac tube. (a) Phase-contrast micrograph of a parasagittal section of an 8-d p.c. mouse embryo with four somites. (b) Dark-field micrograph of the same section as in (a) hybridized to the MLC1A probe. Serial slides were hybridized to the probes for (c) MHC $\alpha$ , (d) MLC1V, and (e) MHC $\beta$ . (The actual sequence in which the sections were cut is MHC $\alpha$ , MLC1A, MLC1V, MHC $\beta$ .) S, somites; H, headfold; CT, cardiac tube. Bar, 300  $\mu$ m.

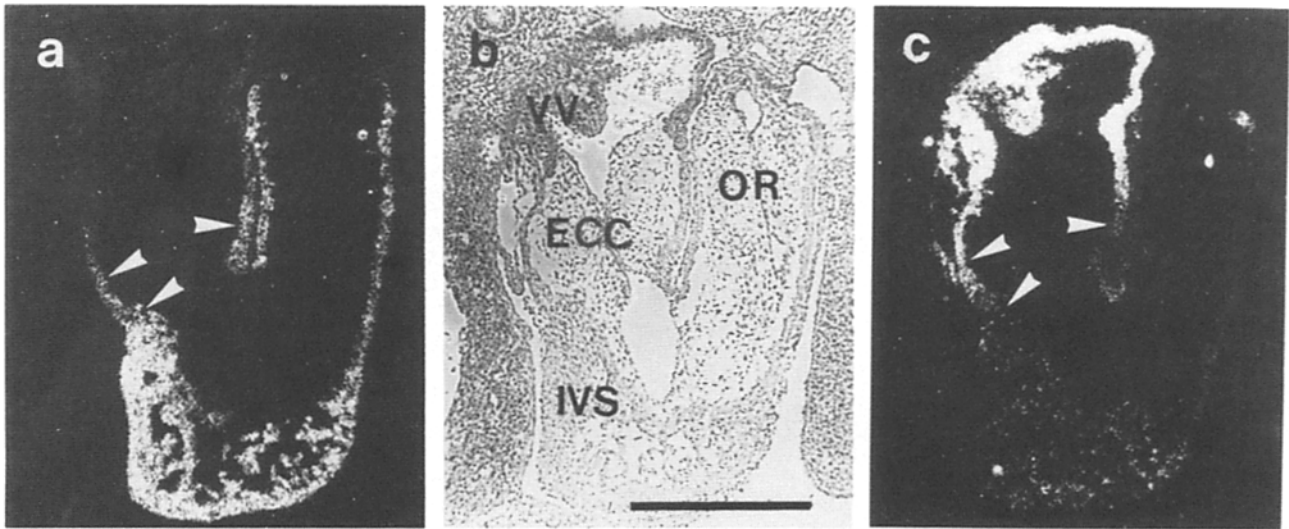


GTGGGTCAGAGAAGCCATGTGAGTCCAATACTCCGTAACAGTAAC-AGCCGCTGTGGATCTCTTGCTTTCACGCAGGCCAAGC3'. These sequence data are available from EMBL/GenBank/DDJB under accession numbers M20773, M19435, and J03932. (2) 3' UTR of the mouse MLC1-Ventricular mRNA (Lyons et al., 1990b) 5'GGAGTCCGAACCACTCC-TTCCCGAAGCTCCAGACATGGTGAGCATCCTGGTTGGGAGATG-CCTGGCCTGCCCTGGGCTTCCTGA3'. These sequence data are available from EMBL/GenBank/DDJB under accession number X53489. (3) 3' UTR of rat MHC<sup>Ventricular</sup> (MHC $\beta$ ) mRNA (Mahdavi et al., 1982) 5'GGTCTCAGGGCTTCACAGGCATTCCTTAGGGTTGGGTAGCACAT-GATCTACTCTTCATTCAGGCCCTTG3'. These sequence data are available from EMBL/GenBank/DDJB under accession number K01463. (4) 3' end of the mouse MHC<sup>Atrial</sup> (MHC $\alpha$ ) mRNA (Weydert et al., 1985) 5'CGAACGTTTATGTTTATTGTGGATTGGCCACAGCGAGGGTCG-CTGGAGAGGTTATTCCTCGTC3'. These sequence data are available from

**Figure 2.** MHC $\beta$  is restricted to ventricular cells at an early stage in cardiac muscle development. (a) Phase-contrast micrograph of a parasagittal section through a 9.5-d.p.c. embryo with 21–24 somites. (b) Dark-field micrograph of the same section as in a hybridized to the MLCIV probe. (c) A parallel section hybridized with the MHC $\beta$  probe. (d) A parallel section hybridized to the MHC $\alpha$  probe. VA, ventriculoarterial portion; A, atrium; H, hyoid arch. Bar, 300  $\mu$ m.



**Figure 3.** Myosin gene transcripts are detected in the developing sinuatrial transition (a–f, arrowheads). (a) Phase-contrast and (b) dark-field micrographs of a parasagittal section through a 10.5-d.p.c. embryonic heart hybridized with the MLCIA probe. Serial slides were hybridized to the probes for (c) MHC $\alpha$ , (d) MHC $\beta$ , and (e) MLCIV. MHC $\beta$  transcripts continue to be expressed in the right side of the sinuatrial transition (d, right arrowhead). (f) A parallel section, 50  $\mu$ m from that in e, was hybridized with the MLCIA probe, showing the sinuatrial transition (arrowhead) and the ventriculoarterial canal (VA; which is indicated by arrows in c and e). (There is a small fold in the section over the atrium in c.) T, trabeculae; A, atrium; V, ventricle. Bar, 300  $\mu$ m.



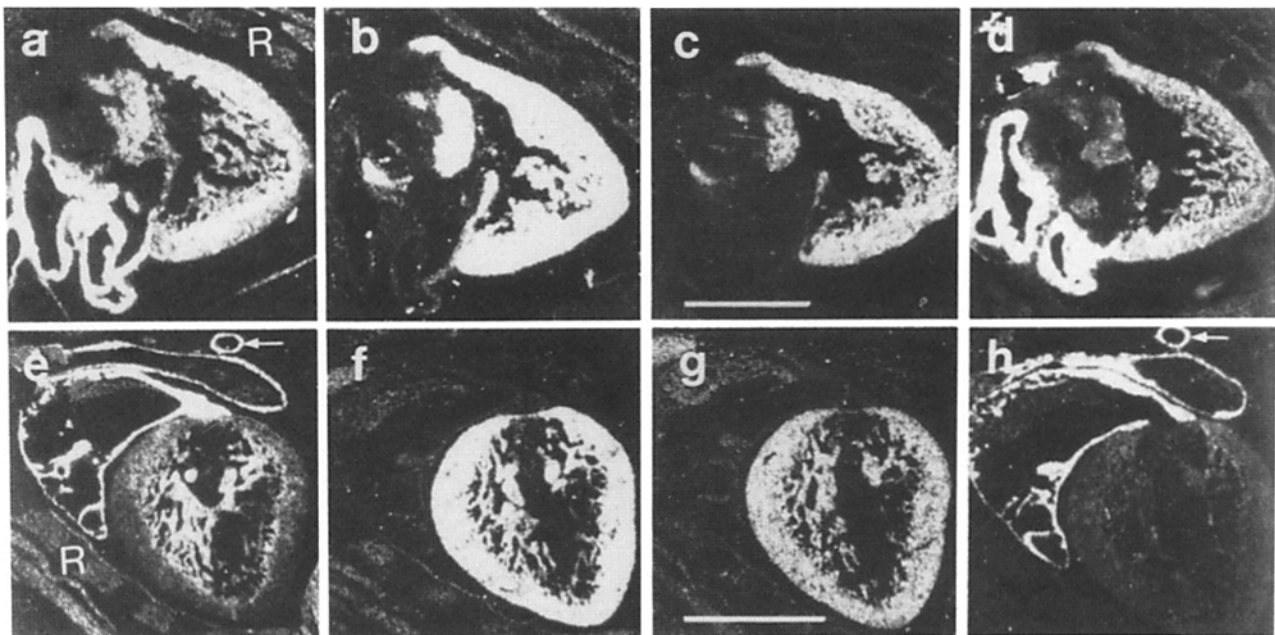
**Figure 4.** Between 10.5 and 15.5 d p.c., MLCIV transcripts gradually become restricted to ventricular cells. Parallel parasagittal sections through an 11.5-d p.c. mouse heart hybridized with (a) the MLCIV probe and with the MHC $\alpha$  probe (b) phase micrograph and (c) corresponding dark-field micrograph. The boundary for restriction of atrial and ventricular transcripts is the atrioventricular transition (a and c, arrowheads). ECC, endocardial cushions; OR, outflow ridges; IVS, interventricular septum, VV, venous valves. Bar, 400  $\mu$ m.

EMBL/GenBank/DDJB under accession numbers M12291 and X53490. The cRNA transcripts were synthesized according to manufacturer's conditions (Stratagene Cloning Systems) and labeled with  $^{35}$ S-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

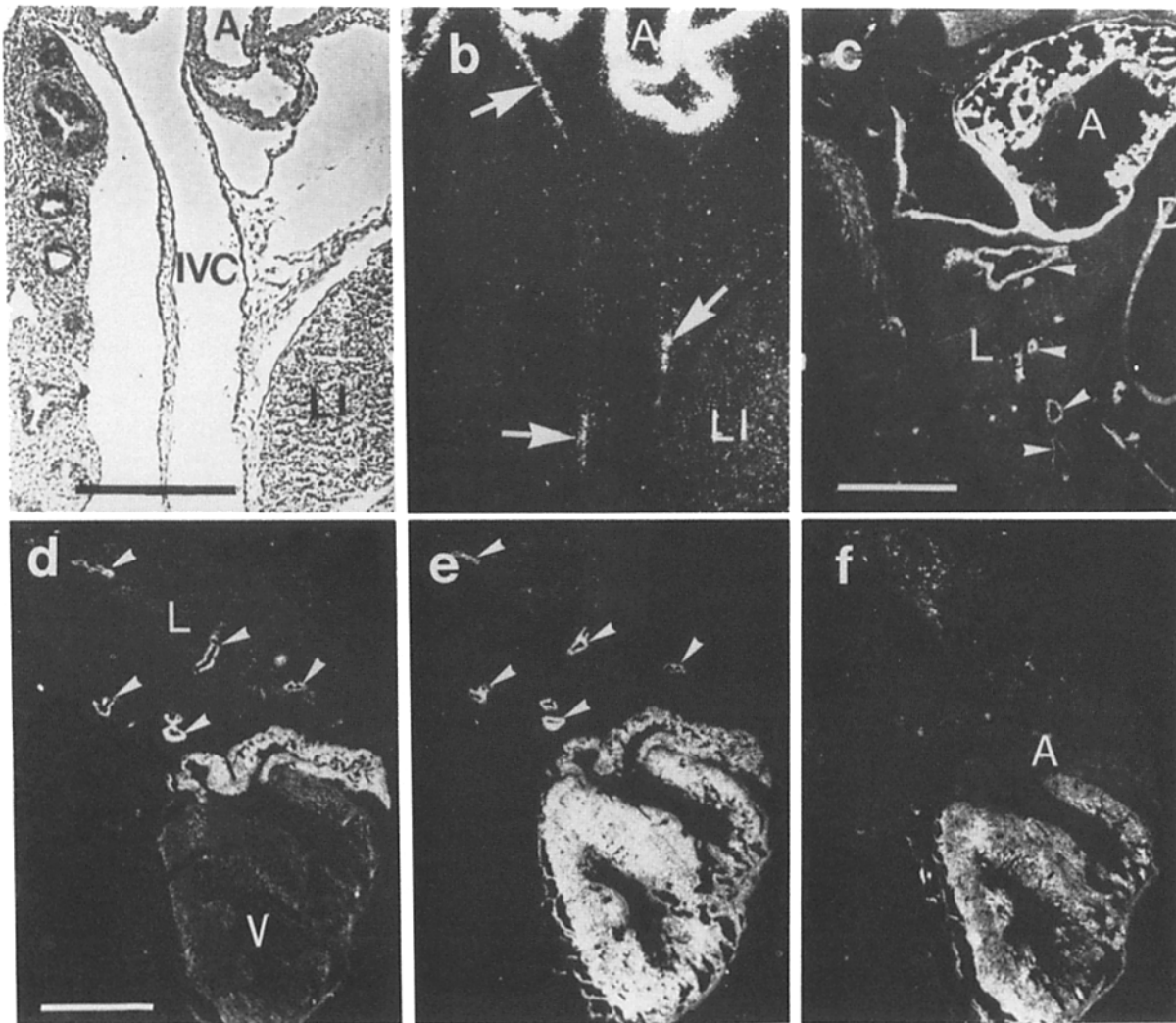
Sections were hybridized overnight at 52°C in 50% deionized formamide,

0.3 M NaCl, 20 mM Tris-HCl, pH 7.4, 5 mM EDTA, 10 mM NaPO $_4$ , 10% dextran sulfate, 1 $\times$  Denhardt's, 50  $\mu$ g/ml total yeast RNA, and 50–75,000 cpm/ $\mu$ l  $^{35}$ S-labeled cRNA probe. The tissue was subjected to stringent washing at 65°C in 50% formamide, 2 $\times$ SSC, 10 mM DTT and washed in PBS before treatment with 20  $\mu$ g/ml RNase A at 37°C for 30 min. Following washes in 2 $\times$ SSC and 0.1 $\times$ 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 dessicant 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.



**Figure 5.** Atrial-specific transcripts disappear from the ventricle in a gradient of expression. (a–d) Dark-field micrographs of parasagittal sections through a 15.5-d p.c. mouse heart. (e–h) Dark-field micrographs of parasagittal sections through a 16.5-d p.c. mouse heart. Serial slides at the two different stages were hybridized with the MHC $\alpha$  probe (d and h), the MLC1A probe (a and e), the MHC $\beta$  probe (c and g), and the MLCIV probe (b and f). R, ribs; pulmonary blood vessel (arrow). Bars: (a–d) 600  $\mu$ m; (e–h) 750  $\mu$ m.





**Figure 6.** At 15 d p.c., MHC $\alpha$  and MLC1A transcripts begin to be detectable around some blood vessels outside the heart. (a) Bright-field micrograph of a 15.5-d p.c. mouse thorax showing the inferior vena cava (IVC), which expresses MLC1A transcripts (b, arrows). At 17.5 d p.c. (c) MLC1A transcripts are detected in a sleeve of atrial muscle that surrounds some pulmonary vessels (arrowheads). Parallel sections of neonatal mouse hearts were hybridized with the probes for (d) MLC1A, (e) MHC $\alpha$ , and (f) MHC $\beta$ . Pulmonary vessels (d and e, arrowheads); LI, liver; A, atrium; L, lung; D, diaphragm. Bars: (a and b) 300  $\mu$ m; (c) 800  $\mu$ m; (d-f) 800  $\mu$ m.

## Results

By 8 d p.c. in the mouse embryo, a tubular heart has formed and the first myocardial contractions occur (Rugh, 1990; Sissman, 1970). At first, the tubular heart is S-shaped. Serial sections from the midsagittal section in Fig. 1 c in the order Fig. 1, c, b, d, and e show the lower half of the S-curve, or the atrioventricular junction (Pexeider et al., 1989), of the developing heart. At this stage, each of the four myosin transcripts that we studied is expressed at a high level in all of the myocardial cells (Fig. 1). This observation suggests that all of the myosin genes in the heart are upregulated initially in a coordinated fashion. These results are consistent with antibody studies of the myofibrillar proteins in the developing rodent heart, which have shown that, at embryonic day 10–11 in the rat, there is coexpression of MHC $\alpha$  and MHC $\beta$  throughout the heart (De Groot et al., 1989). Embryonic day 10 in the rat corresponds to 8 d p.c. in the mouse (Rugh, 1990). Three of the four cardiac myosin transcripts we studied, MHC $\beta$ , MLC1A, and MLC1V, are coexpressed in de-

veloping skeletal muscle. However, these transcripts are detected in cardiac muscle well before they are detected in developing somites (Fig. 1, b, d, and e; Lyons et al., 1990b).

The first major change detected in the pattern of myosin transcript distribution in the developing heart occurs around 9 d p.c. At this stage, a distinct atrium and ventricle have formed (Rugh, 1990; Theiler, 1989). In Fig. 2, parallel parasagittal sections of a 9.5-d p.c. embryo with 21–24 somites demonstrate that MLC1V mRNAs (Fig. 2 b) are detected in all myocardial cells, whereas MHC $\beta$  (Fig. 2 c) is largely restricted to cells in the ventricle and the outflow tract or ventriculoarterial portion (Pexeider et al., 1989). Only a few atrial cells continue to express MHC $\beta$  (Fig. 2 c). MHC $\alpha$  transcripts (Fig. 2 d) are present at high levels in both the atrium and ventricle, but are decreasing in the ventriculoarterial portion. MLC1A mRNAs are expressed in all myocardial cells at this stage (data not shown).

At 9 d p.c., trabeculations are first seen in the ventricles (Sissman, 1970), and they are clearly visible in ventricles of a 10-d p.c. mouse embryo with 28 somites (Fig. 3). In the

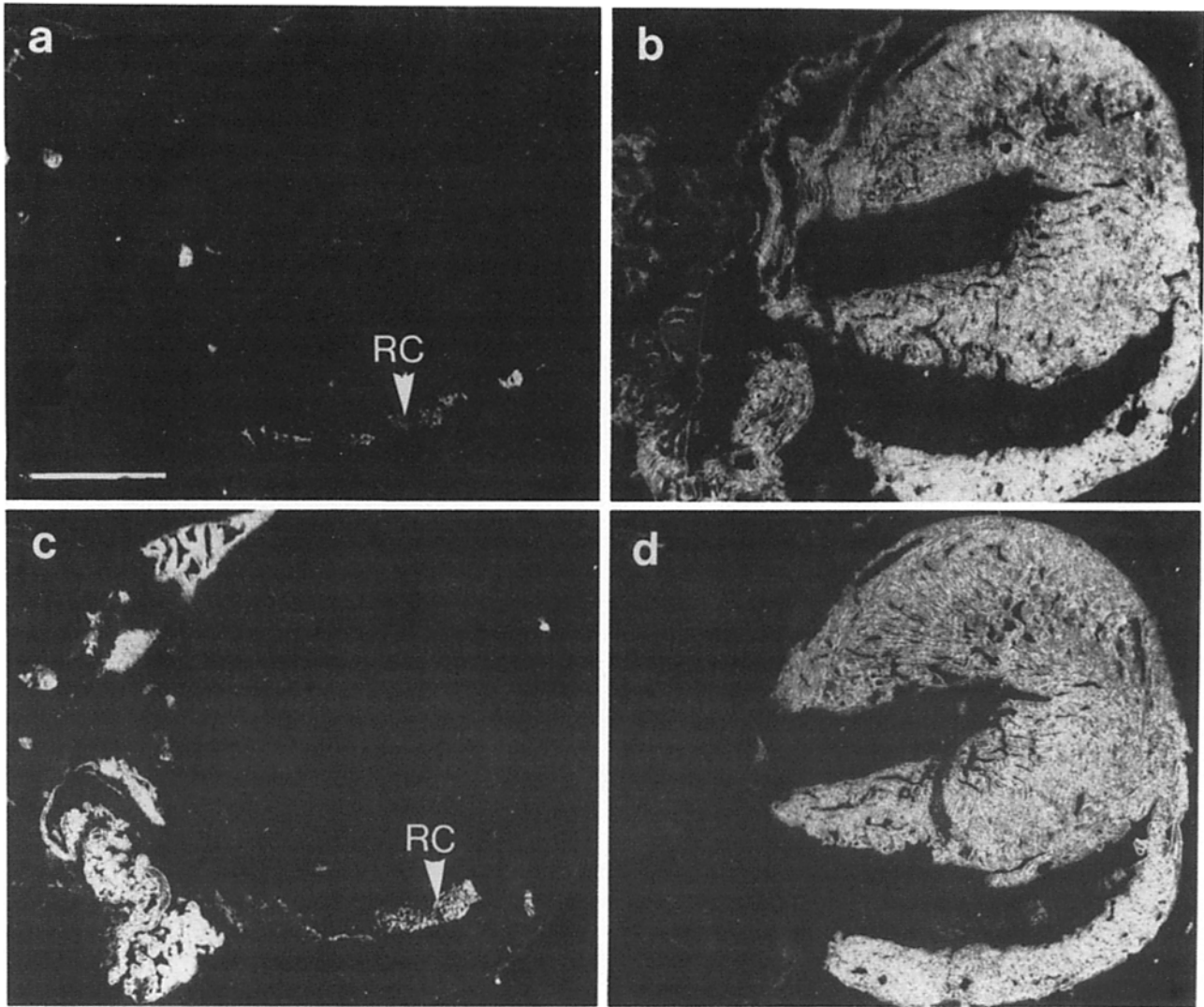


Figure 7. Between 17.5 d p.c. and 7 d after birth, atrial and ventricular cells switch to the adult pattern of myosin gene expression. Dark-field micrographs of parallel sections of a 7-d mouse heart hybridized with the probes for (a) MHC $\beta$ , (b) MHC $\alpha$ , (c) MLC1A, and (d) MLC1V. RC, red blood cells. Bar, 950  $\mu$ m.

Table I. Expression of Actin and Myosin Genes in Developing Mouse Cardiac Muscle

Days p.c.	8.0	9.5	10.5	11.5	12.5	14.5	15.5	17.5	0.0	7.0 p.p.
<b>Ventricular muscle</b>										
$\alpha$ -Cardiac actin	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
MLC1A	+++	+++	+++	+++	+++	+++	++	+	-	-
MLC1V	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
MHC $\alpha$	+++	+++	++	++	++	+	+	++	++	+++
MHC $\beta$	+++	+++	+++	+++	+++	+++	+++	+++	++	-
<b>Atrial muscle</b>										
$\alpha$ -Cardiac actin	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
MLC1A	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
MLC1V	+++	+++	+++	++	+	+	+	+/-	-	-
MHC $\alpha$	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
MHC $\beta$	+++	+	-	-	-	-	-	-	-	-
Sample #	4	3	7	5	7	5	7	5	4	3

developing heart, the growth rate of the trabeculated regions is thought to be greater than the nontrabeculated portions of heart, because in the adult, the portion of the ventricular wall that is trabeculated accounts for >90% of the total ventricular mass (DeHaan and O'Rahilly, 1978). In the trabeculated ventricle, the levels of MHC $\alpha$  mRNAs decrease gradually beginning at 10 d p.c. (Fig. 3 c).

Between 10 and 12 d p.c., another difference in the spatial pattern of myosin gene expression in the embryonic heart is detected in the developing sinuatrial transition (Fig. 3). Transcripts for the atrial isoforms: MLC1A (Fig. 3, a, b, and f) and MHC $\alpha$  (Fig. 3 c), but not MLC1V (Fig. 3 e), even though it is coexpressed in atrial cells at this stage, are detected in the sinuatrial transition. MHC $\beta$  is detected at a low level in one portion of the sinuatrial transition (Fig. 3 d, right arrowhead). This result agrees in part with the observations of De Groot et al. (1989), who noted that MHC $\alpha$  and MHC $\beta$  proteins are coexpressed in the developing sinus node in rat embryos. In the sinuatrial transition, there is a distinct ring or column of cells (Anderson et al., 1978) that forms the primordium of the sinus node. Fig. 3 f is a parallel section, hybridized with the MLC1A probe,  $\sim 50 \mu\text{m}$  from the section in Fig. 3 e, showing the continuity of this ring of cells in the sinuatrial transition.

As development proceeds, the level of MHC $\alpha$  mRNAs decreases in the ventricle (Fig. 4, b and c) and MLC1V transcript levels decrease in the atrium (Fig. 4 a). Fig. 4 contains two parasagittal sections through the atrioventricular junction and ventriculoarterial component of an 11.5-d p.c. mouse embryo. The endocardial cushions, outflow ridges, and a portion of the developing interventricular septum can be seen clearly in the phase-contrast micrograph (Fig. 4 b). The boundary for the expression of the atrial- and ventricular-specific transcripts is the atrioventricular transition (Fig. 4, arrowheads). The atrioventricular conduction system develops both in this transition and in a portion of the interventricular septum (Viragh and Challice, 1982).

The next major change in the spatial distribution of cardiac myosin transcripts in the developing fetal heart is shown in Fig. 5. At 15.5 d p.c., MLC1A transcripts (Fig. 5 a) and MHC $\alpha$  transcripts (Fig. 5 d) begin to disappear from the ventricle in a gradient of expression that runs from very low levels of expression in the ventricular wall to high levels of expression in the papillary muscles and interventricular septum. By 16.5 d p.c., MLC1A transcripts (Fig. 5 e) are only detected in the central portion of the ventricle, and MHC $\alpha$  (Fig. 5 h) transcripts have fallen to a very low level in the ventricle. Both MLC1A and MHC $\alpha$  mRNAs continue to be expressed at high levels in the atria (Fig. 5, a and e and d and h), and both MLC1V mRNAs (Fig. 5, b and f) and MHC $\beta$  mRNAs (Fig. 5, c and g) are expressed at high levels in the ventricles.

In addition to their expression within the heart itself, MLC1A and MHC $\alpha$  transcripts are expressed outside of the heart along the pulmonary and caval vessels. Fig. 6 a is a phase-contrast micrograph of a 15.5-d p.c. mouse showing the inferior vena cava which runs from the liver to the right atrium. Under dark-field illumination (Fig. 6 b), MLC1A transcripts can be seen over portions of the inferior vena cava (arrows) near the liver and close to the atrium. At 17.5 d p.c. (Fig. 6 c), and at birth (Fig. 6 d), MLC1A transcripts are detected around pulmonary vessels at some distance from the

atrium. MHC $\alpha$  transcripts (Fig. 6 e) are also expressed around these vessels. These results are consistent with reports of myocardial extensions or sleeves over pulmonary and caval veins in rodents (Kramer and Marks, 1965; Nathan and Gloobe, 1970). The hybridization of the MHC $\alpha$  and MLC1A probes to the pulmonary and caval vessels is not due to crosshybridization with smooth muscle myosin transcripts since no hybridization was detected to the smooth muscle in other blood vessels, such as the aorta, or in the gut (data not shown).

After decreasing to their lowest level in the fetal ventricle (Fig. 5 h) at 16.5 d p.c., MHC $\alpha$  transcripts begin to increase rapidly. At birth, MHC $\alpha$  transcripts (Fig. 6 e) are present at higher levels than MHC $\beta$  transcripts (Fig. 6 f). This switch continues until MHC $\alpha$  transcripts (Fig. 7 b) have replaced MHC $\beta$  transcripts (Fig. 7 a) in the heart 7 d after birth. This result is consistent with the observation of Lompré et al. (1981), who detected only MHC $\alpha$  protein in 7-d postnatal mouse ventricles. Also at this stage, MLC1A transcripts (Fig. 7 c) are completely restricted to the atrium and only MLC1V transcripts (Fig. 7 d) are detectable in the ventricle.

Table I summarizes the results for in situ hybridization with each of the myosin probes used and lists the number of embryos, fetuses, or postnatal hearts examined at each stage. We did not detect any skeletal muscle-specific MHC or MLC at any stage in the developing heart (Lyons et al., 1990b). We also were unable to detect any MHC embryonic, which has been reported to be expressed in conductive tissue in rodent hearts (Gorza et al., 1988) around the time of birth (data not shown).

## Discussion

We have described the temporal and spatial pattern of expression of four cardiac myosin genes in embryonic and fetal mouse cardiac muscle. Although three of the four myosin gene transcripts we studied are also expressed in skeletal muscle, these genes, encoding MHC $\beta$ , MLC1A, and MLC1V, are regulated differently in the two types of striated muscle (see Lyons et al., 1990b). With the exception of some head muscles, which coexpress developmental isoforms at all stages (Weiczoreck et al., 1985; Soussi-Yanicostas et al., 1990), myosin gene transcripts and proteins are expressed in a distinct sequence (Whalen et al., 1981; Lyons et al., 1983; Lyons et al., 1990a, b) during skeletal muscle development rather than being upregulated together initially and subsequently modulated during development.

Our results suggest that, in cardiac muscle, myosin genes are all expressed at high levels as soon as the cardiac tube forms, and that the expression of each gene is modulated in a different cell-specific manner during development. At 8 d p.c., in the tubular heart, before distinct atrial and ventricular chambers form, each of the myosin gene transcripts we studied are detected throughout the myocardium. This is consistent with the observation that the first myocardial contractions occur at 8 d p.c. in the mouse (Rugh, 1990; Sissman, 1970), suggesting that all of the myosin genes are expressed coordinately. In a similar fashion, cardiac and skeletal  $\alpha$ -actin genes are probably coactivated early in cardiac development (Sassoon et al., 1988), although skeletal  $\alpha$ -actin mRNAs are always detected at a low level in the

heart. The recent results of Rudnicki et al. (1990) concerning the differentiation of cardiac myocytes from DMSO-treated embryonal carcinoma cells in vitro support our findings. These authors found that MHC $\alpha$ , MHC $\beta$ , MLC2A, and MLCIV proteins were coexpressed in mononucleated myocytes, and they suggest that these cells are analogous to cells of the early embryonic myocardium.

Results of antibody studies of MHCs in the embryonic chick heart (Zhang et al., 1986; Sweeney et al., 1987) have shown that the ventricular MHC protein is expressed  $\sim$ 1 d before the atrial MHC protein is detected. We were not able to detect any differences in timing or localization of expression of the contractile protein genes we studied in the embryonic tubular heart of the mouse. In a mouse embryo without somites, at a stage slightly earlier than that shown in Fig. 1, we observed the same pattern of coexpression of the myosin isoforms (data not shown).

As the cardiac tube loops and rotates upon itself to form the distinct atrial and ventricular chambers, MHC $\beta$  transcript levels decrease rapidly in the atrium, but continue to be expressed at high levels in the ventricle. MHC $\beta$  has a lower myosin ATPase than MHC $\alpha$  (Hoh et al., 1978), and cardiac muscle cells expressing MHC $\beta$  have a lower velocity of shortening (Schwartz et al., 1981). These observations suggest that the higher levels of expression of MHC $\beta$  transcripts in the ventricle than in the atrium may correspond to the differences in hemodynamic load in the different chambers of the embryonic heart. This hypothesis is supported by the observation that, after birth, cardiac hypertrophy due to hemodynamic overload is accompanied by a reexpression of MHC $\beta$  transcripts in the rodent ventricle and a decrease in MHC $\alpha$  transcripts (e.g., Izumo et al., 1987). The changes in the pattern of expression of the MHC genes in the embryonic atria and ventricles may also be correlated with changes in the expression of other genes such as those encoding membrane proteins or cytoplasmic enzymes associated with contraction (Ingwall et al., 1985).

In the embryonic chick heart, MHC protein isoform expression is correlated with local differences in the contraction pattern of the heart (de Jong et al., 1987). There is coexpression of MHC $\alpha$  and MHC $\beta$  in parts of the heart with peristaltic contractions, but a single isoform of MHC protein is expressed in synchronously contracting parts of the heart (de Jong et al., 1987). The switch from peristaltic contraction to synchronous contraction in the chick ventricle coincides with the appearance of trabeculations in the ventricular myocardium and with a switch from coexpression of MHC proteins towards expression of MHC $\beta$  alone (de Jong et al., 1987).

The pattern of MLC gene expression in the developing mouse heart is not coordinated with that of the MHC genes. MHC $\beta$  mRNAs are restricted to ventricular cells, except for some cells of the sinuatrial transition, by 10.5 d p.c., but MLCIV mRNAs are detected in atrial cells at a high level until 11.5 d p.c., and at a low level until 15.5 d p.c. This result does not agree with that of Rovner et al. (1990), who noted that MLC1A is the exclusive MLC1 mRNA expressed in the rat atrium throughout development. MLC1A transcripts continue to be expressed at a high level in ventricular cells as the level of MHC $\alpha$  transcripts drops off gradually in the embryonic mouse ventricle. When MHC $\alpha$  transcript levels increase in ventricular cells after 16.5 d p.c., MLC1A transcript levels do not increase, but continue to be restricted to

the atrium. Since MLC1A transcripts are expressed in ventricles at stages where MHC $\beta$  is the predominant MHC isoform, and MLCIV is expressed in atria at stages where MHC $\alpha$  is the only MHC isoform, these MLCs must be able to freely associate with either MHC isoform. Whether this association of atrial and ventricular myosin isoforms plays a role in regulating contractility in the heart during development is not clear. There are conflicting reports on the effect of different MLC combinations with MHC on alterations in myosin ATPase and shortening velocity, which are known to be correlated (Barany, 1967). Wagner and Stone (1983) found that myosin alkali light chain exchange did not increase the ATPase activity of MHC S1 fragments in vitro. However, Sweeney et al. (1988) reported differences in shortening velocities correlated with myosin alkali light chain ratios in isolated skeletal muscle fibers.

The spatial pattern of decrease in the levels of MLC1A transcripts in fetal ventricles is striking. Beginning at  $\sim$ 15 d p.c., MLC1A transcripts begin to disappear in a gradient running from the epicardium toward the endocardium. A similar gradient of expression has been observed for the disappearance of MHC $\beta$  protein in the postnatal developing rat heart (Deschene et al., 1987). These authors also found that MHC $\beta$  protein reappears later in the rat heart in a gradient of expression in the opposite direction to that in which it disappeared, i.e., the endocardium toward the epicardium. Reexpression of MHC $\beta$  protein does not occur in the mature mouse ventricle (Lompré et al., 1981). Hemodynamic factors are known to influence MHC and MLC expression in the adult heart (Swynghedauw, 1986), and have been implicated in the morphogenesis of the chick heart (Clark, 1984). It remains to be established whether developmental changes in the distribution of MHC and MLC isoforms are regulated by such factors as intracardial flow patterns, pressure gradients in various chambers, and transmural gradients of wall stress.

It has been clearly demonstrated that some myosin genes are regulated differently in response to cardiac hypertrophy due to hemodynamic overload. In adult rat hearts, which normally have very low levels of MHC $\beta$  transcripts, MHC $\beta$  mRNAs accumulate in the ventricles within 2 d of the onset of cardiac hypertrophy induced by pressure overload (Izumo et al., 1987; Schiaffino et al., 1989). As MHC $\beta$  expression increases with hypertrophy, MHC $\alpha$  expression decreases. The response to cardiac hypertrophy can vary between species. For example, MLCIV was shown to be induced in overloaded atria in human subjects (Cummins, 1982; Kurabayashi et al., 1988), but not in rats (McNally et al., 1989). Some myosin genes, such as MLC1A, appear to be less responsive to cardiac hypertrophy (Rovner et al., 1990). The mechanisms by which myosin genes are regulated in response to hypertrophy are not known.

The pattern of expression of the MHC $\alpha$  gene in the developing mouse heart is unique in that, after being gradually restricted to atrial cells in the embryo, MHC $\alpha$  transcripts are reexpressed in ventricular cells from 2 d before birth, and replace MHC $\beta$  transcripts by 7 d after birth. Lompré et al. (1984) have suggested that there may be two distinct, developmentally regulated MHC $\alpha$  gene transcripts that differ only at their 3' ends. Our results do not support, but do not rule out, this hypothesis since our MHC $\alpha$  probe is complementary to the 3' UTR of the MHC $\alpha$  gene transcript.

The reexpression of MHC $\alpha$  transcripts may be due to se-



rum thyroid hormone levels in the late fetal mouse, because thyroid hormone (T3) has been shown to upregulate MHC $\alpha$  expression and to downregulate MHC $\beta$  (Lompré et al., 1984). Even though thyroid hormone levels are low at birth (Gambke et al., 1983) in rodents, there may be sufficient T3 supplied by the maternal circulation to begin the switch from MHC $\beta$  to MHC $\alpha$  gene expression in the heart. Chizzonite and Zak (1984) showed that in rats made hypothyroid from 15 d p.c., the amount of MHC $\alpha$  protein in the ventricle at 19 d p.c. is reduced compared to euthyroid controls, and that by 7 d after birth, the hypothyroid ventricle contains only MHC $\beta$ . Thus, hypothyroidism apparently reverses the switch in the two isoforms, which normally occurs during development. Other factors than T3, such as dietary carbohydrate, glucocorticoids (Sheer and Morkin, 1984), and catecholamines, particularly norepinephrine (Bishopric et al., 1989), may also be involved in this transition.

With the exception of thyroid hormone/receptor complexes, which have been shown to interact directly with the MHC $\alpha$  gene promoter (Izumo and Mahdavi, 1988; Glass et al., 1989), differentiation and transcriptional factors implicated in the initiation of the myogenic program and in myosin gene regulation in the heart are still mostly unidentified. Of the factors that have been identified in skeletal muscle, MyoD1 (Davis et al., 1987), myogenin (Wright et al., 1989), myf-5 (Braun et al., 1989), and myf-6 (Braun et al., 1990), none are expressed in cardiac muscle (Sassoon et al., 1989; Lyons et al., 1990a). Thus, the cardiac myosin genes that are coexpressed in skeletal muscle must be regulated by different mechanisms in cardiac and skeletal muscle.

Our results support the hypothesis that the mechanisms of myosin gene regulation in these two types of striated muscle must be quite different, since the developmental patterns of expression of myosin genes in skeletal muscle are unlike those detected in cardiac muscle. After an initial high level of coexpression in the myocardium of the embryonic cardiac tube, the expression of each myosin gene is modulated in a different cell-specific and stage-specific manner in developing atrial and ventricular myocytes.

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## References

- Anderson, R. H., H. Yen, Becker, and J. Gosling. 1978. The development of the sinuatrial node. *In* *The Sinus Node: Structure, Function and Clinical Relevance*. F. Bonke, editor. Martinus Nijhoff Medical Division, The Hague. 166-182.
- Barany, M. 1967. ATPase activity of myosin correlated with speed of muscle shortening. *J. Gen. Physiol.* 50:197-218.
- Barton, P., and M. Buckingham. 1985. The myosin alkali light chain proteins and their genes. *Biochem. J.* 231:249-261.
- Barton, P., A. Cohen, B. Robert, M. Fiszman, F. Bonhomme, J. Guénet, D. Leader, and M. Buckingham. 1985. The myosin alkali light chain of mouse ventricular and slow skeletal muscle are indistinguishable and are encoded by the same gene. *J. Biol. Chem.* 260:8578-8584.
- Barton, P., B. Robert, A. Cohen, I. Garner, D. Sassoon, A. Weydert, and M. Buckingham. 1988. Structure and sequence of the myosin alkali light chain gene expressed in adult cardiac atria and fetal striated muscle. *J. Biol. Chem.* 263:12669-12676.
- Bishopric, N., C. Long, L. Waspe, P. Simpson, and C. Ordahl. 1989. The molecular biology of cardiac myocyte hypertrophy. *In* *Cellular and Molecular Biology of Muscle Development*. L. Keddes and F. Stockdale, editors. A. R. Liss, Inc., New York. 399-412.
- Braun, T., G. Buschhausen-Denker, E. Bober, and H. Arnold. 1989. A novel human muscle factor related to but distinct from MyoD1 induces myogenic conversion in 10T1/2 fibroblasts. *EMBO (Eur. Mol. Biol. Organ.) J.* 8:701-709.
- Braun, T., E. Bober, B. Winter, N. Rosenthal, and H. Arnold. 1990. Myf-6, a new member of the human gene family of myogenic determination factors: evidence for a gene cluster on chromosome 12. *EMBO (Eur. Mol. Biol. Organ.) J.* 9:821-831.
- Chizzonite, R., and R. Zak. 1984. Regulation of myosin isoenzyme composition in fetal and neonatal rat ventricle by endogenous thyroid hormones. *J. Biol. Chem.* 259:12628-12632.
- Clark, E. 1984. Functional aspects of cardiac development. *In* *Growth of the Heart and Disease*. R. Zak, editor. Raven Press, New York. 81-103.
- Cummins, P. 1982. Transitions in human atrial and ventricular myosin light-chain isoenzymes in response to cardiac pressure overload induced hypertrophy. *Biochem. J.* 205:195-204.
- Cummins, P., and S. Lambert. 1986. Myosin transitions in the bovine and human heart: a developmental and anatomical study of heavy and light chain subunits in the atrium and ventricle. *Circ. Res.* 58:846-858.
- Davis, R., H. Weintraub, and A. Lassar. 1987. Expression of a single trans-fected cDNA converts fibroblasts to myoblasts. *Cell.* 51:987-1000.
- Dechesne, C., J. Leger, and J. Leger. 1987. Distribution of  $\alpha$ - and  $\beta$ -myosin heavy chains in the ventricular fibers of the postnatal developing rat. *Dev. Biol.* 123:169-178.
- De Groot, I., W. Lamers, and A. Moorman. 1989. Isomyosin expression patterns during rat heart morphogenesis: an immunohistochemical study. *Anat. Rec.* 224:365-373.
- DeHaan, R., and R. O'Rahilly. 1978. Embryology of the heart. *In* *The Heart: Arteries and Veins*. J. Hurst, editor. McGraw-Hill Inc., New York. 6-18.
- de Jong, F., W. Geerts, W. Lamers, J. Los, and A. Moorman. 1987. Isomyosin expression patterns in tubular stages of chicken heart development: a 3-D immunohistochemical analysis. *Anat. Embryol.* 177:81-90.
- Gall, J., and M. Pardue. 1971. Nucleic acid hybridization in cytological preparations. *Methods Enzymol.* 21:470-480.
- Gambke, B., G. Lyons, J. Haselgrove, A. Kelly, and N. Rubinstein. 1983. Thyroidal and neural control of myosin transitions during development of rat fast and slow muscles. *FEBS (Fed. Eur. Biochem. Soc.) Lett.* 156:335-339.
- Glass, C., S. Lipkin, O. Devary, and M. Rosenfeld. 1989. Positive and negative regulation of gene transcription by a retinoic acid-thyroid hormone receptor heterodimer. *Cell.* 59:697-708.
- Gorza, L., L. Saggini, S. Sartore, and S. Aussoni. 1988. An embryonic-like myosin heavy chain is transiently expressed in nodal conduction tissue of the rat heart. *J. Mol. Cell. Cardiol.* 20:931-941.
- Hoh, J., P. McGrath, and P. Hale. 1978. Electrophoretic analysis of multiple forms of rat cardiac myosin: effect of hypophysectomy and thyroxine replacement. *J. Mol. Cell. Cardiol.* 10:330-334.
- Ingwall, J., B. Marth, M. Kramer, B. Fifer, R. Lorell, R. Shemin, W. Grossman, and P. Allen. 1985. The creatine kinase system in renal and diseased human myocardium. *N. Engl. J. Med.* 313:1050-1055.
- Izumo, S., A. Lompré, R. Matsuoka, G. Koren, K. Schwartz, B. Nadal-Ginard, and V. Mahdavi. 1987. Myosin heavy chain messenger RNA and protein isoform transitions during cardiac hypertrophy: interactions between hemodynamic and thyroid hormone-induced signals. *J. Clin. Invest.* 79:970-977.
- Kramer, A., and L. Marks. 1965. The occurrence of cardiac muscle in pulmonary veins of rodentia. *J. Morphol.* 117:135-150.
- Kurabayashi, M., I. Komuro, H. Tsuchimochi, F. Takaku, and Y. Yazaki. 1988. Molecular cloning and characterization of human atrial and ventricular myosin alkali light chain cDNA clones. *J. Biol. Chem.* 263:13930-13936.
- Lompré, A., J. J. Mercadier, C. Wisnewsky, P. Bouveret, C. Pantaloni, A. D'Albis, and K. Schwartz. 1981. Species- and age-dependent changes in the relative amounts of cardiac isoenzymes in mammals. *Dev. Biol.* 84:286-290.
- Lompré, A., B. Nadal-Ginard, and V. Mahdavi. 1984. Expression of the cardiac ventricular  $\alpha$ - and  $\beta$ -myosin heavy chain genes is developmentally and hormonally regulated. *J. Biol. Chem.* 259:6437-6446.
- Lyons, G., J. Haselgrove, A. Kelly, and N. Rubinstein. 1983. Myosin transitions in developing fast and slow muscles of the rat hindlimb. *Differentiation.* 25:168-175.
- Lyons, G., M. Ontell, D. Sassoon, and M. Buckingham. 1990a. Developmental regulation of muscle gene expression in embryonic mouse muscle. *In* *The Dynamic State of Muscle Fibers*. D. Pette, editor, W. deGruyter, Berlin. 75-89.
- Lyons, G., M. Ontell, D. Sassoon, R. Cox, and M. Buckingham. 1990b. The expression of myosin genes in developing skeletal muscle in the mouse embryo. *J. Cell Biol.* 111:1465-1476.
- Mahdavi, V., M. Periasamy, and B. Nadal-Ginard. 1982. Molecular character-

- ization of two myosin heavy chain genes in the adult heart. *Nature (Lond.)* 297:659-664.
- Mahdavi, V., A. Chambers, and B. Nadal-Ginard. 1984. Cardiac  $\alpha$ - and  $\beta$ -myosin heavy chain genes are organized in tandem. *Proc. Natl. Acad. Sci. USA* 81:2626-2630.
- McNally, E., P. Buttrick, and L. Leinwand. 1989. Ventricular myosin light chain 1 is developmentally regulated and does not change in hypertension. *Nucleic Acids Res.* 17:2753-2767.
- Nadal-Ginard, B., and V. Mahdavi. 1989. Molecular basis of cardiac performance. *J. Clin. Invest.* 84:1693-1700.
- Nathan, H., and H. Gloobe. 1970. Myocardial atrio-venous junctions and extensions (sleeves) over the pulmonary and caval veins. *Thorax* 25:317-324.
- Pexeider, T., A. Wenink, and R. H. Anderson. 1989. A suggested nomenclature for the developing heart. *Int. J. Cardiol.* 25:255-264.
- Rovner, A., E. McNally, and L. Leinwand. 1990. Complete cDNA sequence of rat atrial myosin light chain 1: patterns of expression during development and with hypertension. *Nucleic Acids Res.* 18:1581-1586.
- Rudnicki, M., G. Jackowski, L. Saggin, and M. McBurney. 1990. Actin and myosin expression during development of cardiac muscle from cultured embryonal carcinoma cells. *Dev. Biol.* 138:348-358.
- Rugh, R. 1990. *The Mouse*. Oxford University Press, Oxford, UK. 430 pp.
- Sassoon, D., I. Garner, and M. Buckingham. 1988. Transcripts of  $\alpha$ -cardiac and  $\alpha$ -skeletal actins are early markers for myogenesis in the mouse embryo. *Development (Camb.)* 104:155-164.
- Sassoon, D., G. Lyons, W. Wright, V. Lin, A. Lassar, H. Weintraub, and M. Buckingham. 1989. Expression of two myogenic regulatory factors: myogenin and MyoD1 during mouse embryogenesis. *Nature (Lond.)* 341:303-307.
- Schiaffino, S., J. Samuel, D. Sassoon, A. Lompré, I. Garner, F. Marotte, M. Buckingham, L. Rappaport, and K. Schwartz. 1989. Nonsynchronous accumulation of  $\alpha$ -skeletal actin and  $\beta$ -myosin heavy chain mRNAs during early stages of pressure-overload-induced cardiac hypertrophy demonstrated by in situ hybridization. *Circ. Res.* 64:937-948.
- Schwartz, K., Y. Lecarpentier, J. Martin, A. Lompré, J. Mercadier, and B. Swynghedauw. 1981. Myosin isoenzymic distribution correlates with speed of myocardial contraction. *J. Mol. Cell. Cardiol.* 13:1071-1080.
- Schwartz, K., A. Lompré, P. Bouveret, C. Wisniewsky, and R. Whalen. 1982. Comparisons of rat cardiac myosins at fetal stages in young animals and in hypothyroid adults. *J. Biol. Chem.* 257:14412-14418.
- Sheer, D., and E. Morkin. 1984. Myosin isoenzyme expression in rat ventricle: effects of thyroid hormone analogs, catecholamines, glucocorticoids and high carbohydrate diet. *J. Pharmacol. Exp. Ther.* 229:872-879.
- Sissman, N. 1970. Developmental landmarks in cardiac morphogenesis: comparative chronology. *Am. J. Cardiol.* 25:141-148.
- Soussi-Yanicostas, N., J. Barbet, C. Laurent-Winter, P. Barton, and G. Butler-Browne. 1990. Transition of myosin isozymes during development of human masseter muscle: persistence of developmental isoforms during postnatal stage. *Development (Camb.)* 108:239-249.
- Sweeney, H., M. Kushmerick, K. Mabuchi, F. Sréter, and J. Gergely. 1988. Myosin alkali light chain and heavy chain variations correlate with altered shortening velocity of isolated skeletal muscle fibers. *J. Biol. Chem.* 263:9034-9039.
- Sweeney, L., R. Zak, and F. Manasek. 1987. Transitions in cardiac isomyosin expression during differentiation of embryonic chick heart. *Circ. Res.* 61:287-295.
- Swynghedauw, B. 1986. Developmental and functional adaptation of contractile proteins in cardiac and skeletal muscles. *Physiol. Rev.* 66:710-771.
- Syrový, I. 1985. Atrial and ventricular myosin ATPase-divergence with increasing animal species size. *Pfluegers Arch.* 404:80-82.
- Theiler, K. 1989. *The House Mouse: Atlas of Embryonic Development*. Springer-Verlag, New York. 178 pp.
- Viragh, S., and C. Challice. 1982. The development of the conduction system in the mouse embryo heart. IV. Differentiation of the atrioventricular conduction system. *Dev. Biol.* 89:25-40.
- Wagner, P., and D. Stone. 1983. Myosin heavy chain-light chain recombinations and interactions between the two classes of light chains. *J. Biol. Chem.* 258:8876-8882.
- Weiczorek, D., M. Periasamy, G. Butler-Browne, R. Whalen, and B. Nadal-Ginard. 1985. Coexpression of multiple myosin heavy chain genes, in addition to a tissue-specific one, in extraocular muscle. *J. Cell Biol.* 101:618-629.
- Weydert, A., P. Daubas, I. Lazaridis, P. Barton, I. Garner, D. Leader, F. Bonhomme, J. Catalan, D. Simon, J. Guenet, F. Gros, and M. Buckingham. 1985. Genes for skeletal muscle myosin heavy chains are clustered and are not located on the same mouse chromosome as a cardiac myosin heavy chain gene. *Proc. Natl. Acad. Sci. USA* 82:7183-7187.
- Whalen, R., and S. Sell. 1980. Myosin from fetal hearts contains the skeletal muscle embryonic light chain. *Nature (Lond.)* 286:731-733.
- Whalen, R. G., S. M. Sell, G. Butler-Browne, K. Schwartz, P. Bouveret, and I. Pinset-Harstrom. 1981. Three myosin heavy chain isozymes appear sequentially in rat muscle development. *Nature (Lond.)* 292:803-809.
- Wright, W., D. Sassoon, and V. Lin. 1989. Myogenin, a factor regulating myogenesis, has a domain homologous to MyoD1. *Cell* 56:607-617.
- Zhang, Y., S. Shafiq, and D. Bader. 1986. Detection of a ventricular-specific myosin heavy chain in adult and developing chicken heart. *J. Cell Biol.* 102:1480-1484.