Contraction Accelerates Myosin Heavy Chain Synthesis Rates in Adult Cardiocytes by an Increase in the Rate of Translational Initiation*

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The purpose of this study was to determine the mechanism by which contraction acutely accelerates the synthesis rate of the contractile protein myosin heavy chain (MHC). Laminin-adherent adult feline cardiocytes were maintained in a serum-free medium and induced to contract at 1 Hz via electrical field stimulation. Electrical stimulation of contraction accelerated rates of MHC synthesis 28% (p < 0.05 by 4 h as determined by incorporation of [3H]phenylalanine into MHC. MHC mRNA expression as measured by RNase protection was unchanged after 4 h of electrical stimulation. MHC mRNA levels in messenger ribonucleoprotein complexes and translating polysomes were examined by sucrose gradient fractionation. The relative percentage of polysome-bound MHC mRNA was equal at 47% in both electrically stimulated and control cardiocytes. However, electrical stimulation of contraction resulted in a reproducible shift of MHC mRNA from smaller polysomes into larger polysomes, indicating an increased rate of initiation. This shift resulted in significant increases in MHC mRNA levels in the fractions containing the larger polysomes of electrically stimulated cardiocytes as compared with nonstimulated controls. These data indicate that the rate of MHC synthesis is accelerated in contracting cardiocytes via an increase in translational efficiency.

Hypertrophic growth occurs in terminally differentiated adult cardiocytes by an increase in cellular mass via a relatively coordinate increase in the proteins comprising each of the cellular components (1). This accumulation of cardiocyte proteins occurs by an increase in rates of protein synthesis relative to rates of protein degradation (2). The anabolic changes that occur during hypertrophy of the adult myocardium are generally considered to be a compensatory response to increased mechanical load as defined by adherence of the cardiocytes to the culture dish and establishment of a resting length (8) and in response to passive stretch of cardiocytes adherent to a deformable membrane (9). Thus, the integrity of adult cardiocytes in primary culture is maintained with respect to the ability to transduce changes in mechanical load into an anabolic response such as accelerated protein synthesis rate.

The specific mechanisms by which cardiocyte protein synthesis is regulated probably occur at many levels, including transcriptional, post-transcriptional, and translational processes (10). It is well established, for example, that transcriptional and post-transcriptional mechanisms regulate steady state mRNA levels and are responsible for qualitative changes in gene expression during cardiac hypertrophy (11, 12). At the translational level, protein synthesis rates can be accelerated by changes in efficiency or capacity (2, 13). Translational efficiency refers to the efficiency with which the cardiocyte utilizes translational machinery such as mRNA, ribosomes, initiation factors, and elongation factors; whereas, translational capacity refers to the relative abundance of these translational components in the cardiocyte, in particular the ribosome and translation factor pools. Protein synthesis rates are accelerated by an increased translational capacity during sustained hypertrophic growth of the myocardium (2, 14, 15). In contrast, most of the evidence for changes in translational efficiency is found in acute studies in which protein synthesis rates increased within hours after a load was imposed (5, 16–18). These studies suggest that cardiocyte protein synthesis could be regulated by a temporal sequence in which changes in translational efficiency are followed by sustained changes in translational capacity. In skeletal muscle, alterations in load have a marked effect on translational efficiency, particularly in the acute phase (19–21). For example, unweighting of the soleus muscle in rats results in a reduction in MHC synthesis which has been attributed to a decrease in translational activity (21).

In previous studies employing adult feline cardiocytes in primary culture, we demonstrated that electrically stimulated contractile cardiocytes in primary culture, we demonstrated that electrically stimulated contractation resulted in an acute acceleration of both total protein synthesis and MHC synthesis rates (6). Two lines of evidence suggested that this acceleration occurred via a mechanism involving an increase in translational activity. First, protein synthesis rates were accelerated by contraction, even when transcription was blocked with actinomycin D. Second, because the effects of electrical stimulation were observed by 1 h, the acceleration of protein synthesis preceded any measur-

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1 The abbreviations used are: MHC, myosin heavy chain; Pipes, 1,4-piperazinediethanesulfonic acid; MOPS, 4-morpholinepropanesulfonic acid.
able changes in the amount of translational machinery as reflected by an increase in the ribosomal pool. In this study, MHC was used as a cardio-myocyte-specific marker to determine whether translational mechanisms are involved in the acute acceleration of the rate of protein synthesis in response to electrically stimulated contraction of adult cardiocytes. Comparisons were made between quiescent cardiocytes and cardiocytes electrically stimulated to contract by measuring the synthesis rate of MHC protein, the size of the MHC mRNA pool, and the translational efficiency of MHC mRNA. These studies demonstrated that: 1) rates of MHC synthesis are accelerated by electrical stimulation without a corresponding change in steady state mRNA levels, and 2) the mechanism for accelerating MHC synthesis in contracting cardiocytes is an increase in translational efficiency as reflected by a shift of MHC mRNA into larger polysomes.

MATERIALS AND METHODS

Electrical Stimulation Model—Adult feline cardiocytes were obtained by collagenase digestion in combination with mechanical agitation as described previously (6, 8, 9). Following cell isolation, the Ca++-tolerant cardiocytes were placed in Life Technologies, Inc. M199 serum-free medium with Earle’s salts at a concentration of 50,000 rod-shaped cells/ml. The cells were plated on 245 × 245-mm culture trays (Nuncclone) coated with 20 μg/ml laminin (Upstate Biotechnology, Inc.) to facilitate cell adhesion. After an overnight incubation, the medium was changed to remove nonadherent cells. The M199 medium was modified as before, and adult feline cardiocytes, which are normally quiescent in culture, were induced to contract synchronously via electrical field stimulation as described previously with modification (6). In order to stimulate a larger number of cardiocytes, the system was adapted according to the method of Berger et al. (22) for use with large culture trays in which electrical pulses were delivered to the medium via carbon electrodes submerged at the opposite ends of each tray (Fig. 1). The distance between the electrodes was 23 cm. The polarity of the electrodes was alternating with each electrical pulse to minimize electrolysis at the electrodes. In order to set the threshold voltage for contraction, the lowest voltage required to stimulate greater than 50% of the cells was determined and then exceeded by 10%, resulting in an approximate response ratio of 70%. The resultant pulses of approximately 6 volts/cm between electrodes were delivered as a square wave for a duration of 5 ms at 1 Hz for 4 h.

Measurement of Myosin Heavy Chain Synthesis Rates—Rates of MHC synthesis were determined by measuring incorporation of [3H]Phe into electrophoretically purified MHC. Cells were radiolabeled with 0.4 mm [3H]Phe (20 μCi/ml, Amersham Corp.) over the entire 4 h of electrical stimulation. Total cell protein was scanned in a lysis buffer consisting of: 0.4 M Trizma (Tris base) glycine, 5% sodium dodecyl sulfate (SDS), 20% glycerol, 0.5% β-mercaptoethanol, and 1.5 M phenylmethylsulfonyl fluoride. The lysates were boiled for 5 min and cooled to room temperature. The samples were electrophoresed on 3-mm polyacrylamide-N,N′-diallyltartardiamide 10–13% gradient gels for 24 h at 10 V/cm. The MHC band was identified by Coomassie Brilliant Blue R-250 (Bio-Rad) staining and excised. The band was solubilized in 2% periodic acid, 4% lactic acid, pH adjusted to 3.0 with NH₄OH. The protein was precipitated by adding 70% HClO₄ to a final concentration of 6% and centrifuged 15 min at 10,000 × g. The pellet was washed twice with 6% HClO₄ and dried by vacuum centrifugation. The pellet was solubilized in 0.3 M NaOH by heating to 60 °C for 1 h. Aliquots were assayed for radioactivity by liquid scintillation counting and for MHC protein by the BCA method (Pierce) using bovine serum albumin as a standard. The specific radioactivity of Phe in the medium was determined by scintillation counting of aliquots of culture medium. Previous studies have shown that the Phe-RNA pools equilibrate to 80% of medium Phe-specific radioactivity in both electrically stimulated and quiescent cardiocytes (6).

Probes for MHC mRNA—Two probes were used to measure MHC mRNA levels. For RNase protection assays, [32P]UTP-labeled antisense RNA probes were synthesized by in vitro transcription from a 436-base pair fragment (p-MHC-5) of the rat α-MHC cDNA inserted into a pGEM-3Zf(+) plasmid (23). This region of α-MHC mRNA extends from nucleotides 3459 through 3895 of the coding sequence and is highly conserved as indicated by a 90% sequence similarity to rat β-MHC mRNA and an 89% sequence similarity to human β-MHC mRNA. In the vertebrate of adult cat, it has been demonstrated that the β-MHC isoform is expressed exclusively (24). In order to confirm that the degree of homology of the probe was the same for feline β-MHC mRNA, a partial cDNA clone for feline β-MHC was isolated from an adult feline cardiocyte cDNA library. The identity of the cDNA clone as the β-MHC isoform was based upon the high degree of sequence similarity to the 3′-untranslated regions of the rat and human β-MHC mRNA isoforms. Sequence analysis of the region extending between nucleotides 3459–3714 revealed that the degree of homology of feline β-MHC to rat α-MHC is 92%, the same as for rat β-MHC.2 RNase protection using total RNA extracted from adult cardiocytes yielded a protected band of approximately 83 nucleotides, the same size band observed for rat β-MHC mRNA.

For the slot-blotting analyses, a 215-base pair cDNA clone of feline cardiomyocyte β-MHC mRNA was employed. DNA sequence analysis revealed that the feline species was greater than 92% similar to rat β-MHC mRNA in the region between nucleotides 4028 and 4243 and to human β-MHC in the region between 4017 and 4232. The cDNA insert was subsequently subcloned into a pGEM-3Zf(+) plasmid. [32P]UTP-labeled cDNA probes were generated by the polymerase chain reaction using oligonucleotide primers complementary to the T7 and SP6 promoter regions flanking either side of the cDNA insert.

Quantification of MHC mRNA Expression—Total cellular RNA was extracted from cell cultures via the RNAzol method (Biotex Laboratories Inc.), and MHC mRNA was quantified by an RNase protection assay as follows. The RNA was resuspended in hybridization buffer containing 60% formamide, 400 mM sodium acetate, pH 4.6, 280 mM NaCl, 4.5 mM ZnSO₄, 250 units/ml S1 nuclease (Promega). Reactions were terminated with 50 μl of stop buffer (4 M ammonium acetate, 100 mM EDTA). The samples were phenol/chloroform-extracted and precipitated in ethanol with 20 μg of Escherichia coli tRNA as carrier. The samples were pelleted by centrifugation, washed in 70% ethanol, and resuspended in 80% formamide, 10 μg/ml tRNA. Samples were heated to 65°C and then chilled on ice to rehybridize on 8 μl, 4% polyacrylamide gels. The gels were dried, washed, dried, and processed for autoradiography. The protected MHC mRNA band was quantified by computer-assisted image analysis of autoradiograms and normalized to the corresponding amount of 28 S rRNA/sample as determined by slot blotting.

Quantification of MHC mRNA in Polysonome Fractions—Cardiocyte culture cytoplasm was rinsed three times on ice with ice-cold phosphate-buffered saline to which 100 μg/ml cycloheximide was added to arrest polypeptide chain elongation. Cardiocytes were scraped from the culture trays twice in 10 ml of phosphate-buffered saline/cycloheximide, pelleted by centrifugation, and resuspended in 1 ml of resuspension buffer (10 mM Tris, pH 7.5, 250 mM KCl, 2 mM MgCl₂, 0.5% (v/v) Triton X-100). The resuspended cells were homogenized with 18 strokes of a glass rod. Dounce homogenizer and transferred to a chilled 1.5-ml microtube. 150 μl of a solution containing 10% (v/v) Tween 80, 5% (v/v) deoxycholate was added, and the homogenate was vortexed and incubated on ice for 15 min. Homogenates were centrifuged 10 min at 10,000 × g to pellet nuclei, mitochondria, and insoluble proteins. The polysonome distribution of MHC mRNA was measured by two methods, RNase protection and slot blotting.

To prepare polysons for RNase protection assays, post-mitochondrial supernatants were layered onto 15–50% linear sucrose gradients and centrifuged for 95 min at 32,000 rpm in an SW-41 rotor (Beckman Instruments). Gradients were fractionated into seven fractions of 1.2 ml each from the gradient peak, followed by sucrose/chloroform extraction and ethanol precipitation using tRNA as carrier. MHC mRNA levels in each fraction were determined by the RNase protection assay as described above. Prior to hybridization, aliquots were taken to measure 28 S rRNA by slot blotting.

Polysomes were prepared for the slot blotting method by layering the post-mitochondrial supernatant onto a sucrose gradient and centrifuging for 95 min at 35,000 rpm in an SW-41 rotor. The gradients were fractionated into eight fractions of 1.2 ml each starting with the top of the gradient. The samples were phenol/chloroform-extracted and ethanol-precipitated. The RNA was resuspended in 67% formamide, 7% (v/v) formaldehyde, 25 μM MOPS, pH 7.0, 3 mM EDTA, 0.8 M sodium acetate and heated at 60 °C for 10 min. The RNA was immobilized on...
Translated Regulation of Myosin Heavy Chain Synthesis

Hybond-N membranes (Amersham) by means of a slot blotting apparatus and UV cross-linked. The blots were hybridized at 42°C in a buffer containing 50% formamide, 10× Denhardt’s solution, 50 mM Tris, pH 7.5, 0.1% Na2P2O7, 1% SDS, 100 μg of salmon sperm DNA/ml and 32P-labeled feline MHC cDNA probe in excess. The blots were washed three times over 1 h with 2× SSC, 0.1% SDS at 42°C, followed by three more washes over 1 h at 70°C in 0.1× SSC, 0.1% SDS. The membranes were processed for autoradiography and the optical density of the hybridization signals were measured by computer-assisted image analysis. In order to normalize the MHC signal to the amount of RNA recovered in the polysome fractions, the blots were stripped and hybridized to the 28 S rRNA probe. The 28 S rRNA signal was processed for autoradiography and measured by computer-assisted image analysis.

Quantitation of Subunit RNA—Post-mitochondrial supernatants were prepared from electrically stimulated and quiescent cultures as described above for polysomes. A 150-μl aliquot of the post-mitochondrial supernatant was removed to assay for total RNA and a 900-μl aliquot was layered onto 1 ml aliquot of the post-mitochondrial supernatant. Gradients were centrifuged 18 h in a Sorvall SW-41 rotor at 37,000 rpm, and 40 and 60 S subunit peaks were collected by gradient fractionation and pooled. Total RNA and RNA in subunits were assayed by the method of Munro and Fleck (26), and RNA in subunits was calculated as a percentage of total RNA (15, 26).

Statistical Analysis—Statistical analysis was performed using StatView 4.0 software (Abacus Concepts Inc.). Because the two experimental groups of cardiocytes were derived from the same preparation, differences between nonstimulated and electrically stimulated groups were determined using a paired Student’s t test. A value of p < 0.05 was considered to be a significant difference.

RESULTS

We have demonstrated previously that contraction induced by electrical stimulation accelerated rates of total protein synthesis and fractional rates of MHC synthesis. In the present studies, relatively large numbers of cardiocytes were needed for the preparation of polysomes and RNA. The system was adapted to electrically stimulate large trays of cardiocytes with the use of carbon electrodes (Fig. 1). In order to validate that the same acute anabolic response as observed before (6) was elicited in cardiocytes using this system, rates of MHC synthesis were measured over 4 h of electrically stimulated contraction and compared with nonstimulated controls. In agreement with previous studies, contraction acutely accelerated the rate of MHC synthesis as compared with nonstimulated controls (Fig. 2).

In order to determine whether the acceleration of MHC synthesis was due to an increase in MHC mRNA levels, MHC mRNA expression was quantified using an RNase protection assay. In the present studies, relatively large numbers of cardiocytes were needed for the preparation of polysomes and RNA. The system was adapted to electrically stimulate large trays of cardiocytes with the use of carbon electrodes (Fig. 1). In order to validate that the same acute anabolic response as observed before (6) was elicited in cardiocytes using this system, rates of MHC synthesis were measured over 4 h of electrically stimulated contraction and compared with nonstimulated controls. In agreement with previous studies, contraction acutely accelerated the rate of MHC synthesis as compared with nonstimulated controls (Fig. 2). In order to determine whether the acceleration of MHC synthesis was due to an increase in MHC mRNA levels, MHC mRNA expression was quantified using an RNase protection assay. In the present studies, relatively large numbers of cardiocytes were needed for the preparation of polysomes and RNA. The system was adapted to electrically stimulate large trays of cardiocytes with the use of carbon electrodes (Fig. 1). In order to validate that the same acute anabolic response as observed before (6) was elicited in cardiocytes using this system, rates of MHC synthesis were measured over 4 h of electrically stimulated contraction and compared with nonstimulated controls. In agreement with previous studies, contraction acutely accelerated the rate of MHC synthesis as compared with nonstimulated controls (Fig. 2).

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Figure 1. Schematic diagram of the electrical stimulation apparatus. A physiological stimulator was employed to initiate contraction via electric field stimulation. Electrical pulses of alternating polarity were delivered to the medium via carbon electrodes positioned at the ends of the culture tray. The arrows denote alternating direction of current.

Figure 2. Effect of contraction on MHC synthesis rates. Rates of MHC synthesis were determined after 4 h of labeling in electrically stimulated and quiescent cardiocytes. Asterisk, significant difference, p < 0.05 as determined by a paired Student’s t test. Values are mean ± S.E., n = 8 cardiocyte preparations.

Figure 3. Specificity of probes for feline cardiac MHC mRNA. A, Northern blots showing the specificity of the rat α-MHC cDNA probe (lane 1) and the feline β-MHC cDNA probe (lane 2). 15 μg of total RNA extracted from adult cat ventricle was run per lane. B, linearity of the RNase protection assay for MHC mRNA levels in adult feline cardiocytes. Total RNA was extracted from quiescent cardiocytes and added to the assay in the indicated amounts. The optical density of the protected MHC band on the autoradiogram was measured by computer-assisted digital image analysis. R2 = 0.99.
MHC mRNA recovered in the free (fractions were the same in electrically stimulated and non-stimulated cardiocytes. Total RNA in stimulated (S) and control (C) cultures from six different experimental preparations was assayed. A, summary data of digital image analysis of the autoradiogram in A. Optical densities were normalized to 28 S rRNA in each sample. There was no significant difference between the two groups as determined by Student’s t test. Values are the mean ± S.E., n = 6 cardiocyte preparations.

In Fig. 4, MHC mRNA levels were compared between electrically stimulated (S) and nonstimulated controls (C) in each of six experiments. Fig. 4A is an autoradiogram showing the MHC bands following RNase protection. In order to normalize for the amount of total RNA that was recovered and added to the assay, aliquots of each diluted sample were slot-blotted and probed for 28 S rRNA. The summary data of MHC mRNA normalized to 28 S rRNA are shown in Fig. 4B. These data demonstrate that electrically stimulated contraction did not significantly alter MHC mRNA levels. A difference in the MHC signal, such as that observed in experiment number 2, was the result of a differential recovery of total RNA extracted from the cardiocytes. There was not a difference in MHC levels in this particular experiment when corrected for the amount of 28 S rRNA added to the assay. Thus, the acceleration of MHC synthesis measured after 4 h of electrical stimulation occurred without any significant changes in MHC mRNA levels.

Because rates of MHC synthesis were accelerated without a change in MHC mRNA, we examined whether the existing MHC mRNA pool was utilized more efficiently for protein synthesis in electrically stimulated cardiocytes. In order to determine the relative amount of the MHC mRNA pool active in translation, we quantified the amount of MHC mRNA that was located in the bound, polysome fractions and in the free, non-polysome fractions of the gradients. Post-mitochondrial supernatants prepared from whole cell extracts were layered onto 15–50% linear sucrose gradients and centrifuged. The gradients were fractionated into a lighter fraction containing mRNP particles, 40 S and 60 S subunits and 80 S ribosomes, and a heavier fraction containing polysomes (refer to absorbance tracing in Fig. 6A). As demonstrated in Fig. 5A, the amounts of MHC mRNA recovered in the free (F) and polysome-bound (B) fractions were the same in electrically stimulated and non-stimulated cardiocytes. Summary data from four experiments are shown in Fig. 5B, confirming that approximately equal amounts of total MHC mRNA were present in the actively translating polysome region of the gradients in both electrically stimulated and nonstimulated cardiocytes. The same results were obtained when MHC mRNA signal was normalized to recovered 28 S rRNA. Values are mean ± S.E., n = 4 cardiocyte preparations.

Because the relative amount of the MHC mRNA pool in polysomes was unchanged in electrically stimulated cardiocytes, we investigated whether the distribution of MHC mRNA in the actively translating polysome was changed. Cardiocyte extracts were centrifuged on 15–50% linear sucrose gradients as before and the gradients fractionated into 1.2-ml fractions starting with the 40 S ribosomal peak. Fig. 6A is a representative gradient profile showing the resolution of the fractionation procedure and the content of each fraction. Fig. 6B shows autoradiograms of an RNase protection assay which demonstrated that in nonstimulated cardiocytes, the majority of MHC mRNA was localized to the first three fractions. There were much smaller amounts in fractions 4 through 6, the region of the gradient that contained the larger polysomes. In contrast, electrical stimulation of contraction resulted in a shift of MHC mRNA into gradient fractions 4 and 5 corresponding to larger polysomes.

In Fig. 6C, summary data are presented for six separate experiments using the RNase protection assay to measure MHC mRNA in the gradient fractions. Using absorbance traces such as that in Fig. 6A as a reference, the gradients were divided into the ribosomal subunit and 80 S ribosome region (Gradient Fraction 1), the small polysome region (Gradient Fractions 2 and 3) and the large polysome region (Gradient Fractions 4–6). It should be noted that there was some overlap of the 80 S ribosomal peak between gradient fractions 1 and 2 and of the peak containing four polysomes between gradient...
increased from 0.1 ± 0.05 to 0.34 ± 0.07 in electrically stimulated cardiocytes, a significant increase of 240%. Although the decreases in MHC mRNA in the ribosomal subunits, 80 S, and small polysome fractions of electrically stimulated cardiocytes were not statistically significant, the total decrease of 0.21 accounted for the corresponding increase of 0.24 in the heavy polysome fractions. Thus, a small shift of MHC mRNA from the lighter regions of the gradient resulted in a relatively big increase of MHC mRNA in the larger polysome fractions of electrically stimulated cardiocytes.

In these experiments, recovery of ribosomes and polysomes was accounted for by hybridization to a 28 S rRNA probe. There were no significant differences in the percentage of 28 S rRNA in each region of the gradient between electrically stimulated and nonstimulated gradient fractions (data not shown).

In order to confirm the results obtained by the RNase protection method, the experiments were repeated and MHC mRNA levels in the gradient fractions were measured by slot blotting using a feline β-MHC cDNA probe. The specificity of the cDNA probe is demonstrated in Fig. 3A (lane 2). In the slot-blot method, the entire RNA sample recovered from each gradient fraction was immobilized directly onto the hybridization membrane. Furthermore, the amount of recovered RNA in each fraction was measured by stripping the blot and hybridizing to the 28 S rRNA probe. These experiments also differed from the RNase protection experiments in that the gradients were centrifuged at a faster speed to improve the resolution of the polysome region of the gradients and enhance the signal intensity in the larger polysome fractions.

In Fig. 7A, the distribution of MHC mRNA in gradients of nonstimulated and electrically stimulated cardiocytes are compared. The percentage of MHC mRNA in each gradient fraction was calculated from the sum total of MHC mRNA in all the gradient fractions. The values were divided by the percentage of 28 S rRNA in each corresponding fraction, thereby correcting for any differences in recovery of ribosomes and polysomes. For comparison to the gradients in Fig. 6C, fractions 2 and 3 correspond to the ribosomal subunit and 80 S fraction, fractions 4 through 6 correspond to the small polysome fraction, and fractions 7 and 8 correspond to the large polysome fraction. In nonstimulated cardiocytes, MHC mRNA relative to 28 S rRNA was highest in fractions 4 and 5 and subsequently declined in the fractions containing the larger polysomes. By comparison, there was significantly more MHC mRNA relative to 28 S rRNA in fraction 8 of electrically stimulated cardiocytes. MHC mRNA in this fraction increased significantly from 0.74 ± 0.26 in nonstimulated to 1.33 ± 0.24 in electrically stimulated cardiocytes. Because recovery of 28 S rRNA was the same (Fig. 7B), these data demonstrate that there was a reproducible shift of MHC mRNA from the smaller polysomes into the larger polysomes contained in fraction 8 of electrically stimulated cardiocytes. These data are consistent with the results shown in Fig. 6, demonstrating that small shifts of MHC mRNA from the smaller polysome fractions result in substantial increases in MHC mRNA located in the larger polysomes of electrically stimulated cardiocytes. Furthermore, the absolute amount of MHC mRNA in fraction 8 of electrically stimulated cardiocytes, normalized to 28 S rRNA, increased 215% ± 51%, p < 0.03 over nonstimulated controls (mean ± S.E., n = 4 paired experiments).

In Fig. 7B, it is demonstrated that the changes in the distribution of MHC mRNA in the gradients of electrically stimulated cardiocytes were not due to differential recovery of ribosomes and polysomes. For each gradient, the relative amount of 28 S rRNA per fraction was plotted as a percentage of the total amount of recovered 28 S rRNA. There were no significant
differences in the recovery of 28 S rRNA throughout the gradients of quiescent and electrically stimulated cardiocytes. Furthermore, there were no significant differences in absolute amounts of recovered 28 S rRNA in the gradient fractions between paired samples of electrically stimulated and non-stimulated cardiocytes (data not shown).

The increase in translational efficiency could have resulted from an overall increase in translational initiation in which free ribosomal subunits were recruited into polysomes. In order to test this possibility, the percentage of total RNA labeled to 40 and 60 S ribosomal subunits was quantified. The percentage of RNA in the subunit region of the gradient was 34% ± 10% in electrically stimulated and 33 ± 11% (mean ± S.E.) in non-stimulated cardiocytes. Thus, similar to the MHC mRNA pool, electrical stimulation did not mobilize a significant amount of the free ribosome pool into polysomes.

**DISCUSSION**

During the hypertrophic process in adult myocardium, there is a relatively proportional increase in cardiac protein such that the terminally differentiated phenotype is essentially maintained (1). The synthesis of myofibrillar proteins such as actin and myosin is increased in order to facilitate net accumulation of protein and subsequent assembly into nascent myofibrils (27–29). In the electrical stimulation model used in this study, there was no measurable accumulation of myosin and/or total cell protein over the 4-h time period. However, consistent with other adult models of hypertrophy, we have recently demonstrated that continuous electrical stimulation of adult feline cardiocytes increased protein content and cell size over 7 days without any significant changes in morphology and myofibrillar architecture (7). It is well established in several models of hypertrophy that transcription has a role in regulating the synthesis of contractile proteins, particularly qualitative changes in the expression of contractile protein isoforms in rodent species (11, 30). However, in the ventricles of larger animals, including humans, these changes in isofrom expression occur only to a very limited extent for actin and do not occur for myosin (11, 24, 30). In a quantitative context, changes in contractile protein synthesis rates in response to load do not necessarily correspond to mRNA levels in adult myocardium (18, 31). In perfused heart preparations, contractile arrest over several hours resulted in a decrease in MHC synthesis rate without a change in MHC mRNA levels (18). When an additional load was imposed via an elevation of aortic perfusion pressure, MHC synthesis rate was accelerated in arrested hearts without a change in MHC mRNA. Thus, translational mechanisms could play a major role in regulating contractile protein synthesis rates in adult cardiocytes, especially in the early phase after a change in load.

Because cardiac MHC mRNA has α and β isoforms, the possibility existed that there was induction of the α-MHC isoform and preferential translation after 4 h of electrically stimulated contractile activity. The two isoforms diverge in sequence in the 3'-untranslated region. We were unsuccessful in our attempts to detect an α-MHC mRNA signal in adult feline cardiac RNA using oligonucleotide probes directed against the 3'-untranslated region of either rat or human α-MHC mRNA. Definitive confirmation that the α-MHC isoform was not expressed or induced in adult feline cardiocytes will require the isolation of a feline α-MHC clone. However, the possibility that α-MHC mRNA was induced is unlikely for the following reasons. 1) Adult feline cardiocytes express the β-MHC isoform exclusively, and MHC isoform expression in adult cats did not change in response to pressure overload hypertrophy (24). 2) Contractile activity did not induce α-MHC isoform expression in primary cultures of neonatal rat cardiocytes nor did it induce α-MHC mRNA in perfused rat heart preparations (18, 25, 32). In fact, contractile activity and/or increased workload induce expression of the β-MHC isoform in rodent models of hypertrophy, the isoform already expressed in adult feline cardiocytes (11, 32). 3) Available sequence data from both rat and human α- and β-MHC isoforms predict that the size and pattern of the MHC band obtained by the RNase protection assay would change if α-MHC mRNA was induced by electrically stimulated contraction. No such changes were observed as the protected band remained the same size as the rat β-MHC band. 4) The shift of MHC into larger polysomes was detected using either the rat α-MHC probe or the feline β-MHC probe. Both probes were specific for the highly conserved rod region of the molecule in which there was a sequence similarity of at least 90% between the α- and β-MHC isoforms of rat and human species. Both probes detected α- and β-isoforms with equal affinity so that the total MHC mRNA pool was actually being measured.

The acute acceleration of MHC synthesis in response to electrically stimulated contraction occurred independent of a change in MHC mRNA abundance, suggesting that the rapid
alterations in synthesis rate were due to greater translational efficiency. There are two possible mechanisms to explain the more efficient utilization of the MHC mRNA pool at the level of peptide chain initiation. The first mechanism involves a mobilization of mRNA from translationally inactive mRNP particles to actively translating polysomes, whereas the second mechanism involves an increased rate of ribosome initiation on mRNA in the polysome fraction (33). The data in Fig. 5 indicated that MHC synthesis rates were not accelerated by selective mobilization of MHC mRNA into polysomes, even though there was a large percentage of total cellular MHC mRNA localized in the free mRNP pool. The reason for the relatively high percentage of inactive MHC mRNA with respect to translation is not known, although the percentage of MHC in mRNPs in skeletal myotubes prepared varied between 35 and 90% (34–36). The adult cardiocytes were cultured and maintained in a two-dimensional tissue culture environment and were not mechanically loaded to the same extent as cardiocytes in vivo. In addition, cultures of adult feline cardiocytes in serum-free medium have a slightly decreased rate of protein synthesis as compared with cells maintained in medium supplemented with serum (37). These lower rates may reflect an overall decreased translational efficiency and in part account for the relatively high percentage of MHC mRNA in the mRNP fraction.

The second mechanism to increase translational efficiency is by increasing the rate of initiation of ribosomes onto mRNA relative to the rate of peptide chain elongation, resulting in an increased size of MHC mRNA polysomes (33, 38, 39). The data in Figs. 6 and 7 are consistent with this type of mechanism as there was more MHC mRNA per unit mass of recovered 28 S rRNA in larger polysomes. In these experiments, the 28 S rRNA probe was used to correct for recovery of polysomes from the gradient fractions. Because recovery of 28 S rRNA in the gradient fractions was the same between nonstimulated and electrically stimulated cardiocytes, the shift of MHC mRNA into large polysomes of electrically stimulated cardiocytes is indicative of the fact that ribosomes are initiating faster onto MHC mRNA relative to elongation rate. The result is that the MHC mRNA pool would be more efficient as more polypeptides are synthesized per mRNA molecule. As an alternative possibility, the shift of mRNA into heavier polysomes could have resulted from a decrease in peptide chain elongation, as occurs during treatment with cycloheximide (38, 39). In unloaded soleus muscle, a reduction in protein synthesis rate was the result of a slower rate of peptide chain elongation (40). The possibility of a decreased elongation rate is unlikely, because inhibition of peptide chain elongation would be expected to cause a decrease, rather than an increase, in MHC synthesis rates.

It is not known how selective the increase in translational efficiency was for MHC mRNA. The changes in MHC synthesis rate in contracting cardiocytes may be indicative of a more global increase in translational efficiency. Our previous studies demonstrated that rates of total protein synthesis were acutely accelerated by electrical stimulation, and MHC synthesis rates were accelerated to a similar extent (6). If there was an overall increase in translational efficiency of total protein synthesis, a greater amount of the ribosome pool would be expected to mobilize into the polysome pool and/or shift into larger polysomes. This was not the case as the percentage of total RNA in subunits remained the same in electrically stimulated cardiocytes, and there were no differences in the amount of recovered rRNA in the larger polysome fractions of electrically stimulated as compared with quiescent cardiocytes. However, an overall shift of ribosomes into larger polysomes could have gone undetected because of the relatively small percentage of total rRNA in the large polysome fractions (16% in Fig. 6 and 20–25% in Fig. 7). In a terminally differentiated cell type such as the adult cardiocyte, the synthesis of MHC can increase in proportion to total cell protein because the relative abundance of MHC mRNA is maintained at a constitutively high level. Furthermore, based on steady state synthesis rates, MHC mRNA probably has an intrinsically high rate of initiation. Therefore, relatively rapid changes in MHC synthesis as measured in response to electrically stimulated contraction could occur by the MHC pool more effectively competing for a limited quantity of one of more of the translational components that are involved in accelerating protein synthesis (41).

It is generally recognized that overall changes in translational efficiency probably involve altering the specific activities of translational initiation factors that are rate-limiting for peptide chain initiation (33, 41). In the adult cardiocyte, a translational mechanism for accelerating the rate of protein synthesis would serve to coordinate protein synthesis and maintain the differentiated phenotype during the early phase of hypertrophic growth. The present studies show that protein synthesis can be accelerated prior to an increase in the RNA pool, indicating that transcription was not required. Furthermore, a translational mechanism for regulating the efficiency of protein synthesis rates acutely could be followed by an increase in translational capacity, a well-characterized mechanism for accelerating protein synthesis during sustained hypertrophic growth of the heart (2). Thus, a temporal sequence for regulating cardiocyte protein synthesis rates in response to a hypertrophic stimulus could potentially involve altering the activity of initiation factors initially, followed by a sustained increase in the relative amount of translational machinery.

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Translational Regulation of Myosin Heavy Chain Synthesis

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