Proc. Natl. Acad. Sci. USA
Vol. 83, pp. 3175–3179, May 1986
Biochemistry

Construction and characterization of the α form of a cardiac myosin heavy chain cDNA clone and its developmental expression in the Syrian hamster

(multigene family/gene expression/cardiomyopathy)

CHOONG-CHIN LIEW AND MARK A. JANDRESKI

Department of Clinical Biochemistry, Laboratory for Molecular Cardiology, Banting Institute, University of Toronto, Toronto, ON, M5G 1L5 Canada

Communicated by J. Tuzo Wilson, January 2, 1986

ABSTRACT **A cDNA clone, pVHC1, was isolated from a Syrian hamster heart cDNA library and was compared to the rat α (pCMHC21) and β (pCMHC5) ventricular myosin heavy chain cDNA clones. The DNA sequence and amino acid sequence deduced from the DNA show more homology with pCMHC21 than pCMHC5. This indicates that pVHC1 is an α ventricular myosin heavy chain cDNA clone. However, even though pVHC1 shows a high degree of nucleotide and amino acid conservation with the rat myosin heavy chain sequences, the carboxyl-terminal peptide and the 3′-untranslated region are highly divergent and specific for this cDNA clone. There appears to be an amino acid deletion in the 3′ end of the hamster α myosin heavy chain as compared to the rat α myosin heavy chain. S1 nuclease mapping experiments have shown that the mRNA represented by this cDNA clone is scarcely expressed in neonatal development, but its expression increases with age and reaches maximal levels in adult life. This cDNA clone provides a useful tool to follow the myosin heavy chain mRNA changes during development and during the genesis of a cardiomyopathy, an autosomal recessive defect carried by the Syrian hamster.**

Considerable evidence indicates that several muscle-specific contractile proteins, including the myosin heavy chain, are constituted by multigene families. Differential expression of myosin light and heavy chains have been demonstrated in muscle and nonmuscle cell types as well as in invertebrate and avian muscle tissues by peptide mapping, immunological cross-reactivity, and DNA analysis (1–5). The myosin isozymes in cardiac tissue present a particularly intriguing challenge for the examination of differential expression during development.

The isoforms of ventricular myosin, which are expressed as either homodimers of the α myosin heavy chain (V1 form) or the β myosin heavy chain (V3 form) or as heterodimers of the α and the β myosin heavy chain (V2 form) in the course of myocardial cell development (6–10), are a striking example of specific gene expression; these three forms of ventricular myosin have been extensively studied in rat and rabbit (6, 7, 11–15). The molecular basis for the expression of the ventricular myosin isoforms has been actively investigated in relation to hormone induction (11, 16–18), stimulation by cardiac pressure overload as well as in disease states (19–21). The structure of the α and β forms of the ventricular myosin heavy chain genes in rat have been partially elucidated by Mahdavi and her coworkers (22–24). They have shown that the α and the β forms of the ventricular myosin heavy chain genes in rat are organized in tandem, and the intron positions in the 5′-end region of the genes are conserved.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

The Syrian hamster has several features that can be used to study myocardial cells during normal growth and during the development of heart disease. Some strains of this animal exhibit an autosomal recessive defect, a cardiomyopathy, which appears 30–45 days after birth (25–27). It has been reported that development of the cardiomyopathy is associated with an altered distribution of the three myosin isozymes (28, 29); there is a shift from the V1 form of the ventricular myosin isozymes (which contains a higher ATPase activity than the V3 form) to the V3 form. It has been speculated that this shift in the myosin isozymes to the V3 form and to a lower ATPase activity is a compensatory process that may lead to a more economical working of the myocardial cell (29).

This report deals with the construction of a myocardial cDNA library from Syrian hamster ventricular tissue and the characterization of the α form of a ventricular myosin heavy chain cDNA clone. This specific cDNA clone, through the use of the S1 nuclease mapping technique, provides a feasible means of examining the expression of hamster ventricular myosin isoforms during growth and the course of developing cardiomyopathy.

MATERIALS AND METHODS

Materials. Reverse transcriptase from avian myeloblastosis virus was purchased from Life Sciences (St. Petersburg, FL). Nuclease S1, DNA polymerase I (Klenow fragment), and restriction enzymes were purchased from Boehringer Mannheim and used according to manufacturer's specifications. Low-melting agarose, terminal deoxynucleotidyltransferase (TdT), and pBR322 cut at the *Pst* I site and oligo(dG)-tailed were purchased from Bethesda Research Laboratories. Nitrocellulose paper (0.45-μm pore size) was from Schleicher & Schuell. ^{32}P-labeled nucleotides were from New England Nuclear.

Methods. Total RNA was isolated from the ventricular tissue of 28-day-old random-bred Syrian hamster by using the guanidine hydrochloride method, and oligo(dT)-cellulose chromatography was used to obtain poly(A)$^+$ RNA, as described (30).

Construction of Recombinant Plasmids. Total poly(A)$^+$ RNA from the hamster ventricle was used as a template for the synthesis of single-stranded cDNA by using oligo(dT) primers in the absence of actinomycin D (30). Double-stranded cDNA synthesis was carried out using DNA polymerase I (Klenow fragment) (31). The incubation was done at 16°C for 20 hr followed immediately by a 75-min incubation at 42°C. We found that the additional use of reverse transcriptase was unnecessary. The double-stranded cDNA was then separated from the nucleotides by column chromatography on Sephadex G-50 Fine (Pharmacia) using 100 mM NaCl, 10 mM Tris·HCl (pH 8.0), 1 mM EDTA (TNE buffer). Nuclease S1 digestion, tailing of the cDNA with dCTP and terminal deoxynucleotidyltransferase, and annealing were

completed following standard procedures (31). The recombinant plasmids, containing the oligo(dC)-tailed cDNA and oligo(dG)-tailed pBR322, were used to transform *Escherichia coli* LE392 as described by Hanahan (32). The resultant transformants were selected by their resistance to tetracycline and sensitivity to ampicillin. Myosin heavy chain clones were detected by the method of Grunstein and Hogness (33) using labeled DNA fragments from a rat ventricular myosin heavy chain cDNA clone pCMHC21 (22, 34).

Restriction Endonuclease Mapping of Recombinant Plasmids. Following the restriction endonuclease cleavage of plasmid DNA, fragments were analyzed by 0.8% agarose gel and 10% polyacrylamide gel electrophoresis (31). Alternatively, partial digestions of plasmid DNA were carried out essentially as described by Smith and Bernstiel (35) to delineate the restriction endonuclease sites of the insert.

S1 Nuclease Mapping. S1 mapping was carried out essentially by the rapid and simple method of Burke (36). Fragments obtained by restriction endonuclease (e.g., *Pst* I) digestion were inserted into the M13 mp8 vector (37). The M13 sequencing (17mer) was annealed to the template. [α-^{32}P]dATP (100 μCi; 800 Ci/mmol; 1 Ci = 37 GBq) was added to the mixture containing unlabeled dCTP, dGTP, and dTTP, each at a final concentration of 100 μM. DNA polymerase I (Klenow fragment) was added to extend and copy the cloned fragment at 30°C for 20 min and then immediately chased by adding excess unlabeled dATP. The labeled M13 transcripts were then cleaved with *Eco*RI. This cloned radioactive fragment was isolated by 2% low-melting agarose gel electrophoresis (31).

Total cardiac RNA was isolated from 2- to 3-day-, 20-day-, and 7-month-old hamsters. The radio-labeled cDNA probe (300,000 cpm) and 30 μg of total RNA were precipitated together. The pellet was resuspended in 20 μl of hybridization buffer containing 0.4 M NaCl and 10 mM Pipes, pH 6.5. The samples were heated at 100°C for 3 min and then hybridized at 65°C for 1 hr. At the end of 1 hr, 200 μl of a solution containing 180 mM NaCl, 30 mM sodium acetate (pH 4.5), 4.5 mM zinc acetate and 1 unit (Vogt) of nuclease S1 per μl was added to the mixture and incubated for 30 min at 37°C. EDTA was added to a concentration of 10 mM, and the S1 nuclease-resistant hybrids were precipitated by addition of 3 vol of ethanol and then analyzed on a 6.5% polyacrylamide gel containing 8 M urea. Following gel electrophoresis, the gel was dried and exposed to Kodak XAR x-ray film.

Sequencing of DNA. Fragments, which were obtained from restriction endonuclease digestion (e.g., *Pst* I and *Pvu* II) of the myocardial cDNA clone, were inserted into the M13 mp8 and mp9 vectors (37). DNA sequencing was carried out by the dideoxy chain termination method (38).

RESULTS AND DISCUSSION

Construction and Isolation of Ventricular Myosin Heavy Chain cDNA Clones from Cardiac Muscle. Ventricles of neonatal hearts from 28-day-old hamsters were rapidly collected and kept in liquid nitrogen prior to the extraction of RNA. Poly(A)$^+$ RNA was isolated by oligo(dT)-cellulose column chromatography and synthesized into double-stranded cDNA. A cDNA library was constructed by the G·C tailing method in the *Pst* I site of pBR322. Eight recombinant plasmids, giving a positive hybridization signal to probe pCMHC21 (22), a rat α ventricular myosin heavy chain cDNA clone, were identified. Subsequent characterization of these cDNA clones indicated that there were only four different-sized clones with two distinctive restriction site patterns. One of these clones, pVHC1, has a similar pattern of restriction enzyme sites to the restriction map of pCMHC21, as shown in Fig. 1. pCMHC21 has been identified as an α ventricular myosin heavy chain cDNA clone. However, due to the high proportion of homology between the α and the β forms of the myosin heavy chain, the restriction maps could not be relied on to identify whether pVHC1 was an α or a β clone conclusively.

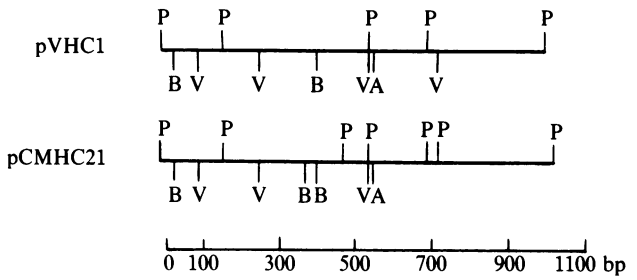

FIG. 1. The restriction enzyme sites of pVHC1 compared to the α form of the rat myosin heavy chain cDNA clone pCMHC21. A, *Ava* I; B, *Bgl* I; P, *Pst* I; V, *Pvu* II. bp, Base pairs.

Nucleotide Sequence Analysis of pVHC1. The recombinant plasmid pVHC1 was cut with either *Pst* I or *Pvu* II (to sequence across *Pst* I sites), inserted into the M13 mp8 and mp9 sequencing vectors, and sequenced by the dideoxy chain termination method. Each strand was sequenced in both directions. The DNA sequence of pVHC1 is shown in Fig. 2 along with the encoded amino acid sequences. Comparison of its encoded amino acid sequence with the published sequences of the rat ventricular α (pCMHC21) and β (pCMHC5) myosin heavy chain is also presented (22). As shown in Table 1, the DNA sequence of pVHC1 and the computer generated amino acid sequence have more homology to pCMHC21 than to pCMHC5, including the 3′-untranslated region. This indicates that pVHC1 is an α ventricular myosin heavy chain cDNA clone.

It should be noted that the fifth amino acid residue from the 3′ end glutamine (codon CAG) is missing in the hamster α myosin heavy chain cDNA clone pVHC1 as compared to the rat α myosin heavy chain clone pCMHC21. However, the fifth amino acid residue from the 3′ end glycine (codon GCC) in the hamster β myosin heavy chain cDNA clone pVHC2 is not missing (unpublished data). This is a significant deviation between the rat and hamster α myosin heavy chain genes. Not only have three base pairs of DNA been removed from the gene but an amino acid has been removed from the protein. However, it does not appear that this amino acid deletion would significantly affect the structure of the myosin heavy chain protein.

Expression of the α Form of the Ventricular Myosin Heavy Chain mRNA During Postneonatal Development. Alterations of the myosin isoforms have been reported to be associated with development, hormonal stimulation, and pressure-overload in the rat and rabbit (6, 7, 11–15). We selected the 28-day-old hamster heart for the purpose of isolating both the α and the β forms of the ventricular myosin heavy chain mRNAs. To follow the phenotypic expression of the α isoform of the myosin heavy chain mRNA, ventricular RNA from 2-day-old, 20-day-old, and 7-month-old hamster heart was isolated. The 3′ end of the *Pst* I restriction fragment from pVHC1 was subcloned into the M13 mp8 sequencing vector, used to generate a probe, and hybridized to equivalent amounts of ventricular RNA isolated from the three different developmental stages and adult soleus muscle RNA. The probe is 350 bases long, maps at the 3′ end of the cDNA insert, and includes the codons for the last 73 amino acids at the carboxyl terminus, the 3′-untranslated region (69 bases), one of the poly(C) tails (13 bases), and 46 bases from the M13 vector. As shown in Fig. 3, when the probe hybridized to the RNA, it gave the expected full length of protection for the mRNA portion of the probe (≈290 bp) and is only seen in the heart RNA samples. The soleus RNA is not protected and is

```
CAG CTC AGC CAG GCC AAC AGA ATA GCC TCA GAG GCC CAG AAG CAC CTG AAG AAC GCC
Gln Leu Ser Gln Ala Asn Arg Ile Ala Ser Glu Ala Gln Lys His Leu Lys Asn Ala
------------------------------------------------------------------------
------------His-------------Met-----Ala-----------------Glu-Val-----Ser-Leu

CAA GCC CAC TTG AAG GAC ACC CAG CTC CAG CTG GAT GAT GCG CTC CAC GCC AAT GAC
Gln Ala His Leu Lys Asp Thr Gln Leu Gln Leu Asp Asp Ala Leu His Ala Asn Asp
-----------------------------------------------------Val-Arg------------
----Ser-Leu---------------------Ile---------------------Val-Arg------------

GAC CTG AAG GAG AAC ATC GCC ATC GTG GAG CGG CGG AAC ACC CTG CTG CAG GCG GAG
Asp Leu Lys Glu Asn Ile Ala Ile Val Glu Arg Arg Asn Thr Leu Leu Gln Ala Glu
------------------------------------------------------------------------
-------------------------------------------------Asn--------------------

CTG GAG GAG CTG CGG GCT GTG GTG GAG CAG ACA GAG CGG TCT CGG AAG CTG GCG GAG
Leu Glu Glu Leu Arg Ala Val Val Glu Gln Thr Glu Arg Ser Arg Lys Leu Ala Glu
------------------------------------------------------------------------
------------------------------------------------------------------------

CAG GAG CTG ATC GAG ACC AGC GAG CGG GTG CAG CTG CTG CAC TCT CAA AAC ACC AGC
Gln Glu Leu Ile Glu Thr Ser Glu Arg Val Gln Leu Leu His Ser Gln Asn Thr Ser
-------------------------------------------------------------Asn----
------------------------------------------------------------------------

CTC ATC AAC CAG AAG AAG AAG ATG GAG GCA GAC CTG ACC CAG CTC CAG ACC GAG GTG
Leu Ile Asn Gln Lys Lys Lys MET Glu Ala Asp Leu Thr Gln Leu Gln Thr Glu Val
--------------------------------Asp-------------Ser-----------------------
--------------------------------His-------------Ser-----------------------

GAA GAG GCG GTG CAG GAG TGT AGG AAT GCG GAG GAG AAG GCC AAG AAA GCC ATC ACA
Glu Glu Ala Val Gln Glu Cys Arg Asn Ala Glu Glu Lys Ala Lys Lys Ala Ile Thr
------------------------------------------------------------------------
------------------------------------------------------------------------

GAT GCC GCC ATG ATG GCG GAG GAG CTG AAG AAG GAG CAG GAC ACC AGC GCC CAC CTG
Asp Ala Ala MET MET Ala Glu Glu Leu Lys Lys Glu Gln Asp Thr Ser Ala His Leu
------------------------------------------------------------------------
------------------------------------------------------------------------

GAG CGC ATG AAG AAG AAC ATG GAG CAG ACC ATC AAG GAC CTG CAA CAC CGG CTG GAC
Glu Arg MET Lys Lys Asn MET Glu Gln Thr Ile Lys Asp Leu Gln His Arg Leu Asp
------------------------------------------------------------------------
------------------------------------------------------------------------

GAA GCC GAG CAG ATC GCG CTC AAG GGT GGC AAG AAG CAG CTG CAG AAG CTG GAG GCC
Glu Ala Glu Gln Ile Ala Leu Lys Gly Gly Lys Lys Gln Leu Gln Lys Leu Glu Ala
------------------------------------------------------------------------
------------------------------------------------------------------------

CGG GTC CGC GAG CTG GAG AAC GAG CTG GAG GCT GAG CAG AAG CGC AAT GCC GAG TCC
Arg Val Arg Glu Leu Glu Asn Glu Leu Glu Ala Glu Gln Lys Arg Asn Ala Glu Ser
------------------------------------------------------------------------
------------------------------------------------------------------------

GTG AAG GGC ATG AGG AGG AGT GAG CGG CGC ATC AAG GAG CTC ACC TAC CAG ACA GAG
Val Lys Gly MET Arg Arg Ser Glu Arg Arg Ile Lys Glu Leu Thr Tyr Gln Thr Glu
--------------------Lys-------------------------------Asn----------------
--------------------Lys-------------------------------Asn----------------

GAG GAC AAG AAG AAC CTG GTG AGG CTG CAG GAC CTG GTA GAC AAG CTA CAG CTG AAG
Glu Asp Lys Lys Asn Leu Val Arg Leu Gln Asp Leu Val Asp Lys Leu Gln Leu Lys
-----------------------------------------------------Asn--------------------
------------------------Leu-----------------------------------------------

GTG AAG ACC TAC AAG CGC CAG GCT GAG GAG GCG GAG GAG CAG GCC AAC ACC AAT CTG
Val Lys Thr Tyr Lys Arg Gln Ala Glu Glu Ala Glu Glu Gln Ala Asn Thr Asn Leu
--------Ala-----------------------------------------------------------
--------Ala-----------------------------------------------------------

TCC AAG TTC CGC AAG GTG CAG CAT GAA CTG GAC GAG GCA GAG GAG AGG GCC GAC ATC
Ser Lys Phe Arg Lys Val Gln His Glu Leu Asp Glu Ala Glu Glu Arg Ala Asp Ile
------------------------------------------------------------------------
------------------------------------------------------------------------

GCC GAG TCC CAG GTC AAC AAG CTG CGG GCC AAG AGC CGT AAC ATC GGT GCC AAG
Ala Glu Ser Gln Val Asn Lys Leu Arg Ala Lys Ser Arg Asn Ile Gly Ala Lys
-----------------------------------------------------Asp-----------------Gln
-----------------------------------------------------Asp-----------------Gly

AAG ATG CAC GAT GAA GAA TGA
Lys MET His Asp Glu Glu END      TGCTGCCCGCAGGCCTTGCTGTTGCTAGCCCACAATAAACACG
-----------------------END CCTGTCCAGCAGAAAGA----C--C-----C-T----------T---
Leu Asn Glu Glu END ATCTTGCTCTACCCAACCCTAAGGATGCCTGTGAAGCCCTGAGACCTGGAGCCTTT

AATGCCTGACTCTG (A)12
----TTC- (A)27
GAAACAGCACCTTCAGGCAGAAACACAATAAAGCAATTTTCCTTCAAGCC (A)37
```

FIG. 2. The DNA sequence of pVHC1. The amino acid sequence was deduced from the DNA sequence, and its amino acid sequence was compared with those of the α (pCHMC21) and β (pCMHC5) forms of the rat ventricular myosin heavy chain. Line 1, nucleotide sequence of pVHC1; line 2, amino acid sequence of pVHC1; line 3, amino acid sequence of pCMHC21; line 4, amino acid sequence of pCMHC5. The dashed lines indicate areas of homology. Amino acids and nucleotides at the 3'-untranslated region that are not homologous are printed out.

Table 1. Homology of hamster myosin heavy chain cDNA (pVHC1) compared to the rat α (pCMHC21) and β (pCMHC5) myosin heavy chain cDNA (excluding the 3'-untranslated regions)

Myosin heavy chain	Homology, % DNA	Homology, % Protein	Protein homology,* %	Protein homology,† %	DNA homology,‡ %	DNA homology in the 3'-untranslated regions, %
α	93	96	99	97	98	55
β	90	92	96	94	96	9

*Allowing conservative amino acid changes.
†Discounting 3'-carboxyl-terminal peptides.
‡Discounting third base changes.

degraded. This finding strongly suggests that the myosin heavy chain in slow-twitch skeletal muscle is distinctly different from that in cardiac muscle, which agrees with reported data (22, 39). The 3'-carboxyl-amino acid codons and the 3'-untranslated regions in the cardiac myosin heavy chain mRNAs appear to be unique (see Table 1, Fig. 2, and refs. 22 and 40). This allows the probe to be used as a specific tool to follow the expression of the α ventricular myosin heavy chain mRNA during development. The results in Fig. 3 clearly show that the α form of myosin heavy chain mRNA is expressed during all stages of development. However, the amount of mRNA expressed at 2 days after birth is far less than at 20 days after birth, which in turn, is less than at 7 months. This pattern corresponds very closely to the ventricular myosin protein pattern seen during maturation of both rat and hamster heart (28, 41); these data suggest that the mRNA represented by pVHC1 is coding for the α ventricular myosin heavy chain.

In conclusion, we have constructed and isolated a cDNA clone that codes for the α form of myosin heavy chain mRNA from Syrian hamster heart. The expression of the α form of the ventricular myosin heavy chain mRNA is age dependent and tissue specific. Based on the nucleotide sequence and the deduced amino acid sequence of pVHC1, this cDNA clone is highly homologous to the rat α cardiac cDNA clone pCMHC21 with some distinct differences at the 3' end of the clone. It can be concluded from these results that the myosin heavy chain mRNA coded for in pVHC1 represents the α ventricular myosin heavy chain mRNA from the Syrian hamster.

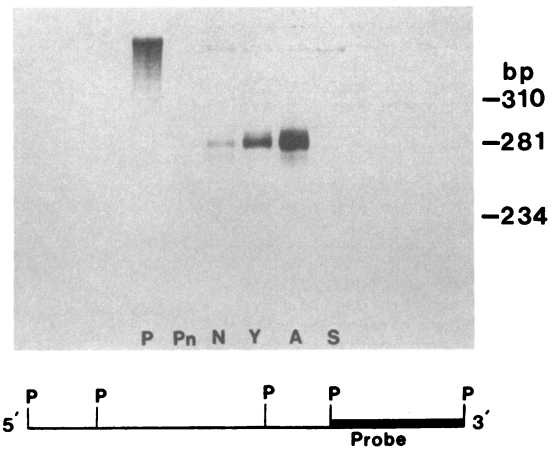

FIG. 3. S1 nuclease mapping. RNA obtained from 2-day-old (lane N), 20-day-old (lane Y), and 7-month-old (lane A) hamster heart and hamster soleus muscle (lane S) were hybridized with a 350-base probe (P) that maps at the 3' end of pVHC1. This probe was obtained by digestion of pVHC1 with *Pst* I and labeling the probe using the M13 mp8 vector. The expected size of a fully protected fragment (≈290 bp) is seen in the heart RNA samples and not in the soleus muscle sample. As a control the probe with no RNA hybridized to it is completely degraded by S1 nuclease (lane Pn). bp, Base pairs.

This work is generously supported by grants from the U.S. Public Health Service (HL-18824-01), the Ontario Heart and Stroke Foundation, Ontario, and the Medical Research Council (Canada). M.A.J. holds a University of Toronto Open Fellowship. Initial construction of the cDNA library was carried out in Dr. B. Nadal-Ginard's laboratory while on a sabbatical leave (C.C.L.) at the Children's Hospital, Harvard Medical School. We thank Drs. V. Mahdavi, B. Nadal-Ginard, and M. Periasamy for providing us with the rat cardiac myosin heavy chain clones and their valuable advice. We also thank Mr. W. Hagan for his technical assistance in the DNA sequence analysis of pVHC1.

1. Whalen, R. G. & Sell, S. M. (1980) *Nature (London)* **286,** 731–733.
2. Whalen, R. G., Sell, S. M., Erickson, A. & Thornell, L. E. (1982) *Dev. Biol.* **91,** 478–484.
3. Winkelmann, D. A., Lowey, S. & Press, J. L. (1983) *Cell* **34,** 295–306.
4. Nadal-Ginard, B., Medford, R. M., Nguyen, H. T., Periasamy, M., Wydro, R. M., Horning, D., Gubits, R., Garfinkel, L. I., Wieczorek, D., Bekesi, E. & Mahdavi, V. (1982) in *Muscle Development: Molecular and Cellular Control*, eds. Person, M. L. & Epstein, H. F. (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY), pp. 143–168.
5. Masaki, T. & Yoshizaki, C. J. (1974) *J. Biochem. (Tokyo)* **76,** 123–131.
6. Lompre, A. M., Mercardier, J. J., Wisnewsky, C., Bouveret, P., Pantaloni, C., D'Albis, A. & Schwartz, K. (1981) *Dev. Biol.* **84,** 286–290.
7. Clark, W. A., Jr., Chizzonite, R. A., Evereth, A. W., Rabinowitz, M. & Zak, R. (1982) *J. Biol. Chem.* **257,** 5449–5454.
8. Hoh, J. F. Y., McGrath, P. A. & White, R. J. (1976) *Biochem. J.* **157,** 87–95.
9. Whalen, R. G., Schwartz, K., Bouveret, P., Sell, S. M. & Gros, F. (1979) *Proc. Natl. Acad. Sci. USA* **76,** 5197–5201.
10. Schwartz, K., Lompre, A. M., Bouveret, P., Wisnewsky, C. & Whalen, R. G. (1982) *J. Biol. Chem.* **257,** 14412–14418.
11. Malhorta, A., Penpargkal, S., Fein, F. S., Sonnenblick, E. N. & Scheuer, J. (1981) *Circ. Res.* **49,** 1243–1251.
12. Umeda, P. K., Kavinsky, C. J., Sinha, A. M., Hsu, H. J., Jakovic, S. & Rabinowitz, M. (1983) *J. Biol. Chem.* **258,** 5206–5214.
13. Mercardier, J. J., Lompre, A. M., Wisnewsky, C., Samuel, J. L., Bercovici, J., Swynghedauw, B. & Schwartz, K. (1981) *Circ. Res.* **49,** 525–532.
14. Gorza, L., Pauletto, P., Pessima, A. C., Sartore, S. & Shiaffinao, S. (1981) *Circ. Res.* **49,** 1003–1009.
15. Scheuer, J., Malhorta, A., Hirsch, C., Capasso, J. & Schaible, T. F. (1982) *J. Clin. Invest.* **70,** 1300–1305.
16. Hoh, J. F. Y., McGrath, P. A. & Hale, P. T. (1978) *J. Mol. Cell. Cardiol.* **11,** 1053–1076.
17. Flink, I. L., Rader, J. H. & Markin, E. (1979) *J. Biol. Chem.* **254,** 3105–3110.
18. Pagani, E. D. & Solero, R. J. (1983) *Am. J. Physiol.* **245,** 713–720.
19. Lompre, A. M., Schwartz, K., D'Albis, A., Lacombe, G., Theirn, N. Y. & Swynghedauw, B. (1979) *Nature (London)* **282,** 105–107.
20. Litten, R. Z., Martin, B. J., Low, R. B. & Albert, N. R. (1982) *Circ. Res.* **50,** 856–864.
21. Rupp, H. (1981) *Basic Res. Cardiol.* **76,** 79–88.

22. Mahdavi, V., Periasamy, M. & Nadal-Ginard, B. (1982) *Nature (London)* **297**, 659–665.
23. Mahdavi, V., Chambers, A. P. & Nadal-Ginard, B. (1984) *Proc. Natl. Acad. Sci. USA* **81**, 2626–2630.
24. Strehler, E. E., Mahdavi, V., Periasamy, M. & Nadal-Ginard, B. (1985) *J. Biol. Chem.* **260**, 468–471.
25. Hamburger, F., Nixon, C. W., Eppenbeger, M. & Baker, J. R. (1962) *Med. Exp. (Basel)* **6**, 339–345.
26. Strobeck, J., Factor, S. M., Bhan, A., Sole, M. J. & Liew, C. C. (1979) *Ann. N. Y. Acad. Sci.* **317**, 59–88.
27. Bishop, S. P., Tilley, I. P. & Sole, M. J. (1979) in *Spontaneous Animal Models of Human Disease* (Academic, New York), pp. 59–64.
28. Wiegand, V., Stroh, E., Hennings, A., Lossnitzer, K. & Kreuzer, H. (1983) *Basic Res. Cardiol.* **78**, 665–670.
29. Malhotra, A., Karell, M. & Scheur, J. (1985) *J. Mol. Cell. Cardiol.* **17**, 95–107.
30. Jandreski, M. & Liew, C. C. (1984) *Can. J. Biochem.* **62**, 185–190.
31. Maniatis, T., Fritsch, E. F. & Sambrook, J. (1982) *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY).
32. Hanahan, D. (1983) *J. Mol. Biol.* **166**, 557–580.
33. Grunstein, M. & Hogness, D. S. (1975) *Proc. Natl. Acad. Sci. USA* **72**, 3961–3965.
34. Medford, R. M., Wydro, R. M., Nguyen, H. T. & Nadal-Ginard, B. (1980) *Proc. Natl. Acad. Sci. USA* **77**, 5749–5753.
35. Smith, H. O. & Bernstiel, M. L. (1976) *Nucleic Acids Res.* **3**, 2387–2398.
36. Burke, J. F. (1984) *Gene* **30**, 63–68.
37. Messing, J. & Vieira, J. (1982) *Gene* **19**, 269–276.
38. Sanger, F., Nichlen, S. & Coulson, A. R. (1977) *Proc. Natl. Acad. Sci. USA* **74**, 560–564.
39. Lompre, A. M., Nadal-Ginard, B. & Mahdavi, V. (1984) *J. Biol. Chem.* **259**, 6437–6446.
40. Periasamy, M., Wieczorek, D. F. & Nadal-Ginard, B. (1984) *J. Biol. Chem.* **259**, 13573–13578.
41. Whalen, R. G., Sell, S. M., Butler-Browne, G. S., Schwartz, K., Bouveret, P. & Pinset-Harstrom, I. (1981) *Nature (London)* **292**, 805–809.