Sequential Activation of α-Actin Genes during Avian Cardiogenesis: Vascular Smooth Muscle α-Actin Gene Transcripts Mark the Onset of Cardiomyocyte Differentiation

Deborah L. Ruzicka,* and Robert J. Schwartz*

*Department of Cell Biology, Baylor College of Medicine, Houston, Texas 77030; and
†Department of Surgical Research, The Children's Hospital, Harvard Medical School, Boston, Massachusetts 02115

Abstract. The expression of cytoplasmic β-actin and cardiac, skeletal, and smooth muscle α-actins during early avian cardiogenesis was analyzed by in situ hybridization with mRNA-specific single-stranded DNA probes. The cytoplasmic β-actin gene was ubiquitously expressed in the early chicken embryo. In contrast, the α-actin genes were sequentially activated in avian cardiac tissue during the early stages of heart tube formation. The accumulation of large quantities of smooth muscle α-actin transcripts in epimyocardial cells preceded the expression of the sarcomeric α-actin genes. The accumulation of skeletal α-actin mRNAs in the developing heart lagged behind that of cardiac α-actin by several embryonic stages. At Hamburger-Hamilton stage 12, the smooth muscle α-actin gene was selectively down-regulated in the heart such that only the conus, which subsequently participates in the formation of the vascular trunks, continued to express this gene. This modulation in smooth muscle α-actin gene expression correlated with the beginning of coexpression of sarcomeric α-actin transcripts in the epicardium and the onset of circulation in the embryo. The specific expression of the vascular smooth muscle α-actin gene marks the onset of differentiation of cardiac cells and represents the first demonstration of coexpression of both smooth muscle and striated α-actin genes within myogenic cells.

The high degree of sequence conservation between the actin proteins of a eucaryotic species suggests that this multigene family arose by duplication and subsequent divergence from a common ancestral gene (44). In the course of these events, certain regulatory and structural structures of the actin loci diversified to produce the specialized actin genes observed today (5). In birds and mammals amino acid sequence analysis (42, 43) and gene isolations (3, 5, 9, 12, 14) have revealed the presence of three distinct types of actin isoforms, which can be classified as "cytoplasmic," "striated," or "smooth muscle" in origin based on their predominant distribution in adult tissues. Each of these three actin types is represented by at least a pair of cognate actin genes. For example, the cytoplasmic actins, including β and γ (43), as well as the novel avian type 5 (3) isoforms, are enriched in nonmuscle cells where they participate in the formation of the cytoskeletal apparatus and cellular microfilaments that function in cell motility, mitosis, and related processes (44). In contrast the striated (α-cardiac and α-skeletal) and smooth muscle (α-smooth and γ-smooth) actins compose the sarcomeres and myofibrils of warm-blooded vertebrate contractile tissues (42-44).

In adult muscle tissues, skeletal α-actin represents the predominant sarcomeric form in skeletal muscle, while α-cardiac is the most abundant striated actin in heart tissue (42, 45). Likewise, smooth muscle α-actin is the major isof orm of vascular tissue, such as the aorta, while γ-smooth muscle actin predominates in the gastrointestinal and genital tracts (42). Although adult muscle tissue preferentially expresses a single actin isoform, coexpression of the various actin gene pairs occurs during muscle cell differentiation in vivo and in vitro (2, 13, 17, 25, 28, 31, 32). Cytoplasmic and striated actins may be present within a single myogenic cell. For instance, β- and γ-cytoplasmic actins and the α-sarcomeric actin (α-skeletal and α-cardiac actin) mRNAs are coexpressed in developing avian heart and limb tissues (2, 17). Similarly, the smooth muscle and cytoplasmic actin pairs have been identified in embryonic chicken gizzard (35) and rat aorta and vena cava (43). The coexpression of certain actin pairs during early developmental processes is not unexpected, considering that these genes might still share some evolutionarily conserved regulatory sequences. However, to date a coexpression of both striated and smooth muscle actins in the same tissue has not been reported. In particular, little information has been available about the smooth muscle α-actin gene activity in early myogenic cell lineages in develop-
ing embryos when coexpression of α-striated actins has been observed.

The recent isolation and delineation of the structures of the avian muscle α-actin genes (5, 8, 9) have allowed us to map a dynamic pattern of actin gene activities during the early stages of heart formation in the chick embryo. Previous analysis of actin profiles in embryonic avian heart tissue by two-dimensional PAGE indicated that muscle-specific α-actin biosynthesis is initiated during the early stages of cardiacogenesis (46). However, this technology could not differentiate between the three different α-actin isoforms. Instead, we have constructed specific actin mRNA hybridization probes from the 3′ transcribed but untranslated regions of the avian actin genes (6, 17) for quantitation and in situ localization of individual actin transcripts. We report that the smooth muscle α-actin gene is broadly expressed in the heart during the earliest phases of cardiogenesis. α-Cardiac and α-skeletal actin gene transcripts accumulate sequentially during later phases of heart formation. When α-cardiac actin mRNA levels predominate in the beating heart, the smooth muscle α-actin gene activity becomes down-regulated and restricted in a region-specific manner. Our data indicates that the specific expression of the smooth muscle α-actin gene marks the earliest appearance of embryonic cardiac cells and the first demonstration of coexpression of both smooth muscle and striated α-actin genes within myogenic cells.

Materials and Methods

Animal Material

Fertilized White Leghorn chicken eggs were purchased from Texas A & M University (Poultry Sciences, College Station, TX) and were incubated at 37°C. Developing embryos were carefully staged on the basis of morphological criteria described by Hamburger and Hamilton (15).

Preparation of 32P-labeled M13 Probes

We previously described the construction of messenger RNA-specific probes from the 3′-untranslated regions of chicken actin genes cloned into M13 vectors (6, 17). Uniformly labeled, single-stranded DNA probes were synthesized from M13 templates by primer extension reactions using [α-32P]dCTP and [α-32P]dTTP (3,000 Ci/mmol; ICN Biomedicals Inc., Irvine, CA) according to the methods of Bergsma et al. (3) with a specific activity of 1 × 106 cpm/μg DNA. The single-stranded 32P-labeled DNA fragments, ~200 bases long, were eluted from polyacrylamide gel as previously described (3). Probes for dot blot analysis were added directly to the hybridization buffer (6× SSC, 50 mM sodium phosphate, pH 7.0, 0.5% SDS, 0.02% Ficoll, and 0.02% polyvinyl pyrroliodine). For in situ hybridization experiments, probes were ethanol precipitated overnight at −20°C after the addition of 0.1 vol 3 M sodium acetate, 100 μg/ml RNA, and 25 μg/ml sheared, calf thymus DNA. Probes were dissolved in deionized formamide at 68°C and diluted 1:1 with a 2× buffer consisting of 4× SSC, 20 mM vanadyl sulfate, 20% dextran sulfate, and 2% bovine serum albumin, as described by Lawrence and Singer (22).

RNA Isolation and Analysis

Staged embryos were explanted under sterile conditions, quick frozen in liquid nitrogen, and stored at −80°C. RNA was isolated according to the methods of Chirgwin et al. (10). RNA samples used for dot blots were glyoxylated (39), sample volumes were adjusted to 200 μl with 15× SSC, and the samples were spotted onto moistened nylon membranes under low vacuum in a Minifold apparatus (Bio-Rad Laboratories, Richmond, CA). Wells were rinsed with 200 μl of 20× SSC and the blots were baked for 2 h at 80°C. Glyoxal was dissociated from bound RNA by boiling the nylon membranes in 20 mM Tris-HCl (pH 8.0) for 5 min, after which they were air dried. The blots were hybridized with 1 × 106 cpm/ml of a 32P-labeled, single-stranded DNA probe as previously described (17). Washed blots were exposed to Kodak XAR-5 film at −20°C with a Cronex Lighting Plus intensifying screen (DuPont Co., Wilmington, DE).

In Situ Hybridization Methods

Staged embryos were fixed for 30 min at room temperature in 4% paraformaldehyde in PBS, pH 7.4, dehydrated in an ethanol series, cleared in xylene, and embedded in Paraplast II (Fisher Scientific Co., Allied Corp., Pittsburgh, PA). Tissue sections (4 μm) were cut on a conventional (AO) microtome, mounted on sterile, polylysine-coated slides, and baked for 1–2 h in a 57°C oven to insure adhesion of the sections to the slides. Slides were stored in a desiccated chamber at 4°C until required for in situ hybridization experiments.

The in situ hybridization methods used in these studies were modifications of those described by Cox et al. (11) and Lawrence and Singer (22). Briefly, histological sections were deparaffinized in xylene and rehydrated in an ethanol series. Samples were rinsed in PBS containing 2 mM EDTA and digested with 1 μg/ml proteinase K in 100 mM NaCl, 10 mM Tris-HCl, pH 7.4, 2 mM EDTA for 15 min at 37°C. The enzyme was inactivated by postfixation in PBS-buffered, 4% paraformaldehyde, pH 7.4, and sections were washed for 10 min in 200 mM Tris-100 mM glycine, pH 7.6. To reduce nonspecific binding of the probes, sections were treated with 0.25% (vol/vol) acetic anhydride in triethanolamine buffer for 10 min, according to the methods of Hayashi et al. (46). After a brief rinse in 2× SSC, tissue sections were prehybridized in 50% formamide-2× SSC for 30 min at 37°C before application of the 3′-specific actin mRNA probes. In some cases, the treated slides were dehydrated in a graded series of ethanol, air dried, and stored at 4°C for use on the next day. In those cases, tissue sections were rehydrated in PBS-2 mM EDTA before prehybridization.

10 μl of the hybridization buffer containing 1 × 106 cpm of a given 32P-labeled M13 probe were applied to each tissue section on a slide. Sections were covered with small pieces of parafilm, and the slides were transferred to a humidified, 37°C incubator for 18–20 h. After hybridization, slides were washed at 37°C in copious volumes of 50% formamide-2× SSC over a 60-min period, followed by another 30-min wash in 50% formamide-1× SSC. A final wash in 1× SSC was performed at room temperature, after which the slides were dehydrated through an ascending series of ethanol (70–99%) containing 50 mM ammonium acetate, and then air dried. Hybridization signals were localized by autoradiography using Kodak NTB-2 nuclear track emulsion. Sections were stained with hematoxylin and eosin, and coverslips were mounted in permount before examination under brightfield and darkfield optics on a Zeiss photomicroscope.

Results

Specific hybridization probes composing the 3′-noncoding regions of the β-cyttoplasmic, and α-cardiac, α-skeletal, and α-smooth muscle actin genes have been reported (6, 17, 47) and schematically represented in Fig. 1. These uniformly 32P-labeled, single-stranded DNA probes prepared from M13 vectors enabled us to analyze and compare the temporal and spatial patterns of expression of four major actin genes in developing avian embryos.

Sequential Appearance of Actin mRNAs

Total RNA extracted from early chicken embryos (~6–100 h in ovo (Hamburger-Hamilton [HH] stages 1–24) was analyzed by RNA dot blots (Fig. 2). The morphogenetically active, prespreak blastoderm (HH stage 1/2) contained significant quantities of β-cyttoplasmic actin gene transcripts in its cellular RNA pools (Fig. 2 a). In addition, the relative abundance of β-cyttoplasmic actin mRNA per microgram total RNA remained essentially constant through at least 5 d in ovo (HH stage 27), during which time the rudiments of the major organ systems have formed (Fig. 2). Therefore, β-cyttoplasmic mRNAs are a major component of the overall actin

1. Abbreviation used in this paper: HH, Hamburger-Hamilton.

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Figure 1. Chicken actin gene structures and 3'-specific non-coding M13 probes. Exons are shaded black, introns are white, and the untranslated regions are stippled. The sizes of the indicated 3'-noncoding sequences inserted into the listed M13 phages are: (a) cytoplasmic β-actin, 213 nt; (b) cardiac α-actin, 164 nt; (c) skeletal α-actin, 221 nt; and (d) smooth muscle α-actin, 140 nt.

Figure 2. Dot blots of actin mRNAs in staged chicken embryos. Serial dilutions of total RNA isolated from Hamburger and Hamilton (15) staged chicken embryos were spotted onto nylon filters and hybridized with 32P-labeled, single-stranded 3'-specific M13 probes for (a) β-cytoplasmic, (b) α-cardiac, (c) α-skeletal, or (d) α-smooth actin mRNAs. RNA was isolated from embryos (E), muscle (M), heart (H), gizzard (G), brain (B), and liver (L).
gene expression profile in both early and later stages of development.

In contrast to the β-cytoplasmic actin gene which appears to be constitutively expressed during early development, the appearance of transcripts from the three α-actin genes occurs late, after the formation of the primary embryonic axis (Fig. 2, b–d). Lower, but consistent signals were first detected for smooth muscle and cardiac α-actin transcripts at HH stages 8 and 9, respectively, at ~30 h in ovo (Fig. 2, b and d). However, skeletal muscle α-actin mRNAs were not detected in samples of total RNA isolated from embryos before HH stage 12, ~45–50 h in ovo (Fig. 2 c). Thus, the onset in expression of these three genes appears to represent at least two discrete regulatory events.

Comparison of RNA dot blot data on selected tissues on HH stage-36 and ~45 embryos indicates that profiles of 10-d embryos are similar, but not identical, to those of the pre-hatching, 19-d chick. The patterns of expression of the actin genes exhibit stage- and tissue-dependent variations. For example, cardiac and skeletal α-actin mRNAs are coexpressed in the hearts of HH stage-36 embryos, while a more mature tissue profile for breast muscle, in which α-skeletal transcripts predominate, does not emerge until later in development (Fig. 2). This observation is consistent with a previous report by Hayward and Schwartz (31) in which actin mRNAs in embryonic and posthatching chicken thigh muscles were quantified. Although less dramatic than the α-actin scenario, the β-cytoplasmic actin mRNA profile suggests its expression is also modulated during the course of growth and differentiation in various tissues. The relative abundance of these transcripts per microgram total RNA in both heart and breast muscle declines between 10 and 19 d in ovo.

**In Situ Hybridization Analysis in the Early Avian Embryo**

Although RNA blot analysis of whole embryos illustrated the temporal appearances of α-actin gene mRNAs during early development, these studies did not allow identification of specific tissue or tissue components, which were responsible for the observed hybridization signals. As a result, we conducted in situ hybridization experiments on paraffin sections to determine the localization and identity of actin gene transcripts expressed during early embryogenesis. Fig. 3, a and b show a representative autoradiogram on a section through the primitive streak region of an HH stage-5 embryo, photographed under brightfield and darkfield optics, respectively. β-Cytoplasmic actin gene transcripts were observed in all regions of the embryonic blastoderm, confirming the results of our RNA blot analyses. The hybridization signal is strong and confined to the cellular layers of the gastrulating embryo. This image contrasts strongly with tissue sections hybridized with either the α-cardiac (Fig. 3, c and d) or α-skeletal (Fig. 3, e and f) probes, in which little or no binding was observed under otherwise identical conditions. Similarly, no significant accumulation of α-smooth actin transcripts was found prior to HH stage 7/8 (Fig. 3, g and h).

**α-Smooth Muscle Actin mRNA First Appears in the Tubular Heart**

The expression profile of α-actin genes at HH stage 9/10 was dramatically different from that of earlier embryos (Fig. 4). We observed the first appearance of smooth muscle α-actin transcripts along the entire length of the developing heart. This strong signal was superimposed over the tubular heart proper, including anterior structures corresponding to the presumptive conus arteriosus and ventral aorta, as well as cardiogenic cells located along the roots of the omphalomesenteric veins which contribute to the formation of the atrial and sinus venosus regions of the heart (Fig. 4, g and h). α-Cardiac transcripts were first detected at this time as a discrete, localized weaker signal in the presumptive ventricular myocardium of the fusing heart primordia (Fig. 4, c and d). Occasionally we observed a very weak hybridization signal with the α-cardiac probe in HH stage-8 embryos in the thickened splanchnic mesoderm which forms the proximal wall of the amniocardiac vesicle (data not shown). As with earlier stages, skeletal muscle α-actin transcripts showed no appreciable accumulation over background and no apparent localization by 30 h in ovo (Fig. 4, e and f), while significant quantities of β-cytoplasmic actin mRNAs were detected in all embryonic tissues (Fig. 4, a and b). In contrast, the endocardium showed no affinity for any of the α-actin mRNA probes at any stage. This is the first demonstration of the broad expression of the smooth muscle α-actin gene during the early phases of cardiogenesis and blood vessel formation in higher vertebrates.

**α-Cardiac and α-Skeletal Actin mRNAs Appear Sequentially**

In later stages of cardiac morphogenesis, the pattern of α-actin gene expression is further modified. As predicted from the RNA blot studies in Fig. 2, the in situ data indicated that cardiac α-actin transcripts accumulate over time to significant levels throughout the epimyocardium of the tubular heart (compare Figs. 4, c and d, 5, a–f, and 6, c and d). The spread of the cardiac α-actin mRNA signal, initially localized in the ventricular myocardium at HH stage 9, to other regions of the epimyocardium, spatially mimicked the regional progression of myofibrillogenesis in the developing chicken heart described by Hiruma and Hirakow (19). Using 32P-labeled probes, we found no evidence of regional differences in silver grain density along the length of the tubular heart which might correlate with cardiac looping. However, in contrast to other regions of the heart, little or no α-cardiac signal was apparent at the posterior boundary of the sinuatrial region (Fig. 5, c–f). Nevertheless, this trend of increased α-actin mRNA accumulation correlates well with the ongoing histogenesis and functional differentiation of the heart and agrees with studies on contractile protein profiles of embryonic avian striated muscle which indicate that actin and myosin content of the embryonic chicken heart rise linearly during ontogeny (23, 46).

Concurrent with a marked increase in the cardiac α-actin mRNA signal in the heart at HH stage 12 (Fig. 5, c and d, and Fig. 6, c and d), significant changes in the expression of the two other α-actin genes were also observed at this stage. Skeletal muscle α-actin transcripts were detected in the heart tissue at this time (Fig. 6, e and f). Although the α-skeletal signal was relatively low at this stage, its spatial distribution was largely identical to that of α-cardiac mRNAs. Under darkfield optics the epimyocardium was outlined by silver grains (Fig. 6, e and f), however, the conus region ex-
Figure 3. Actin gene expression in the early chicken embryo. Brightfield (a, c, e, and g) and darkfield (b, d, f, and h) micrographs of embryonic tissue sections hybridized in situ with 3' specific probes for β-cytoplasmic (a and b), α-cardiac (c and d), α-skeletal (e and f), or α-smooth (g and h) actin mRNAs. Transverse sections through the primitive streak region of HH stage-5 embryos (a-f) or neural fold region of an HH stage-8 embryo (g and h). Bar, 250 μm.
Figure 4. Appearance of smooth muscle and cardiac α-actin RNA transcripts in the paired heart tube. In situ localization of β-cytoplasmic (a and b), α-cardiac (c and d), α-skeletal (e and f), or α-smooth (g and h) actin transcripts in HH staged-9/10 embryonic tissue sections. The autoradiographs have been photographed under brightfield (a, c, e, and g) and darkfield (b, d, f, and h) optics. Arrows indicate the location of the presumptive embryonic myocardium. H, head ectoderm; P, anterior intestinal portal; S, somites; VV, omphalomesenteric veins. Bar, 250 μm.
Figure 5. Cardiac α-actin gene RNA transcripts accumulate during the formation of the heart before its expression in somites. (a and b) Frontal section of an HH stage-10 chicken embryo through the tubular heart. Arrows indicate the developing epimyocardium. B, forebrain; G, foregut; VV, omphalomesenteric vein. (c and d) Representative section through the tubular heart of an HH stage-12/13 embryo. C, conus; SA, sinoatrial region; V, ventricle. (e and f) Sagittal section through cardiac and somitic tissues of an HH stage-12 embryo. Arrows indicate the location of somitic myoblasts exhibiting a low level α-cardiac signal. S, somites; SA, sinoatrial region of the heart. (g and h) Higher magnification of the somite region is shown in g and h. Bars: (a-d) 250 μm; (e-h) 100 μm.
Figure 6. Skeletal α-actin transcripts appear last, while smooth muscle α-actin gene expression becomes regionally localized after the formation of heart. Tissue sections were hybridized with 32P-labeled M13 probes specific for β-cytoplasmic (a and b), α-cardiac (c and d), α-skeletal (e and f), or α-smooth muscle (g and h) actin mRNAs in HH stage-12/13 embryos. C, conus; SA, sinoatrial region; V, ventricle; VA, ventral aorta. Bar, 250 μm.

Subsequently, the dermomyotome component of the more advanced, anterior somites in HH stage-13/14 embryos showed a slightly higher affinity for the α-skeletal probe than the surrounding tissues. Similarly, the α-smooth actin mRNA signal was negligible in somitic cells of embryos before HH stage 14. Thus, in the avian embryo, the expression of the sarcomeric α-actin genes in the somites lagged significantly behind that in the heart.

**Down-Regulation of α-Smooth Muscle Actin Gene Activity**

The smooth muscle α-actin signal profile at later stages is

Hibited a somewhat higher signal than other areas of the epimyocardium. This result was reproducible, and this local or regional difference persists at least through HH stage 14/15 (Fig. 7, a and b).

We observed that α-actin transcripts were not detected in the somites before HH stage 12, although β-cytoplasmic actin mRNAs were abundant in these structures, as elsewhere in the embryo (Fig. 5). In parasagittal sections low level α-cardiac signals were occasionally observed in a small population of longitudinally oriented, elongated cells immediately underlying the dorsal somitic epithelium in anterior regions of HH stage-12 and -13 embryos (Fig. 5, e–h).
characterized by a secondary localization. Although widely distributed in the epimyocardium of the heart between HH stages 8 and 10, smooth muscle α-actin transcripts were restricted to cells residing in the anteriormost regions of the heart, including the conus arteriosus and its extension into the ventral aorta by HH stage 12 (Fig. 6, g and h). Smooth muscle α-actin mRNAs were also detected in the developing extraembryonic vasculature at this stage (data not shown). This secondary localization of the α-smooth mRNA signal is apparently stable in subsequent stages (Fig. 7, c and d), and ultimately the signal was confined to differentiating smooth muscle of the vascular system (Fig. 8). Thus, the smooth muscle α-actin gene appears to be down-regulated by HH stage 12 in avian epimyocardial cells which will not participate in the formation of vascular structures (compare Figs. 4, g and h, and 6, g and h).

Discussion

In recent years the notion that the sarcomeric α-actin genes are coordinately expressed in striated muscle tissue has gained wide acceptance (13, 25, 31, 32, 45). Coordinate expression of the two striated α-actin genes is thought to be an early marker of myogenic commitment or determination. Mohun and co-workers (30) have observed that the cardiac and skeletal α-actin genes are transcribed simultaneously in skeletal muscle lineages at the early neurula stage in *Xenopus laevis* embryos. Our data indicates that the α-actin genes are not coordinately regulated during early heart formation (Fig. 9). Smooth muscle α-actin gene activity appears first between HH stages 8 and 9 and demarcates the beginning of cardiomyocyte differentiation in the avian embryo. RNA blots and in situ hybridization analysis revealed the dominance of the smooth muscle α-actin transcripts over those of the other α-actins and agrees temporally with the observation that α-actin biosynthesis is initiated in the cardiac mesoderm of HH stage-8/9 avian embryos before fusion of the cardiac vesicles and formation of the definitive tubular heart (46). The activation of the sarcomeric α-actin genes in heart tissue follows subsequently the formation of primary organ rudiments in the chicken embryo, with expression of the cardiac isotype preceding skeletal by two to three HH stages. Thus, the early appearance of the three actin mRNA isotypes during organogenesis is sequential, rather than coordinate in nature which contrasts with the situation in *Xenopus* embryos. Consistent with these findings, sequential and inde-
Figure 8. The expression of smooth muscle α-actin gene transcripts appears in the vasculature in later stage chicken embryos. In situ localization of smooth muscle α-actin RNA in transverse sections through the heart-associated blood vessels (asterisks) of a 6-d embryo (a and b).

Figure 9. α-Actin gene expression during early avian development. The temporospatial localization of α-actin gene transcripts in the heart tissue of chicken embryos at various stages during morphogenesis is indicated. Diagrammatic representations of different stages of avian cardiogenesis have been adapted for Patten (33).
dependent activation of the sarcomeric α-actin genes has also been reported in embryonic avian skeletal myoblast cultures (17, 18). Furthermore, the observation that the order of appearance and rate of accumulation of these transcripts is species specific and differs in cardiac and somitic tissues suggests that the interaction of different tissue primordia and the consequent variations in the local inductive environment are important factors in the expression of the α-actin genes.

The observed accumulation of significant quantities of vascular smooth muscle α-actin transcripts beginning at HH stage 8 correlates more closely with the estimated onset of α-actin protein biosynthesis (between HH stages 8 and 9) in the avian cardiac mesoderm (46) than do the expression profiles of the two sarcomeric α-actin genes. Furthermore, differences in the tryptic maps of α-actins isolated from a pooled sample of HH stage 9-13 embryonic chicken cardiac tissue versus adult avian hearts (46) are suggestive that the smooth muscle isoform is present together with sarcomeric α-actin in the early embryonic myocardium. In addition, ultrastructural differentiation of the embryonic avian epicardium begins approximately at HH stage 9, at which time microfilament-like bundles are first visible in the cytoplasm (19, 20, 23, 25, 26). Since the earliest myocardial filament bundles are reminiscent of the organization in smooth muscle (4), it is possible that the primitive contractile apparatus of differentiating avian cardiomyocytes may in fact be largely composed of the smooth muscle α-actin isoform during this early transitional phase. Such a filament system could be important in supporting the early contractile functions of the immature heart at a time when sarcomerogenesis is just beginning (20, 21, 38, 48). The recent immunohistochemical identification of significant quantities of the vascular smooth muscle α-actin protein isoform in embryonic rat myocardium (Lessard, J. L., and N. M. Sawtell, personal communication) indicates that early expression of this gene is a general characteristic of warm-blooded vertebrate cardiogenesis. In retrospect, the induction of this gene during early heart formation is not surprising since the embryonic heart is derived from paired endothelial tubes within the splanchnic mesoderm, thus in part resembling the developmental origin of vascular primordia. Taken together, these observations suggest that smooth muscle α-actin may be broadly classified as an embryonic sarcomeric actin during heart development.

The nature of the mechanisms regulating the timing and coordination of isotypic shifts in expression of actin and other contractile protein genes during ontogeny remains largely undefined. Although contractile protein expression is regulated at many points along the expression pathway, transcriptional controls appear to play a major role in regulating the overall level of expression of muscle-specific genes and their translational products. Indeed, a strong correlation between the relative abundance of contractile protein mRNAs and the ratios of contractile protein isoforms synthesized or accumulated by embryonic and adult striated muscle in vivo and in vitro has been reported (27, 34, 37, 45). Recently, Minty et al. (29) have proposed that cell- and stage-specific activation and modulation of the transcriptional activity of these genes involves separate mechanisms. This model fits reasonably well with the observation that the steady-state levels of the various actin transcripts in striated muscle are modulated in a tissue-specific manner at defined stages during ontogeny. However, the additional cellular or regional specificity of contractile protein expression profiles in striated muscle tissues suggests that localized cellular factors play a significant role in these events (1). For example, a correlation between stretch or contractile activity and expression of muscle-specific proteins in cardiac and skeletal muscle tissues and cell cultures has been observed (7, 24, 40). Thus, it may be physiologically relevant that a striking rise in expression of the sarcomeric α-actin genes in the avian heart correlates approximately with the establishment of the intra- and extra-embryonic circulations of the chicken embryo at HH stage 12 (33). Analogously, an increase in α-actin gene expression in the somites occurs at the time of decondensation of the tissue blocks and myotomal cell elongation and mobilization (Ruzicka, D., and R. Schwartz, manuscript submitted for publication).

The early activation followed by permanent down-regulation of smooth muscle α-actin gene expression in those epimyocardial cells that are not destined to form vascular elements, and the later increase of these transcripts in the extramembranous vasculature further implicates the cellular microenvironment, cell-cell interactions, or physiological factors in the regulation of this and other muscle-specific genes. The recent identification of a negative regulatory element and a modulatory sequence, upstream of the constitutive core promoter region of the smooth muscle α-actin gene (6) suggests a mechanism by which cell-specific factors could control actin gene expression. Specific trans-acting cellular factors that control these regulatory elements could themselves be differentially expressed or modified as a result of the variation in the local tissue environment and therefore be the regionally specific factors that modulate smooth muscle α-actin gene expression as observed during early avian cardiovascular development.

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