Increased expression of eukaryotic initiation factor 4E during growth of neonatal rat cardiocytes in vitro

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Maklhouf, Antoine A., and Paul J. McDermott. Increased expression of eukaryotic initiation factor 4E during growth of neonatal rat cardiocytes in vitro. Am. J. Physiol. 274 (Heart Circ. Physiol. 43): H2133–H2142, 1998.—Eukaryotic initiation factor 4E (eIF-4E) is rate limiting for translational initiation. The purpose of this study was to determine whether eIF-4E levels are increased during cardiocyte growth produced by increased load in the form of electrically stimulated contraction. Neonatal rat cardiocytes were cultured on a matrix of aligned type I collagen. The cardiocytes aligned in parallel to the direction of the collagen fibrils and exhibited an elongated, rod-shaped morphology. Cardiocytes were electrically stimulated to contract at 3 Hz (alternating polarity, 5-ms pulse width). Nonstimulated cardiocytes were quiescent and used as controls. Electrically stimulated contraction produced hypertrophic growth as determined by the following criteria: 1) increased protein content, 2) increased RNA content, 3) accelerated rate of protein synthesis, and 4) threefold increase in promoter activity of the atrial natriuretic factor gene. Cardiocyte growth was associated with an increase in eIF-4E mRNA levels that reached 48 ± 9% after 2 days of electrically stimulated contraction. eIF-4E protein levels were increased by more than twofold over the same period. We conclude that an adaptive increase in eIF-4E is an important mechanism for maintaining translational efficiency during cardiocyte growth.

myocyte; hypertrophy; collagen; translation; initiation factors
lular KCl or the calcium channel blocker verapamil. Plating the cardiocytes at a relatively low density reduces the number of intercellular contacts, thereby minimizing spontaneous contraction (13). Second, neonatal cardiocytes aligned on collagen could be electrically stimulated to contract for prolonged periods of time at relatively low voltages, even when plated at a sparse density. This increase in responsiveness to electrical stimulation is facilitated by the combination of an elongated morphology and the ability to align the cardiocytes in parallel with the direction of the electrical field (34, 45). We utilized cardiocytes aligned on collagen for two primary purposes. First, the anabolic effects of increased load in the form of electrically stimulated contraction were established by direct comparison with quiescent cardiocytes maintained without any arresting agents. Second, the model was used to test the hypothesis that eIF-4E levels increase in parallel with the ribosome pool to accelerate the rate of protein synthesis during hypertrophic growth.

**MATERIALS AND METHODS**

Cell culture of neonatal rat cardiocytes. Ventricle cardiocytes were isolated from the hearts of 1- to 4-day-old Sprague-Dawley rats using a combination of enzymatic digestion and mechanical dissociation as described previously (11, 24). The cells were plated on an aligned collagen matrix as prepared by the method of Simpson et al. (44) with modifications. A neutral solution of 0.9 mg/ml collagen type I was prepared by mixing 1.5 ml of 10 Eagle's minimum essential medium (MEM, Gibco BRL), 600 µl of 0.5 M 2-hydroxyethylpiperazine-N′-2-ethanesulfonic acid, 19 ml MEM, and 9 ml collagen type I (3.0 mg/ml collagen; Collagen). The solution was brought to pH 7.4 and incubated on ice. An amount of 500 µl of the solution was applied to the edge of each 16-cm² well of a four-well culture tray (Nunclon) and spread unidirectionally with a cell scraper. The trays were tilted at 45°, and the excess collagen was aspirated. The collagen matrix was incubated at 37°C for 2 h, air dried, and rehydrated with serum-supplemented medium. The collagen matrix consisted of an array of parallel collagen fibrils on which the cells were plated at a density of 1 × 10⁵ cells/cm². After we allowed an overnight incubation period, the adherent cells were rinsed and maintained in serum-free media as described before (24).

On day 3 in culture, contractile activity was initiated by electrical stimulation with carbon electrodes (9, 15). Electrical pulses of alternating polarity were delivered to the culture medium at a frequency of 3 Hz and pulse width of 5 ms.

**Protein and RNA determination.** Protein-to-DNA ratios were determined for each individual culture well as described previously (11). Cells were harvested in a solution consisting of standard sodium citrate (300 mM NaCl and 30 mM sodium citrate) and 0.25% sodium dodecyl sulfate (SDS). Protein concentration was assayed by the bicinchoninic acid method (BCA, Pierce). To account for any contribution of the collagen to the protein assay, we determined the contribution of collagen to the protein assay was negligible.

The protein was solubilized in 0.3 N NaOH. Radioactivity of total protein was determined by liquid scintillation counting, and the mass of total protein was assayed by the BCA method. The rate of protein synthesis (nmol · mg⁻¹ · h⁻¹) was calculated by dividing the rate of leucine incorporation into total cell protein (dpm · mg⁻¹ · h⁻¹) by the specific activity of leucine in the culture medium (dpm/nmol).

Determination of eIF-4E protein levels. Total cell homogenates were prepared by rinsing each culture well twice in ice-cold PBS and scraping in LCB buffer (47). The material was homogenized in a Dounce homogenizer and centrifuged at 12,000 g. An aliquot of the supernatant was saved for

**Determination of myosin heavy chain mRNA expression.** Total RNA was isolated using the Ultraspec method (Biotec). Aliquots (5 µg) of total RNA were subjected to the slot blot method onto a nylon membrane and probed with a 32P-labeled myosin heavy chain (MHC) probe generated by the polymerase chain reaction as described previously (10). Blots were stripped and reprobed for 28S rRNA using a 32P-labeled probe generated by nick translation of a 28S rDNA clone (24). The expression of α-MHC and β-MHC isoforms was determined using oligonucleotide probes that were end labeled with 32P using T4 polynucleotide kinase (11). As confirmed by Northern blot analysis, these probes did not bind nonspecifically to ribosomal RNA under the hybridization and washing conditions that we employed. The ratio of MHC mRNA to 28S rRNA was calculated using computer-assisted digital image analysis.

**Determination of eIF-4E mRNA levels.** eIF-4E mRNA levels were measured by an RNase protection assay. Total RNA was extracted from a four-well plate using the Ultraspec method, and the precipitated RNA was suspended in 30 µl of RNase hybridization buffer (80% formamide; 40 mM piperazine-N,N′-bis(2-ethanesulfonic acid), pH 6.4; 0.4 M sodium acetate; 1 mM EDTA). Radiolabeled glyceraldehyde phosphate dehydrogenase (GAPDH) and eIF-4E antisense cRNA probes were added to each sample and hybridized for 16 h at 37°C.

**Digestion of unhybridized RNA was performed by addition of 300 µl of digestion buffer [10 mM tris(hydroxymethyl)aminomethane (Tris), pH 7.5; 5 mM EDTA; 200 mM sodium acetate] containing 10 U of RNase ONE (Promega). The RNase was inactivated by the addition of 5 µl of a 10% SDS solution containing 5 mg/ml tRNA as carrier. The RNA was precipitated in 70% ethanol, washed, and then resuspended in gel-loading buffer. The samples were resolved on 4% polyacrylamide-urea gels (10).

The antisense eIF-4E cRNA probe was made as follows. A 556-base pair fragment of the rat eIF-4E coding sequence was generated by polymerase chain reaction amplification of a λ-Zap II rat cDNA library (Stratagene) with eIF-4E-specific primers. The fragment was subcloned into pGEM3Zf, and its identity and orientation were confirmed by DNA sequencing.

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**Measurements of rate of protein synthesis.** Cardiocytes were pulse labeled with 10 µCi/ml 3,4,5-3H(N) leucine (180 Ci/mmol, NEN Products) for 4 h, washed in phosphate-buffered saline (PBS), and scraped in 1 N perchloric acid (PCA). The protein pellet was washed three times in 1 N PCA, solubilized in 0.3 N NaOH, and pelleted again in 1 N PCA. The protein was solubilized in 0.3 N NaOH. Radioactivity of total protein was determined by liquid scintillation counting, and the mass of total protein was assayed by the BCA method. The rate of protein synthesis (nmol · mg⁻¹ · h⁻¹) was calculated by dividing the rate of leucine incorporation into total cell protein (dpm · mg⁻¹ · h⁻¹) by the specific activity of leucine in the culture medium (dpm/nmol).

To determine the specific radioactivity of leucine in the culture medium, samples were directly counted in a scintillation counter, and the results were divided by the final concentration of leucine in the culture medium. In an additional set of experiments, the specific radioactivity of leucine in the culture medium and in the leucyl-tRNA pool was measured by the dansyl-Cl method as described before (9).

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protein determination, and the remainder of the supernatant was mixed with an equal volume of 2x sample buffer (20% glycerol; 4% SDS; 125 mM Tris, pH 6.8; 5% 2-mercaptoethanol; 50 mM dithiothreitol) and denatured by boiling for 5 min. Aliquots of each sample were resolved by SDS-PAGE using 15% acrylamide, and Western blot analysis was performed as described previously (47). To confirm that equal amounts of protein were loaded onto each lane, the membranes were stripped after immunoblotting and stained for total protein. There were no differences in the staining intensity between individual samples.

Immunofluorescent staining of actin and myosin. Cardiocytes were rinsed three times with ice-cold PBS and fixed for 30 min in PBS containing 2% paraformaldehyde. The fixative was removed by washing with PBS, and the cardiocytes were permeabilized with 0.3% Triton X-100 for 3 min. The plates were rinsed with PBS and maintained in 0.02% sodium azide until the staining procedure was performed. For actin staining, the cells were incubated for 30 min in 10% donkey serum blocking solution, rinsed with PBS, and incubated for 20 min in a 1:50 dilution of rhodamine-labeled phalloidin (Molecular Probes). For myosin immunostaining, the cells were incubated in the blocking solution for 30 min followed by an overnight incubation with a 1:2,000 dilution of anti-myosin antibody (2). The cardiocytes were rinsed with PBS and incubated for 2 h in a solution of fluorescein isothiocyanate-labeled anti-mouse IgG (Jackson ImmunoResearch).

Assay of atrial natriuretic factor reporter gene activity. To measure promoter activation of the atrial natriuretic factor (ANF) gene, cardiocytes were transiently transfected with pANF638L, a reporter gene construct consisting of the luciferase coding sequence linked to the proximal 638 bases of the rat ANF promoter (18). pGL2 (Promega), a promoterless luciferase construct, was used as a negative control. pRSVL, a RSV promoter-linked luciferase, was used as a positive control. To normalize for transfection efficiency, cardiocytes were cotransfected with pON249, a construct consisting of the β-galactosidase gene attached to the human cytomegalovirus promoter. Two-day-old cardiocyte cultures were prepared for transfection by a 4-h incubation in fresh medium supplemented with either 4% horse or neonatal calf serum. To each culture well, 500 µl of a solution containing 16 µg pON249, 16 µg pANF638L, 0.25 M CaCl2, and 25 mM N,N-bis-(2-hydroxyethyl)-2-aminoethanesulfonic acid (BES, Calbiochem) were added. After a 17- to 20-h incubation period in 3% CO2, the cardiocytes were rinsed several times with serum-free medium until no precipitate was visible under phase-contrast microscopy. The cardiocytes were harvested after 48 h in reporter lysis buffer (Promega). Luciferase activity was assayed in a Luminat luminometer (EG & G).

RESULTS

The collagen matrix was prepared so that the fibrils polymerized along the longitudinal axis of each culture well. Cardiocytes that were cultured on an aligned type I collagen matrix displayed an elongated morphology and were aligned along the direction of the collagen fibrils (Fig. 1). Because the cardiocytes were plated at low density, they did not develop extensive intercellular contacts (Fig. 1A), and there was essentially no spontaneous contractile activity. However, isolated cardiocytes could be stimulated to contract by electrical stimulation, providing the opportunity to compare contracting cardiocytes with a stable population of quiescent controls. For electrical stimulation, carbon electrodes were positioned on either end of each culture well (15). As a result of this configuration, the longitudinal axis of the elongated cardiocytes was in parallel with the direction of the electrical field. To determine the effect of alignment on the excitation threshold, cardiocytes were plated at equal densities on either aligned or randomly oriented (nonaligned) collagen. The cardiocytes were electrically stimulated under standard parameters (70 V, 3 Hz) for 24 h. We then determined the excitation threshold by varying the voltage intensity and monitoring for the presence or absence of synchronous beating as assessed by phase-contrast microscopy. Of the 21 fields of randomly oriented cardiocytes plated on nonaligned collagen, 3 fields failed to exhibit synchronous contractions altogether under the maximum field intensity of 100 V allowed by the stimulation apparatus. The average threshold in the remaining fields of nonaligned cardiocytes was 76 ± 3 V (mean ± SE, n = 18 fields; 2 experiments). The threshold voltage for cardiocytes plated on aligned collagen was 45 ± 2 V (n = 21 fields; 2 experiments), a threshold significantly lower compared with randomly oriented cardiocytes. These results are consistent with studies showing that responsiveness to electrical field stimulation is enhanced when elongated cardiocytes are aligned in parallel with the direction of the electrical field (45).

The data in Fig. 1 illustrate two primary morphological differences between electrically stimulated and quiescent cardiocytes. First, the size of the electrically stimulated cardiocytes was greater than the quiescent controls (compare Fig. 1, A vs. B). More intercellular contacts developed in association with the increase in size (Fig. 1B). The second morphological difference is the development of a myofibrillar architecture in electrically stimulated cardiocytes (Fig. 1, C-F). In contracting cardiocytes, the number of myofibrils was increased. Sarcomeres were present throughout the cell extensions and were arranged in lateral registry. This pattern was observed using probes for either actin or myosin (Fig. 1, D and F). In contrast, staining of quiescent controls with either of these probes showed significantly less myofibrillar development with myofibrils localized mainly in the cell periphery (Fig. 1, C and E).

To better define the effect of contraction on cardiocyte growth, cellular protein and RNA content relative to DNA were measured over time during continuous electrical stimulation and were compared with quiescent controls (Fig. 2). There was a time-dependent increase in the protein-to-DNA ratio of 16 ± 5 and 31 ± 3% after 2 and 4 days of electrically stimulated contraction, respectively. Translational capacity was increased in contracting cardiocytes as shown by a significant increase in the RNA-to-DNA ratio that reached 34 ± 9% after 4 days of electrical stimulation. By comparison, nonstimulated cells did not show any significant change in protein or RNA content. There were no significant differences in DNA content between control and electrically stimulated cultures, indicating that...
cell number remained constant. In addition, when cardiocytes were plated at 1.5 times the regular density to facilitate the development of intercellular contacts and spontaneous contractile activity, there was an increase in protein content comparable to that observed in response to electrical stimulation of more sparsely plated, quiescent cardiocytes (data not shown).

To determine whether the increase in protein content reflected a change in protein synthesis, rates of total protein synthesis were measured by the pulse-labeling method (Fig. 3). After 24 h, electrically stimulated contraction caused a significant increase in the rate of protein synthesis compared with time 0 or with the same-day control. The accelerated rate of protein synthesis was maintained between 24 and 48 h of electrically stimulated contraction. However, the rate of protein synthesis measured after 48 h was not significantly different compared with time 0. Total protein synthesis per cardiocyte (nmol leucine·mg DNA⁻¹·h⁻¹), calculated as the product of the rate of protein synthesis (nmol leucine·mg protein⁻¹·h⁻¹) and the corresponding protein-to-DNA ratio, did increase by 35% after 48 h of electrically stimulated contraction compared with time 0. Total protein synthesis per cardiocyte takes into account the expanding size of the total protein pool as reflected by the increase in the protein-to-DNA ratio (Fig. 2). A small decline in the rate of protein synthesis in nonstimulated cardiocytes was observed in relation to time 0, but the decrease was not statistically significant.

To validate the measurements of protein synthesis, we measured the extent to which the specific radioactivity of leucine in the culture medium equilibrated with the leucyl-tRNA pool, the immediate precursor pool for protein synthesis. The specific radioactivity of leucyl-tRNA equilibrated to 89 ± 6% and 82 ± 6% of the leucine-specific radioactivity in the media in control and electrically stimulated cardiocytes, respectively (3 experiments). These values were not significantly different, thereby confirming that measured increases in the rate of protein synthesis in contracting cells were not due to a change in the specific radioactivity of the leucyl-tRNA pool.

To determine whether quantitative changes in contractile protein gene expression occurred during cardiocyte growth, MHC mRNA levels were assayed by the slot blot method of total RNA, and the total RNA was probed with a cDNA complementary to both the α-MHC and β-MHC mRNA isoforms. The slot blots were stripped and probed for 28S rRNA to normalize for...
changes in the total RNA pool. The ratio of MHC mRNA to 28S rRNA was calculated as an index of relative abundance. Figure 4 shows that MHC mRNA levels did not exhibit any significant changes relative to 28S rRNA in either electrically stimulated cardiocytes or quiescent controls.

In a separate set of experiments, the expression of $\alpha$-MHC and $\beta$-MHC isoforms was determined using oligonucleotide probes that recognize specific sequences in the 3'-untranslated region of each respective MHC mRNA isoform (11). Figure 5 shows that $\alpha$-MHC mRNA levels declined significantly over the 4-day duration of the experiments to 33 ± 7% of the time 0 value in the control cardiocytes. Electrically stimulated contraction did not reverse this decline as $\alpha$-MHC levels remained lower (43 ± 5%). Conversely, $\beta$-MHC levels increased by 30 ± 18 and 80 ± 34% in the control and stimulated groups, respectively. Because total MHC levels were unchanged (Fig. 4), these data indicate that the decrease in $\alpha$-MHC levels was offset by a corresponding increase in $\beta$-MHC. Furthermore, these data show that shifts in $\alpha$-MHC and $\beta$-MHC isoforms in long-term culture were not affected by electrically stimulated contraction.

The induction of ANF mRNA is a marker of the cardiac hypertrophic response in neonatal rat cardiocytes (18, 26). ANF expression was assayed by transfecting 2-day-old cardiocytes with a construct containing the luciferase reporter gene linked to a 638-base pair region of the ANF promoter (Fig. 6). When normalized for $\beta$-galactosidase to control for transfection efficiency, electrically stimulated cardiocytes showed a 3.2 ±
0.3-fold increase in the level of ANF promoter activation compared with controls. In contrast, electrical stimulation had no effect on the activity of a control construct containing a constitutively active RSV promoter linked to luciferase (Fig. 6).

To determine whether a positive correlation exists between eIF-4E levels and hypertrophic growth, steady-state eIF-4E mRNA levels were measured using RNase protection assays. Figure 7A shows an autoradiogram obtained from one experiment. The eIF-4E signal was abolished when antisense-eIF-4E cRNA probe was replaced with a sense eIF-4E cRNA probe, indicating that the band was not the result of nonspecific hybridization (data not shown). The eIF-4E band was also absent when 20 µg of tRNA was used as input RNA, indicating that the unprotected probe was completely digested.

Similarly, substitution of sense GAPDH probe for antisense probe completely abolished the GAPDH signal (data not shown). The GAPDH signal appeared as a double band as a result of mismatches between the
human and rat GAPDH. The summary data in Fig. 7B show that eIF-4E mRNA was increased by 38 ± 6% after 24 h of electrically stimulated contraction compared with quiescent controls. This increase was sustained at 48 h (48 ± 9% higher in the electrically stimulated group) but declined on day 4. To confirm that the increase in eIF-4E mRNA relative to GAPDH mRNA reflected a change in eIF-4E mRNA abundance and not changes in GAPDH levels, 28S rRNA content was determined in a subset of the RNAse protection experiments. This was performed on aliquots of each RNA by the slot blot method and the blots were probed for 28S rRNA. The results showed that eIF-4E mRNA levels were increased relative to 28S rRNA (data not shown).

To determine whether there was a corresponding increase in eIF-4E protein levels, a Western blot was performed using cardiocyte homogenates harvested from control and electrically stimulated cardiocytes at the same time points used for mRNA analysis (Fig. 8). Contracting cardiocytes had 115 ± 11% more eIF-4E per protein mass than controls at the 48-h time point (Fig. 8B). This indicates that eIF-4E content per cell was also increased significantly, since there was an overall increase in protein content as reflected by an increase in the protein-to-DNA ratio in response to electrically stimulated contraction.

Phenylephrine (PE) is a well-known growth-promoting agent for neonatal rat cardiocytes in primary culture. Because PE treatment of neonatal cardiocytes induces a growth response similar to that observed with electrically stimulated contraction, we determined the effect of 24 h PE stimulation on eIF-4E levels. As shown in Fig. 9, PE treatment led to a detectable increase in both eIF-4E mRNA (Fig. 9A) and eIF-4E protein (Fig. 9B). It should be noted, however, that PE treatment was sufficient for the initiation of contractile activity in the cardiocyte cultures.

**DISCUSSION**

The model of neonatal rat cardiocytes aligned on a matrix of type I collagen had several advantages compared with more conventional primary culture preparations. First, when the cardiocytes were plated at relatively low densities, spontaneous contraction was minimized because there were relatively fewer intercellular contacts. Spontaneous contractile activity was limited to occasional beats in a small percentage of the cardiocytes. This enabled us to avoid using arresting agents such as high extracellular KCl or the calcium...
channel blocker verapamil to produce a quiescent control. Second, the cardiocytes developed an elongated, rod-shaped morphology that more closely resembles cardiocytes in vivo (44). The myofibrils were organized in parallel with the longitudinal axis of the cardiocytes. Third, the cardiocytes aligned as a population along the direction of the collagen fibrils, even when plated at a low density. This feature provided the ability to align the longitudinal axis of the cardiocytes in parallel with the direction of the electrical field. Because this parallel alignment decreased the excitation threshold necessary for triggering action potentials in rod-shaped cardiocytes (45), it was possible to stimulate contraction at relatively low voltages. Consequently, current flow was markedly reduced, avoiding damage to the cardiocytes. It is not possible to stimulate contraction using electrical stimulation at low voltages when neonatal cardiocytes are plated at low density on a conventional substrate such as gelatin (11). Under these conditions, the cardiocytes are randomly situated and have a stellate morphology, making them quiescent although refractory to electrical stimulation.

The conclusion that electrically stimulated cardiocytes underwent growth characteristic of hypertrophy is based on the following four criteria: 1) an acceleration of the rate of protein synthesis, 2) an increase in total cell protein as measured by the protein-to-DNA ratio, 3) an increase in total RNA as measured by the RNA-to-DNA ratio, and 4) an increase in ANF promoter activity as measured by a luciferase reporter gene assay. These results extend our previous studies showing that increased load produced by contractile activity is a sufficient stimulus for sustained hypertrophic growth of neonatal rat cardiocytes in vitro (11, 24). The growth-promoting effects of contractile activity in the present study are based on a direct comparison with a control population of cardiocytes that are essentially quiescent in the absence of electrical stimulation, obviating the need for arresting agents. Even though the cardiocytes were quiescent, they displayed a marked reduction in myofibrils similar to that observed following prolonged contractile arrest with verapamil (1). This loss of myofibrils probably resulted from an accelerated rate of contractile protein degradation, since contractile arrest implemented by chronic treatment with verapamil has been shown to accelerate myosin heavy chain and actin degradation (41, 43). Byron et al. (1) have shown that the accelerated rate of myofibrillar protein degradation in arrested cardiocytes is not caused by a reduction in mechanical loading in the form of contraction but rather can be attributed to a loss of calcium transients during excitation-contraction coupling. Interestingly, Fig. 1 shows that myofibrils were still present in quiescent cardiocytes, indicating that the process of myofibrillar assembly remained intact despite an apparent increase in myofibrillar protein degradation.

The increase in RNA-to-DNA ratio that occurred in response to electrically stimulated contraction reflects an overall increase in the ribosome pool. This increase in translational capacity was accompanied by a parallel increase in eIF-4E levels. Generally, most of the initiation factors are present in approximately proportional amounts to the number of ribosomes (0.5–1.0 copies per ribosome) and are therefore probably not rate limiting for protein synthesis (7). In contrast, eIF-4E is present in much lower abundance, the values ranging between 0.02 and 0.2 copies per ribosome. The functional significance of expressing eIF-4E in limiting amounts is apparent from its role in translational initiation, namely facilitating the binding of mRNA to the ribosome. Because the mRNA pool is not rate limiting for protein synthesis, the eIF-4E-to-ribosome ratio is of critical importance for determining the efficiency of protein synthesis, because individual mRNAs must compete for a limited pool of eIF-4E. Thus an increase in eIF-4E levels may be an essential step for maintaining high levels of translational efficiency as the ribosome pool expands during hypertrophic growth of the cardiocyte.

Studies have shown that eIF-4E expression is upregulated in response to growth-promoting stimuli, although mostly in the context of proliferative growth. For example, platelet-derived growth factor stimulation of NIH 3T3 cells cultured in low-serum media led to an increase in eIF-4E levels (38). Experimental overexpression of eIF-4E in HeLa or NIH 3T3 cells caused loss of growth control and malignant transformation (4, 19). Conversely, depletion of eIF-4E through expression of antisense RNA in HeLa cells led to a decreased rate of protein synthesis and slower growth (3). The relationship between proliferative growth and eIF-4E expression is underscored by studies showing that eIF-4E levels are increased in a variety of transformed cell lines and in the more invasive forms of breast carcinomas (29, 33). A hypothesized mechanism by which increased eIF-4E levels can trigger growth is by selective translation of mRNAs encoding proteins that are critically involved in regulating growth (20, 36). Overexpression of eIF-4E increases the translational efficiency of growth-related mRNAs such as fibroblast growth factor, cyclin D1, and ornithine decarboxylase (17, 37, 42). These mRNAs require the helicase activity of the eIF-4F complex to melt excessive secondary structure in the 5′-untranslated region and thereby remove inhibitory constraints for translation. Thus increased expression of eIF-4E in contracting cardiocytes could have an additional function of selectively upregulating the expression of proteins involved in regulating hypertrophic growth.

The expression of eIF-4E appears to be regulated at several levels. Evidence for transcriptional regulation is found in studies showing that eIF-4E mRNA levels were increased following growth induction by c-Myc (38). Analysis of the upstream elements of the human eIF-4E gene indicates that there are two E box motifs that serve as potential targets for c-Myc binding and transactivation of the gene (12). We have recently cloned 2.1 kilobases of the rat eIF-4E gene and confirmed these findings (unpublished observations). Evidence for posttranscriptional and/or translational regulation of eIF-4E expression is found in studies of
activated T lymphocytes. The expression of elf-4E mRNA was markedly increased following activation of quiescent T cells (~16-fold), yet elf-4E protein levels increased by only twofold (22). Furthermore, the increase in elf-4E mRNA levels of activated T cells was not due to a change in the rate of transcription. Thus, transcriptional, posttranscriptional, and/or translational mechanisms may all be involved in regulating elf-4E expression during hypertrophic growth of the cardiocyte, thereby ensuring that elf-4E levels are maintained in the proper ratio with the rest of the translational machinery. Studies are now underway to determine the mechanisms that regulate elf-4E expression in response to increased load of the cardiocyte.

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