Expression of the Sarcomeric Actin Isogenes in the Rat Heart With Development and Senescence

Lucie Carrier, Kenneth R. Boheler, Catherine Chassagne, Diane de la Bastie, Claudine Wisnewsky, Edward G. Lakatta, and Ketty Schwartz

Sarcomeric actin genes, α-cardiac and α-skeletal, are coexpressed in neonatal rodent hearts and are regulated in response to hormonal and hemodynamic stimuli; however, their precise developmental pattern of expression has not been determined, and it is unknown whether they are coexpressed during senescence. We have, therefore, investigated the accumulation of sarcomeric actin transcripts in rat heart during fetal and postnatal development and with senescence by two different techniques: primer extension analysis with an oligonucleotide common to both sarcomeric actins and RNA hybridization with specific cardiac α-actin cRNA probes. We found that at 17–19 days in utero both isoforms are coexpressed and α-skeletal actin mRNAs represent 28.0 ± 0.8% of the sarcomeric actin mRNA total. Skeletal actin mRNAs increase to 40% of the total 1 week after birth (NS, p = 0.15), remain constant for 3 weeks, and decrease to less than 20% of the total in ventricles and atria of 1-month-old rats. The α-skeletal actin transcripts further decline to less than 5% of the total at 2 months of age and do not reaccumulate in senescent animals. There was no significant difference between male and female rat ventricles. By comparison with the known accumulations of α- and β-myosin heavy chain mRNAs, our results demonstrate that whatever the developmental stage the kinetics of expression for the sarcomeric myosin and actin multigene families are independent. (Circulation Research 1992;70:999–1005)

KEY WORDS • α-skeletal actin • α-cardiac actin • development • aging • rat

In mammals, six different actin isoforms are known to exist, two of which, α-skeletal and α-cardiac, are found only in striated muscles.1 Among animal species and muscle types, the quantity of the actin isoforms varies dramatically with development or physiological and pathological conditions.2 These two isoforms are almost identical, differing by only four amino acids over 375 residues.1 Two of the four differences are located at the amino terminus, the region of the protein that binds myosins. Because of the close similarity between the sarcomeric actin isoforms and, consequently, the difficulty in measuring the abundance of these proteins in striated muscle, it has proven advantageous to study the relative mRNA accumulations of the actin isoforms. These transcripts are the products of two different genes whose nucleic acid sequences are highly conserved,3,4 excluding their 5' and 3' untranslated regions, which markedly differ5,6 and can be used to unambiguously distinguish between the two mRNA isoforms. A number of studies have taken advantage of these differences to show that the amounts of each isoactin mRNA, like that for the proteins, vary with species, muscle type, and development; however, most studies were conducted with skeletal muscle, and less is known for cardiac muscle.

In humans, both sarcomeric actin mRNAs are coexpressed4,7; the α-skeletal actin mRNA isoforms represent a minor part of the sarcomeric actins in fetal hearts that increase after birth and accumulate to higher levels than those for the α-cardiac actin mRNAs in adults.8 In chick heart, the sarcomeric actin transcripts accumulate in similar proportions as early as 2.5 days in ovo, and in adults, the α-cardiac mRNA isoforms predominate substantially over the skeletal form.9 In mouse hearts, the two sarcomeric actins are coexpressed in utero (7.5 days postcoitus)10 and the α-skeletal actin declines after birth.3 In rat heart, coaccumulations of the two sarcomeric actin mRNAs have been demonstrated in newborn muscle,3,6,11 and the α-skeletal actin mRNA isoforms are hardly detectable in adult ventricles.6,12 Like the α-skeletal actin mRNA isoforms, β-myosin heavy chain (β-MHC) transcripts predominate in the rat heart during late fetal stages. They are rapidly downregulated after birth13 and reaccumulate with senescence.14 If by loose analogy the sarcomeric actins share common regulatory pathways with those for the β-MHCs, then one could hypothesize that α-skeletal actin transcripts would predominate in late fetal rat hearts and would...
accumulate with senescence. It was therefore the purpose of this study to precisely measure the amounts of the sarcomeric actins with ontogeny and aging and by indirect comparisons determine if the accumulations of α-skeletal actin and β-MHC mRNAs share common regulatory pathways. Because the expression of the isoactin transcripts in atria was unknown, it was also analyzed at different developmental stages.

To address these questions, the proportions of the two sarcomeric actin transcripts in rat hearts have been measured by primer extension assays that simultaneously allow a precise quantification of each mRNA isoform, and the total amount of sarcomeric actins was determined by RNA hybridization techniques. Our results demonstrated that both sarcomeric actin mRNAs were present in late fetal, neonatal, and young rat ventricles and decreased at age 1 month relative to rRNA and total poly(A+) mRNA, that an important contributing factor to this diminution was the decrease in the α-skeletal actin mRNA isoform, and that the α-skeletal actin mRNAs did not reaccumulate with aging.

### Materials and Methods

#### Preparation of Total Cellular RNA

Wistar rats ranging in age from 17 days in utero to 24 months after birth were used in this study. Rats older than 2 months were obtained from the Gerontology Research Center, Baltimore, Md. Total RNA was prepared from atria, ventricles, liver, and skeletal muscle by guanidine isothiocyanate extraction, cesium chloride gradient sedimentation, and two consecutive ethanol precipitations in the presence of sodium or potassium acetate. The RNA concentrations were determined using standard spectrophotometric techniques. One absorbance unit at 260 nm in a 1.0-cm light path cuvette was assumed to be equal to 40 μg/ml RNA. RNA was stored until used as a suspension in 70% ethanol containing 0.3 M sodium or potassium acetate (pH 5.5) at −20°C.

#### Primer Extension Assay

The relative proportions of rat α-skeletal and α-cardiac actins were measured simultaneously in the same sample of RNA by the technique of primer extension. This method takes advantage of differences in length of the 5′ noncoding region of the sarcomeric α-actin mRNA in the rat. An 18-base oligonucleotide complementary to a region of exon 2 (codons 31–37) common to both rat α-skeletal and α-cardiac actin genes and differing by one nucleotide from α-smooth actin and by four nucleotides from γ-smooth actin was synthesized at the Pasteur Institute, Paris. The primer was purified on a 20% denaturing polyacrylamide gel, 5% end-labeled with [γ-32P]ATP (New England Nuclear, Boston) by using T4 polynucleotide kinase (Boehringer) for 1 hour at 37°C, and then separated from the free nucleotides by chromatography on a NENSORB 20 column (New England Nuclear). One picomole of the purified and labeled primer was then added to 10 μg total RNA in the reverse transcriptase reaction (Moloney murine leukemia virus reverse transcriptase, Bethesda Research Laboratories) and incubated exactly as described previously. The oligonucleotide serves as a primer for the reverse transcriptase, which yields fragments of 186 and 195 bases for the rat skeletal and cardiac α-actins, respectively. The precipitated and denatured (85% formamide) reaction products were loaded and separated on 6% (vol/vol) denaturing polyacrylamide-urea gels (1,800 V for 2–3 hours). The RNAs were analyzed several times on different gels to ensure the reproducibility of the results. The gel was then dried and exposed to X-Omat film (Kodak) at −70°C for several hours. Autoradiograms were scanned with a Shimadzu CS-9000 densitometer and the relative densities of the bands determined. The data are reported as the percent of α-skeletal/sarcomeric actin mRNA. Results are expressed as mean±SD, and statistical significance between each group was evaluated by one-way analysis of variance and compared by Scheffé F test. The threshold of significance was chosen as values of p < 0.05.

#### RNA Hybridization

Slot blots of total rat cardiac, skeletal, and liver RNAs were prepared after the RNAs were denatured at 65°C in 15× SSC (1× SSC is 0.15 M sodium chloride and 0.015 M sodium citrate) for 15 minutes and quenched on ice. Serial dilutions (1, 2, and 4 μg) from rat hearts at birth to age 24 months were spotted onto Hybond N membranes (Amersham) by using a minifold apparatus (Schleicher & Schuell, Inc., Keene, N.H.) and the RNAs fixed to the membranes by ultraviolet illumination (0.6 J) according to the manufacturer’s instructions (Vilber Lourmat, Marne-le-Vallee, France). A double-strand cDNA probe (pBSα,) that contains the first noncoding exon of the mouse α-cardiac actin gene (128 nucleotides) and cross-hybridizes with rat α-cardiac actin mRNAs was kindly provided by M. Buckingham. The radioactive α-cardiac cRNA probe (1.2×10^6 dpm/μg) was obtained by promoter-driven transcription by using the endogenous T3 promoter of pBS. In brief, the plasmid was linearized (EcoRI, Boehringer) and the cRNA transcribed according to the manufacturer’s protocols (Stratagene, San Diego, Calif.): 1 μg linearized pBSα, was incubated for 30 minutes at 37°C with 40 units of T3 RNA polymerase (Boehringer) in the presence of 0.4 mM (for each) ATP, CTP, and GTP and 0.02 mM UTP with 200 μCi [α-32P]UTP (800 Ci/mmol, New England Nuclear) in a solution containing 40 mM Tris (pH 8.0), 8 mM MgCl₂, 2 mM spermidine, 50 mM NaCl, 40 units RNasin (Promega Corp., Madison, Wis.), and 0.03 mM dithiothreitol. The transcription reaction products were then diluted 10-fold with 40 mM Tris (pH 7.5), 6 mM MgCl₂, and 10 mM NaCl in the presence of 1 unit RNase-free DNase (Bethesda Research Laboratories) and 40 units RNasin. The DNase treatment was performed for 15 minutes at 37°C, followed by phenol–chloroform extraction and precipitation with 2.5 vol ethanol in the presence of 0.3 M sodium acetate (pH 5.5) for 15 minutes in 100% ethanol–dry ice. After centrifugation, the pellet was dried and resuspended in 10 mM Tris (pH 8.0) and 1 mM EDTA. Prehybridization and hybridization of the membranes were at 42°C in a buffer containing 150 μg/ml salmon sperm DNA, 5× SSC, 0.2% (wt/vol) Ficoll, 0.2% (wt/vol) polyvinylpyrrolidone, 1% (vol/vol) bovine serum albumin, 0.1% (vol/vol) tetrasodium pyrophosphate, 50% (vol/
Vol) formamide, 5 mM EDTA, and 50 mM Tris (pH 7.5). After the membrane was hybridized in the presence of the radioactive cardiac α-actin cRNA probe for ~20 hours, the blots were washed at 65°C two times in 2x SSC and 0.1% sodium dodecyl sulfate for 15 minutes and one time in 0.1x SSC and 0.1% sodium dodecyl sulfate for 20 minutes. The membranes were then exposed to Cronex 4 film (DuPont) with Quanta III intensifying screens (DuPont) at -40°C or -70°C for several hours. Autoradiograms were scanned as described above. Membrane dehybridizations were performed in 0.4% sodium dodecyl sulfate and 0.1% tetrasodium pyrophosphate for 30 minutes at 90°C. Total poly(A⁺) for each RNA was determined by hybridization of the membranes to a T4 polynucleotide kinase [γ-32P]ATP end-labeled oligo(dT) (25-30-mer, Pharmacia, Inc.) probe with a specific activity of 4×10⁶ dpm/µg. Finally, as a control for the amount of RNA effectively bound to the filter from which the α-cardiac actin and poly(A⁺) contents could be normalized, the dehybridized membranes (0.1% sodium dodecyl sulfate at 90°C) were hybridized to a T4 polynucleotide kinase [γ-32P]ATP 5' end-labeled rRNA 18S oligonucleotide probe¹orida diluted with unlabeled oligonucleotide to a specific activity of 5.5×10⁵ dpm/µg. Hybridization with the two oligonucleotides was as previously described,²0 and the membranes were washed two times in 5x SSC for 10 minutes at room temperature for the 18S probe and two times in 5x SSC for 15 minutes at room temperature for the oligo(dT). Membranes were then autoradiographed and the signals quantitated.

For each RNA preparation analyzed by primer extension, the data are reported as the relative percentage of α-skeletal actin mRNA to total sarcomeric actin. For RNA hybridizations, the data are reported in arbitrary units as a function of α-cardiac actin mRNA normalized to rRNA. From these data, the relative amounts of skeletal actin were analytically determined for the slot blots.

Results
Skeletal Actin mRNAs Are Accumulated Transiently After Birth and Then Downregulated With Age

To precisely determine the developmental regulation of actin mRNA isofrom accumulations, the percentage of α-skeletal to sarcomeric actin mRNAs in rat ventricles and atra was quantitated by primer extension. Figure 1 shows an autoradiogram obtained from total liver, skeletal muscle, and cardiac RNA samples; the cardiac RNAs were isolated from ventricles of developing, adult, and aged rats. Only one signal with an apparent length of 186 nucleotides was detected from rat skeletal muscle RNAs. No signals were detected from liver RNAs, indicating the specificity of our assay for sarcomeric actins. With cardiac RNA, two signals were detected, one of which comigrates with that from skeletal muscle and a second one that has an apparent length of 195 nucleotides. These two signals represent α-skeletal and α-cardiac actin mRNAs, respectively. As can be readily distinguished in the figure, the signals for α-skeletal actin relative to α-cardiac actin decrease with age.

Complete analysis of the primer extension assays indicates that the sarcomeric actins are nearly always coexpressed in rat ventricles but that the relative level of expression varies dramatically with age (Figure 2). At 17 and 19 days in utero (−0.5 weeks), α-skeletal actin mRNAs represent 28.1±0.8% of the total, its proportion increases 2 days after birth to 44±7.6% (NS, p=0.15) and remains constant for 3 weeks, decreases by 1 month of age to 15.2±2.9%, and represents in aged rat ventricles only 3.4±3.0% of the total. Thus, in late fetal and neonatal rat ventricles, α-skeletal actins can account for as much as 50% of the total and in senescent rat ventricles almost never more than 5% of the total. This greater than 10-fold decrease with age corresponds

FIGURE 1. Primer extension analysis of rat cardiac ventricular RNAs during development and aging. Primer extensions were performed with 10 µg total RNA from rat skeletal muscle (Sk), liver (L), and ventricles at the indicated ages. The molecular weight markers (MW) are indicated in the first column. No signals were generated from liver RNA. In skeletal muscle, only one product, which has a length of 186 nucleotides, was detected. From cardiac RNA, two fragments were generated, one corresponded to α-cardiac actin (α-card, 195 nucleotides) and the other to α-skeletal actin (α-skel, 186 nucleotides).

FIGURE 2. α-Skeletal actin mRNA accumulations with development and aging. Data for this graph were obtained from the primer extension analysis of rat ventricular RNAs. The results are presented as the percentage of α-skeletal actin mRNA to total sarcomeric (α-cardiac+α-skeletal) actin mRNAs and include samples from fetal (17 and 19 days in utero), neonatal, juvenile, and adult rats. Results are mean±SD.
well with the decrease of 11-fold reported by Mayer et al. but more importantly demonstrates that α-skeletal actin transcripts never constitute a majority of the sarcomeric actins in late fetal to adult rat ventricles. To ensure that these results were not skewed by sex-related factors, RNAs were analyzed from male and female rats aged 3–6 weeks (Figure 3). During this time, the rats develop into sexually mature adults. No significant difference in the percentage of α-skeletal to sarcomeric actin mRNA could be demonstrated between the two groups at any of these ages. These data indicate that the accumulation of the α-skeletal actin mRNAs are not linked to the sex of the animal or, more precisely, to potential hormonal changes occurring during this period of development.

To determine whether the downregulation of the α-skeletal actin mRNA is specific to the ventricles or is a common feature of the whole heart, the developmental regulation of the atria (left and right) was also studied, but only from rats between 3 weeks and 24 months of age because of the difficulty in physically isolating this tissue from fetal and neonatal rat hearts. The results are presented in Table 1 along with the corresponding values obtained from the ventricles. The calculated percentages of the α-actins in the two tissues roughly correspond at all ages studied. It is thus probable that the sarcomeric actins are developmentally regulated in the atria; i.e., α-skeletal actin mRNAs are downregulated with age. However, it is impossible to say from our results if α-skeletal actins represent a significant fraction of the total in fetal and neonatal atria.

**Sarcomeric Actin mRNAs Decrease With Development**

Although we demonstrated that the α-skeletal actin mRNAs transiently accumulate during early development and subsequently decrease with age, it was important to know whether these changes were due to a reduced accumulation of both sarcomeric α-actin mRNAs or a relative increase in α-cardiac actin transcripts. For this determination, a labeled cRNA probe specific for α-cardiac actin was prepared from pBSαc and used to determine the amount of α-cardiac actin transcripts in total ventricular RNA samples with development. Typical data from this experiment are shown in Figure 4. The cardiac cRNA probe strongly hybridized with cardiac RNA, whereas no detectable signals were seen from liver- or skeletal muscle–derived RNAs. The relatively homogeneous intensity of expression for all the neonatal and aged rat heart samples suggested that α-cardiac actin accumulations are unchanged with de-

### Table 1. Comparison of the Skeletal Actin mRNA Accumulations in the Ventricles and Atria of Rats

<table>
<thead>
<tr>
<th>Age</th>
<th>Ventricles</th>
<th>Atria</th>
</tr>
</thead>
<tbody>
<tr>
<td>3 weeks</td>
<td>38.0±8.5</td>
<td>28.1±13.5</td>
</tr>
<tr>
<td>2 months</td>
<td>4.5±2.7</td>
<td>0</td>
</tr>
<tr>
<td>8 months</td>
<td>3.9±3.7</td>
<td>1.8±2.6</td>
</tr>
<tr>
<td>24 months</td>
<td>3.4±3.0</td>
<td>8.4±6.2</td>
</tr>
</tbody>
</table>

Values are mean±SD of the percentage of skeletal/sarcomeric actin mRNA. Primer extension analysis was performed on 10 μg total RNA from total ventricles and atria of rats aged 3 weeks to 24 months. In the atria at 2 months of age, no signals for the α-skeletal actin were detectable.
TABLE 2. Sarcomeric Actin mRNA Accumulations With Development and Aging

<table>
<thead>
<tr>
<th>Age</th>
<th>α-Cardiac</th>
<th>α-Skeletal</th>
<th>Total sarcomeric</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 days</td>
<td>2.80±0.41</td>
<td>1.06±0.29</td>
<td>3.86±0.70</td>
</tr>
<tr>
<td>8 days</td>
<td>2.49±0.10</td>
<td>1.18±0.45</td>
<td>3.68±0.62</td>
</tr>
<tr>
<td>15 days</td>
<td>2.31±0.07</td>
<td>0.98±0.10</td>
<td>3.29±0.18</td>
</tr>
<tr>
<td>23 days</td>
<td>1.97±0.25</td>
<td>0.52±0.11</td>
<td>2.50±0.36</td>
</tr>
<tr>
<td>2 months</td>
<td>2.00</td>
<td>0.08</td>
<td>2.08</td>
</tr>
<tr>
<td>3 months</td>
<td>1.61</td>
<td>0.06</td>
<td>1.68</td>
</tr>
<tr>
<td>8 months</td>
<td>1.87±0.17</td>
<td>0.52±0.11</td>
<td>2.39±0.18</td>
</tr>
<tr>
<td>24 months</td>
<td>1.55</td>
<td>0.12</td>
<td>1.68</td>
</tr>
</tbody>
</table>

Values (in arbitrary units) are the percentage of skeletal/sarcomeric actin mRNA and are the mean±SD obtained from two to four different RNA samples at the indicated ages. Data for this graph were obtained from slot blot analyses of total rat ventricular RNAs after hybridization with the pS35c cRNA probe specific for α-cardiac actin mRNA. The results were normalized to the amounts of 18S rRNA loaded per slot.

Development; however, when normalized with an 18S RNA ribosomal probe, it became apparent that the α-cardiac actins are somewhat more abundant in neonatal and young rat hearts than when compared with more aged hearts (~70% of neonatal values). Furthermore, the amounts of the poly(A+) mRNA do not change with development when compared with 18S rRNA, indicating that the decrease in sarcomeric actins with aging is not due to a net decrease in total mRNA.

Table 2 and Figure 5 summarize the developmental changes in expression for both sarcomeric α-actins. Data were calculated from the percentages of a-actins determined by primer extension analyses and from the normalized values obtained from slot blot analysis. As can be seen, both α-skeletal and α-cardiac actin transcripts decreased with postnatal development and senescence relative to neonates at 23 days by 2.0- and 1.4-fold, at 2 months by 12.5- and 1.4-fold, and at 24 months by 8.3- and 1.8-fold, respectively. With time, the accumulations of total sarcomeric actin mRNAs decrease by 1.5-fold at age 23 days, 1.9-fold at age 2 months, and 2.3-fold at age 24 months. These results are in contrast with those of Mayer et al., who indicated a 3.4-fold increase in accumulation of the α-cardiac transcripts with heart development. This discrepancy could be partially explained by differences in the techniques used for the analyses. Finally, the slight decrease of total sarcomeric actin transcripts with aging cannot alone account for the ~12-fold decrease of α-skeletal actin seen with senescence and demonstrates that the decrease in α-skeletal actin mRNAs results from a down-regulation of its expression.

**Discussion**

In this study we have determined the precise time course of expression of the α-cardiac and α-skeletal actin isogenes in the rat heart during ontogeny and aging, and we have shown that α-skeletal actin transcripts do not reaccumulate with aging. We also showed that both these transcripts are present in the atria and ventricles, that their expressions do not appear to be dependent on sex-related factors, and that the accumulations for both sarcomeric actins decrease with aging.

These findings extend our understanding of the developmental and age-related regulations of the actin gene products and suggest important differences in the regulation of the genes coding for the sarcomeric proteins of heart muscle. To emphasize these differences, we have compared the relative percentages of α-skeletal/sarcomeric actin mRNAs with those of β-MHC/α+β-MHC mRNAs (Figure 6). It is immediately apparent that the accumulations for these transcripts with ontogeny and senescence are distinct and independent. For example, just after birth the α-skeletal actin transcripts accumulate to ~40% and then slowly decrease to <5% of the total at age 2 months (this study). Meanwhile, the β-MHC transcripts decrease rapidly from ~50% to <2% of the total by age 3–4 weeks; afterward, they reaccumulate to eventually attain levels of ~85% with senescence. With cardiac hypertrophy induced by thoracic aortic constriction, these two mRNAs are also known to accumulate in a spatially and temporally distinct manner.

In humans, α-skeletal
actin transcripts increase after birth, accumulate to levels greater than those for the cardiac actin mRNAs in adults, and remain at these same adult levels even with end-stage heart failure, whereas β-MHC remains predominant at all stages of development, age, and disease.21 These results clearly demonstrate differences in the patterns of regulation of the actin and myosin multigene families in cardiac tissue, whatever the animal species.

It is currently unknown why the sarcomeric α-actins are coexpressed or what functional differences if any exist between these two isoforms. It has been suggested during periods of rapid growth (e.g., early development and cardiac hypertrophy in vitro and in vivo) that the expression of the α-cardiac actin gene may not be sufficient for the synthesis of adequate amounts of translatable α-actin mRNA isoforms, hence the reactivation of the α-skeletal actin gene.6 This reactivation does not, however, explain the slight and constant coexpression of these two genes nor the functional significance of their coaccumulations in adult rats. In BALB/c mice containing a duplication of the 5' end of the α-cardiac actin gene and expressing abnormally high levels of the α-skeletal actin transcripts, no phenotypic changes are readily apparent1 nor have precise hemo-
dynamic studies thus far been performed. Single point mutations in the sarcomeric actin genes can also lead to discernible differences in their crossbridge kinetics;22 however, functional differences between the two wild-type sarcomeric actin isoforms have yet to be demonstrated. Perhaps in vitro motility assays could be used to resolve this issue.

The findings of the present study do not indicate what controls the normal expressions of these genes, although a considerable body of evidence is now available from which one can form some hypotheses. In adult rat hearts, injections of thyroxine rapidly increase α-skele-
tal actin transcripts16 and to a lesser extent those of α-cardiac actin,16,23 suggesting that a thyroid hormone responsive element is active in these genes in cardiac tissues. No such element has thus far been identified for these α-actin genes. Because endogenous thyroid hor-
none is released just before birth, some of the develop-
mental changes seen with the sarcomeric actins in neonates could be attributed to this hormone; however, this is probably not the case because neonatal to age 15 day hypothyroid rats have the same proportions of ventricular skeletal and cardiac α-actins as controls.24 Clearly, a number of other factors and regulatory pathways must be required. α-Skeletal mRNAs accumulate in response to a number of different stimuli: hemodynamic overload of rat hearts,12,25 passive stretch,26 or administration of transforming growth fac-
tor-β1, basic fibroblast growth factor,27 or α-adrenergic agonists31 via protein kinase C28 to neonatal cardiac cell cultures. In response to acidic fibroblast growth factor, both α-cardiac and α-skeletal actin are downregu-
lated,27 and α-cardiac actin mRNAs are not changed by transforming growth factor-β1 or basic fibroblast growth factor.27 At least some of these developmental and adrenergic-stimulated changes are regulated at the transcriptional level.29,30 With the possible exception of thyroxine via the thyroid hormone receptor, the ultimate step in any sarcomeric actin transcriptional activation process is completely unknown. Potential candi-
date molecules including c-fos, c-jun, c-myc, JunB or JunD (for review, see Reference 31), and Egr-1, a "zinc finger" early response gene,32 have been suggested to play important regulatory roles.

On the other hand, the promoter regions of the human cardiac and skeletal α-actins have been extensively analyzed and are known to be highly conserved with those from the mouse.33,34 The cardiac α-actin gene has been shown to require at least three factors (MyoD1, CARG-box binding factor, and Sp1) for muscle-specific expression;35 however, MyoD1 does not appear to be implicated in the cardiac-specific regulation of this gene since it is not expressed in cardiac myocytes.36,37 Other promoter regions of importance include the distal regulatory elements 1, 2, and 3.38 Likewise, the skeletal α-actin gene contains a number of distal regulatory elements critical for skeletal muscle-specific regulation, and it has been recently shown that a defined upstream regulatory region of the α-skeletal actin promoter is regulated by β- or α-adrenergic stimulation during myocardial cell hypertrophy.39 The α- and β-MHC gene promoter regions also contain a number of distinct regulatory sites. Those for the β-MHC, for example, include the AP5, NFκB, E box, MCAT, and negative TRE binding domains,40,41 which do not exactly correspond to those of the sarcomeric α-actins. These differences may, therefore, at least partially explain the distinct patterns of expression of these genes during growth and development.

In conclusion, our results provide additional evidence of independent regulations for the sarcomeric α-actins and the α- and β-MHCs. Measurement of RNA accumulations alone are insufficient for one to determine at what level these multigene families are regulated. Transcrip-
tional events are undoubtedly involved,30 but a number of posttranscriptional and translational controls must also be considered before one will be able to understand the complex regulations necessary for the coordinated expression of the functioning sarcomere.

Acknowledgments

We are indebted to M. Buckingham for the gift of the α-cardiac actin clone and wish to thank S. Alonso and A. Barrieux for fruitful discussions.

References

5. Garner I, Minty AJ, Alonso S, Barton PJ, Buckingham M: A 5' duplication of the α-cardiac actin gene in BALB/c mice is associated with abnormal levels of α-cardiac and α-skeletal actin mRNAs in adult cardiac tissue. EMBO J 1986;5:2539–2547


31. Parker TG, Schneider MD: Growth factors, proto-oncogenes, and plasticity of the cardiac phenotype. Annu Rev Physiol (in press)


Expression of the sarcomeric actin isogenes in the rat heart with development and senescence.
L Carrier, K R Boheler, C Chassagne, D de la Bastie, C Wisnewsky, E G Lakatta and K Schwartz

Circ Res. 1992;70:999-1005
doi: 10.1161/01.RES.70.5.999

Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1992 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7330. Online ISSN: 1524-4571

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circres.ahajournals.org/content/70/5/999

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation Research can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Circulation Research is online at:
http://circres.ahajournals.org/subscriptions/