Myotrophin is a soluble-12 kilodalton protein isolated from hypertrophied spontaneously hypertensive rat and dilated cardiomyopathic human hearts. We have recently cloned the gene coding for myotrophin and expressed it in Escherichia coli. In the present study, the expression of myotrophin gene was analyzed, and at least seven transcripts have been detected in rat heart and in other tissues. We have further analyzed the primary structure of myotrophin protein and identified significant new structural and functional domains. Our analysis revealed that one of the ankyrin repeats of myotrophin is highly homologous specifically to those of IκBα proteins, were identified in myotrophin. To verify the significance of these homologies,xB gel shift assays were performed with Jurkat T cell nuclear extract proteins and the recombinant myotrophin. The results indicate that the recombinant myotrophin has the ability to interact with NF-κB/rel proteins as revealed by the formation of ternary protein-DNA complexes. While myotrophin-specific antibodies inhibited the formation of these complexes, rel-specific p50 and p65 antibodies supershifted these complexes. Thus, these results clearly indicate that the myotrophin protein to be a unique rel/NF-κB interacting protein.

Cardiac myocyte cell hypertrophy has been used as an in vitro model for studying cardiac hypertrophy. Cardiac myocytes respond to hemodynamic overload by altering the expression of specific set of genes, which are needed for hypertrophy. Our laboratory has been studying the molecular basis of myocardial hypertrophy using spontaneously hypertensive rat as an animal model (1–3). Earlier, Sen et al. (1,2) isolated a novel 12-kilodalton protein, which we named myotrophin, from the hypertrophied ventricles of spontaneously hypertensive rat (1) and dilated cardiomyopathic human hearts (2) based on its ability to stimulate protein synthesis specifically in cardiac myocytes (1). Recently, we have isolated the cDNA clones encoding rat myotrophin (4) and found that the cardiac myotrophin is identical to a previously reported rat brain v1 protein (Northeastern Ohio affiliate) Grant-in-aid 4847 (to N.S.) and National Institutes of Health Grant HL 47794 (to S.S.). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Myotrophin is highly homologous specifically to those of IκBα proteins, which were observed in xBα proteins, identified in myotrophin. To verify the significance of these homologies, xB gel shift assays were performed with Jurkat T cell nuclear extract proteins and the recombinant myotrophin. The results indicate that the recombinant myotrophin has the ability to interact with NF-κB/rel proteins as revealed by the formation of ternary protein-DNA complexes. While myotrophin-specific antibodies inhibited the formation of these complexes, rel-specific p50 and p65 antibodies supershifted these complexes. Thus, these results clearly indicate that the myotrophin protein to be a unique rel/NF-κB interacting protein.

Cardiac myocyte cell hypertrophy has been used as an in vitro model for studying cardiac hypertrophy. Cardiac myocytes respond to hemodynamic overload by altering the expression of specific set of genes, which are needed for hypertrophy. Our laboratory has been studying the molecular basis of myocardial hypertrophy using spontaneously hypertensive rat as an animal model (1–3). Earlier, Sen et al. (1,2) isolated a novel 12-kilodalton protein, which we named myotrophin, from the hypertrophied ventricles of spontaneously hypertensive rat (1) and dilated cardiomyopathic human hearts (2) based on its ability to stimulate protein synthesis specifically in cardiac myocytes (1). Recently, we have isolated the cDNA clones encoding rat myotrophin (4) and found that the cardiac myotrophin is identical to a previously reported rat brain v1 protein (Northeastern Ohio affiliate) Grant-in-aid 4847 (to N.S.) and National Institutes of Health Grant HL 47794 (to S.S.). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

EXPERIMENTAL PROCEDURES

Northern Analysis of Myotrophin mRNAs—RNA transcripts specific for myotrophin were analyzed in various rat tissues. Total RNA was first isolated from 9-day-old rat hearts. Poly(A)-enriched RNA was isolated using the oligo(dT) method. Briefly, total RNA was applied to poly(A) RNA isolated from various rat tissues in our laboratory also analyzed this multiple tissue Northern blot and is described above. Poly(A) RNA eluted by this method was fractionated on 1% agarose formaldehyde gels, transferred to a ZetaProbe membrane, and hybridized with radiolabeled RNA probe using T7 RNA polymerase-directed in vitro transcription system. The hybridization experiment was done using very high stringency and wash conditions for both the myotrophin cDNA (42 °C, 5 × SSPE, 10 × Denhardt’s, 50% formamide, 2% SDS, and 100 μg/ml salmon sperm DNA) and RNA (50 °C, 1.5 × SSPE, 1% SDS, 0.5% BLOTTO, 50% formamide, tRNA (0.2 mg/ml), and salmon sperm DNA (0.5 mg/ml)) probes. Approximately 5 μg of pure poly(A) RNA (labeled in lane 2 of Fig. 1B) was added to each lane of the Northern blot containing the poly(A) RNA from various rat tissues was obtained from Clonetech (no. 7764-1). Approximately 2 μg of pure poly(A) RNA from each tissue was fractionated on the agarose gel according to Clonetech. The high stringency hybridization and wash conditions recommended by Clonetech were used to analyze this Northern blot. The Northern blot containing poly(A) RNA isolated from various rat tissues was hybridized with the myotrophin cDNA probe using pCRII-8-Myo cDNA clone (4). Utilizing the same clone, single-stranded myotrophin-specific radiolabeled RNA probe was made using T7 RNA polymerase-directed in vitro transcription system. The hybridization experiment was done using very high stringency and wash conditions for both the myotrophin cDNA (42 °C, 5 × SSPE, 10 × Denhardt’s, 50% formamide, 2% SDS, and 100 μg/ml salmon sperm DNA) and RNA (50 °C, 1.5 × SSPE, 1% SDS, 0.5% BLOTTO, 50% formamide, tRNA (0.2 mg/ml), and salmon sperm DNA (0.5 mg/ml)) probes. Approximately 5 μg of pure poly(A) RNA (from lane 2 of Fig. 1B) was added to each lane of the Northern blot containing the poly(A) RNA from various rat tissues was obtained from Clonetech (no. 7764-1). Approximately 2 μg of pure poly(A) RNA from each tissue was fractionated on the agarose gel according to Clonetech. The high stringency hybridization and wash conditions recommended by Clonetech were used to analyze this multiple tissue Northern blot and is described above. Poly(A) RNA isolated from various rat tissues in our laboratory also revealed the same results (data not shown).

Expression of Myotrophin in E. coli—Myotrophin was expressed in E. coli using the T7 promoter-based vector, pET3a (Novagen Inc.) (1). The myotrophin recombinant pET3a-S1 vector was introduced into E. coli BL21(DE3) LysS strain, which harbors a T7 RNA polymerase coding gene. The recombinant myotrophin was expressed by growing the E. coli cells to early log phase and was later induced with 0.1 mM isopropyl-1-thio-β-D-galactopyranoside for 16 h. Overnight induced cells were harvested and lysed in 50 mM Tris-HCl, pH 8.0, 75 mM NaCl by freeze thawing three times. The lysed E. coli cell debris was removed by centrifugation at 10,000 × g, and the soluble supernatant was used to purify the recombinant myotrophin. The soluble form of recombinant myotrophin was highly abundant in the supernatant and was separated from the rest of the E. coli proteins using a Centriprep-30 (30-kDa cutoff) Amicon cartridge. Later, the purified recombinant myotrophin was concentrated using a Centriprep-10 (10-kDa cutoff) cartridge. On a 12% Tris-Tricine SDS-PAGE,2 the purified recombinant myotrophin

*This work was supported in part by American Heart Association (Northeastern Ohio affiliate) Grant-in-aid 4847 (to N.S.) and National Institutes of Health Grant HL 47794 (to S.S.). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

1N. Sivasubramanian, P. Sil, G. Adhikary, and S. Sen, manuscript in preparation.

2The abbreviations used are: PAGE, polyacrylamide gel electrophoresis; kb, kilobase(s); EMSA, electromobility shift assay.
migrated as a single band at the 12-kDa region. Protein concentration was estimated using Bio-Rad protein assay reagent, and appropriate quantities of recombinant myotrophin were used in gel shift assays. The recombinant myotrophin was further tested for its immunoreactivity using native myotrophin-specific antibodies (5). Native myotrophin-specific antibodies were generated against a synthetic peptide containing the 17 amino acid residues of the T26 tryptic peptide of native myotrophin (5). Since Western immunoblot analysis clearly showed that the recombinant myotrophin was immuno reactive to myotrophin-specific antibodies (data not shown), it was used for functional studies.

Electrophoretic Mobility Shift Assays—Phorbol ester-treated human Jurkat T cell nuclear extract, ≈B, consensus double-stranded oligonucleotide substrate (5′-AGTTGAGGGGACCTTTCCAGG-3′), Oct-1 consensus double-stranded oligonucleotide substrate (5′-TGTCAGAGTC AAATCTAGAA-3′), and p50 and p65 supershift antibodies were purchased from Santa Cruz Biotechnology Inc. Poly(dI-dC)poly(dI-dC) was purchased from Pharmacia Biotech Inc. Partially purified recombinant myotrophin (4) and native peptide myotrophin-specific antibody (1-3) were used in the gel shift assays. DNA-protein binding reactions were carried out in 12 mM HEPES-NaOH (pH 7.9), 4 mM TrisCl (pH 7.9), 60 mM KCl, 1 mM EDTA, and 1 mM dithiothreitol, 2 µg of poly(dI-dC)poly(dI-dC) and 10% glycerol in a final volume of 15 µl. The reactions contained 10 µg of Jurkat cell nuclear extract, varying amounts (1-3 µl, containing 200 ng/µl) of bacterially expressed recombinant myotrophin, and 10,000 cpm of end-labeled NF-κB consensus double-stranded DNA probe. After incubating at room temperature for 30 min, the reactions were run on a 4% PAGE using 0.25 TBE as the running buffer and 1 X TBE as the running buffer. The gel was electrophoresed at 160 volts for 2 h. Later, the gel was dried and autoradiographed overnight at ~70 °C. Purified myotrophin-specific antibodies (IgG) (Ref. 5) and preimmune antibodies (IgG) were preincubated with myotrophin for 1 hour in ice before the binding reactions were carried out.

RESULTS

Distribution of Myotrophin mRNA in Rat Tissues—A multiple tissue Northern blot containing poly(A) RNA from various tissues of rat was obtained from Clonetech. Myotrophin-specific double-stranded cDNA probe was used to identify myotrophin-specific transcripts. The blot was hybridized and washed at very high stringency conditions. The results of the experiment are shown in Fig. 1A. In total, at least five myotrophin-specific transcripts were detected in these tissues. Among them, two high molecular weight transcripts (4.3 and 3.5 kb) were detected in almost all tissues. These transcripts were most abundant in brain and least in skeletal muscle compared to other tissues. In addition, three transcripts of 2.4, 1.8, and 1.0 kb in size were also detected in some tissues, although at different levels. These were detected more abundantly in certain tissues like testis and liver compared to other tissues. Based on its ubiquitous distribution, it appears that the myotrophin protein may be playing a very important role in the basic functions of various tissues. We have recently obtained several myotrophin cDNA clones through direct screening of a rat heart 5′-stretch cDNA library (Clonetech), and the preliminary characterization reveals that the size of the clone inserts correspond to the sizes of these multiple transcripts. Based on the initial nucleotide sequence data from few cDNA clones as well as data from rapid amplification of cDNA ends-polymerase chain reactions (4,1), it appears that the heterogeneity in the length of 3′-untranslated regions contributes to the observed heterogeneity in the multiple transcripts. The observation of multiple types of cDNA clones with different 3′-untranslated regions (4, B) suggests the present observation of multiple transcripts in the northern hybridization experiment. Southern analysis of rat genomic DNA also suggests that myotrophin is coded by a single copy gene as revealed by our observation of a single 4.3-kilobase pair HindIII genomic DNA fragment hybridizing to the myotrophin coding region probe,3 and hence these multiple transcripts arise from the single copy myotrophin gene. Similar types of multiple transcripts have been observed for other genes like opsin in mouse, rat, human, and frog (20).

Myotrophin Gene Expression in Rat Heart—Expression of myotrophin gene specifically in rat hearts was analyzed in more detail using Northern blot analysis. Using the coding region of myotrophin gene, both double-stranded DNA probe (lane a in Fig. 1B) as well as single-stranded antisense RNA probe (lane b in Fig. 1B) was used (see “Experimental Procedures”) in different Northern blot experiments. Very high stringency hybridization and wash conditions were followed for this experiment. Initially, only the 4.3- and 3.5-kb transcripts were detected when pure poly(A) RNA was used (lane a in Fig. 1B). Since low molecular weight myotrophin transcripts were not detected significantly in this poly(A) RNA, we included a low-salt wash step in our oligo(dT) purification procedure (10 mM Tris-HCl, pH 7.5, 1 mM EDTA, 0.1 mM NaCl) before eluting the poly(A) RNA with no-salt buffer. The RNA from this low salt wash fraction was ethanol precipitated and analyzed for myotrophin-specific transcripts. Interestingly, the majority of the myotrophin-specific low molecular weight transcripts (2.4, 1.0, 0.7, and 0.4 kb) were observed mostly in the low salt-eluted RNA when compared to pure poly(A) RNA (lane b in Fig. 1B). It is possible that poly(A) tracts in these transcripts may be either shorter in length or totally devoid of and thus eluted in the low-salt buffer. The observation of several myotrophin-specific transcripts in the low salt wash RNA fraction also suggests that these are degraded products of the myotrophin mRNAs after translation or translationally silenced mRNAs ready to be translated upon receiving the physiological signal (21, 22).

Primary Structure Analysis of Myotrophin Protein—The primary structure of the myotrophin protein was analyzed thor-

G. Adhikary, unpublished observations.
Cardiac Myotrophin Exhibits \( \kappa \)B/rel/NF-\( \kappa \)B Interacting Activity

Fig. 2. A, ankyrin repeats and putative phosphorylation sites for protein kinase C and casein kinase II are highlighted on the indirectly predicted amino acid sequence of myotrophin. B, homology of myotrophin ankyrin repeat 2 to I\( \kappa \)B\( \alpha \) ankyrin repeats. It should be noted that in addition to \( \kappa \)B-associated pp40, the BLASTP analysis identified other I\( \kappa \)B\( \alpha \) members (MAD3, RL/\( \kappa \)F-1, and ECI-1) on the same ankyrin repeats 2 and 4 with similar Poisson values.

Electrophoretic Mobility Shift Assays with Recombinant Myotrophin—Because of the above structural observations, electrophoretic mobility shift assays (EMSAs) were performed with J urkat T cell nuclear extract proteins to identify whether the NF-\( \kappa \)B/rel is a target for myotrophin binding. The results are shown in Fig. 3A. Interestingly, we observed that with increasing concentrations of myotrophin, ternary complexes are formed between myotrophin and NF-\( \kappa \)B/rel. Two types of ternary complexes (lanes 5–7 in Fig. 3A) were demonstrated by PAGE. The slower migrating (SC) complexes appear to be heterotrimeric (myo-NF-\( \kappa \)B/rel), and the faster migrating (FC) complexes appear to be devoid of one of the subunits of the NF-\( \kappa \)B/rel complex (Fig. 3A).

Further, the preimmune serum IgG (lane 9 in Fig. 3A) did not prevent this binding, whereas the myotrophin-specific IgG (lane 8 in Fig. 3A) inhibited specifically the formation of these ternary complexes. However, myotrophin by itself did not bind to \( \kappa \)B DNA substrate probe (lane 4 in Fig. 3A). Unlike other known I\( \kappa \)B proteins, myotrophin did not inhibit the DNA binding activity of the NF-\( \kappa \)B complex; instead, it formed ternary complexes. These results were also confirmed by preliminary EMSAs with cardiac myocyte nuclear extracts (data not shown). To confirm the specificity of myotrophin interaction with the NF-\( \kappa \)B/rel complex, Oct-1 EMSAs (14) were also carried out using the same J urkat T cell nuclear extract (Fig. 3B). With the same increasing concentrations of myotrophin, myotrophin (lanes 2–4 in Fig. 3B) did not affect any of the Oct-1-DNA complexes at all. These results clearly show that the specific target for myotrophin is the subunits of the NF-\( \kappa \)B/rel complex.

To further confirm the myotrophin interaction with \( \kappa \)B/rel factors, EMSAs were performed in presence of p50 and p65 antibodies. The results are shown in Fig. 4. When incubated with either p50 (lane 6) or p65 (lane 4) antibodies, the myotrophin-shifted \( \kappa \)B complexes were supershifted to slower migrating ternary complexes (SSC-50 and SSC-65 in Fig. 4), and the intensity of these complexes increased when more myotrophin was present in the reaction. These results clearly show that myotrophin-shifted protein complexes actually contain \( \kappa \)B/rel factors. It should also be noted that phorbol 12-myristate 13-acetate-induced J urkat T cells probably contain a sufficient amount of endogenous myotrophin since myotrophin-shifted \( \kappa \)B complexes were also detected at a lower level in the control experiments (lanes 1 and 2 in Fig. 4).
Cardiac Myotrophin Exhibits rel/NF-κB Interacting Activity

Fig. 3. Electrophoretic mobility shift assays analyzing the effect of recombinant myotrophin on the NF-κB/rel/κB DNA (A) and Oct-1/oct DNA (B) complexes. Phorbol ester-treated Jurkat T cell nuclear extracts were used as source of NF-κB/rel and Oct factors. Bacterially expressed recombinant myotrophin was added ("+" = 1 μl = 200 ng) to the binding reactions, and its effect was analyzed on 4% PAGE. J NE-P, phorbol ester-treated Jurkat T cell nuclear extract; κB, radiolabeled κB DNA probe; α-myo, native myotrophin-specific antibody IgG (5); α-p65, antibody to p65 of NF-κB (supershifting); PI, preimmune serum IgG; Oct, radiolabeled Oct DNA probe; SC, myotrophin-shifted slower migrating complexes; FC, myotrophin-shifted faster migrating complexes; NF-κB, rel-κB heterodimeric protein-DNA complexes.

Fig. 4. Electrophoretic mobility shift assays analyzing the effect of rel-specific p50 and p65 antibodies on the myotrophin-shifted NF-κB rel/κB DNA complexes. Phorbol ester-treated Jurkat T cell nuclear extracts were used as a source of NF-κB/rel factors. Rel-specific p50 and p65 antibodies were added to the appropriate κB binding reactions. Bacterially expressed recombinant myotrophin was added ("+" = 1 μl = 200 ng) to the binding reactions along with appropriate antibodies, and its effect was analyzed on 4% PAGE. J NE-P, phorbol ester-treated Jurkat T cell nuclear extract; κB, radiolabeled κB DNA probe; myo, recombinant myotrophin; α-p65, antibody to p65 of NF-κB complex; α-p50, antibody to p50 of NF-κB complex; SC, myotrophin-shifted slower migrating complexes; NF-κB, rel-κB heterodimeric protein-DNA complexes; SSC-50, supershifted complex by α-p50; SSC-65, supershifted complex by α-p65.

DISCUSSION

In the present study, we have shown that the myotrophin gene is expressed in various rat tissues and as much as seven myotrophin-specific transcripts have been detected in rat heart and in other tissues. These transcripts were most abundant in brain and least in skeletal muscle compared to other tissues. Based on its ubiquitous distribution, it appears that the myotrophin protein may be playing a very important role in the basic functions of various tissues.

Our analysis on the primary structure of the myotrophin protein also revealed the homology between one of the ankyrin repeats of myotrophin and to those of IκBα/rel ankyrin repeats. Furthermore, our analysis showed putative consensus phosphorylation sites for protein kinase C and casein kinase II in myotrophin protein, which were also observed in IκBα proteins. The significance of these homologies were experimentally confirmed with κB gel shift assays. The results of these gel shift assays clearly show that the recombinant myotrophin has the ability to interact with NF-κB/rel proteins in vitro. In vivo experiments are currently being conducted to further confirm these results. Thus, these results clearly indicate that the 12-kDa myotrophin protein is a unique rel/NF-κB interacting protein.

It has been very well documented that upon exposure to a variety of external stimuli, NF-κB/rel proteins are involved in the rapid induction of genes whose products play a central role in the immune responses, inflammation, and cell proliferation (15–19). The most obvious characteristic of NF-κB is its rapid translocation from cytoplasm to nucleus in response to extracellular signals. They are kept dormant in the cytoplasm by the members of the IκB family of proteins. Many signals inactivate the inhibitor IκB, thereby allowing the NF-κB to enter nuclei and rapidly induce coordinate sets of defense-related genes. It is possible that upon exposure to chronic hemodynamic over-load signals, cardiac myocytes respond through their NF-κB rapid response system to alter myocardial gene expression. In the present preliminary study, we have shown by its ability to interact with NF-κB in vitro that myotrophin is probably a component of such a rapid response system, which might in-

...
fluence the transcription of hypertrophy-specific genes. Based on the present study, we speculate that myotrophin is probably involved in regulating the expression of hypertrophy-specific genes in the myocardium through rel factors and κB DNA sites. It should be noted that no transcription regulatory factor has been reported so far to be involved in cardiac hypertrophy. Further studies are in progress to determine the exact mechanism of action of myotrophin.

Acknowledgments—We thank Vijaya Kandaswamy and David Young for technical help during this project.

REFERENCES

1. Sen, S., Kundu, G., Mekhail, N., Castel, J., Misono, K. & Healy, B. (1990) J. Biol. Chem. 265, 16635–16643
2. Sil, P., Misono, K. & Sen, S. (1993) Circ. Res. 73, 98–108
3. Mukherjee, D. P., McTiernan, C. F. & Sen, S. (1993) Hypertension 21, 142–148
4. Sivasubramanian, N., Adhikary, G. & Sen, S. (1994) Circulation 90, 637 (abstr.)
5. Sil, P., Mukherjee, D. P. & Sen, S. (1995) Circ. Res. 76, 1020–1027
6. Altschul, S. F., Gish, W., Miller, W., Myers, E. W. & Lipman, D. J. (1990) J. Mol. Biol. 215, 403–410
7. Tadka, M., Yamakuni, T., Song, S. Y., Yamakawa, Y., Seta, K., Okuyama, T. & Isobe, T. (1992) Eur. J. Biochem. 207, 615–620
8. Tadka, M., Isobe, T., Okuyama, T., Watanabe, M., Kondo, H., Yamakawa, Y., Ozawa, F., Hishinuma, F., Kubota, M. & Minegishi, A. (1994) J. Biol. Chem. 269, 9946–9951
9. Davis, N., Gho, S., Simmons, D. L., Tempst, P., Liou, H. C., Baltimore, D. & Bose, H. J. (1993) Science 253, 1268–1271
10. Haskell, S., Beg, A. A., Tompkins, S. M., Morris, J. S., Yurochko, A. D., Sampson, J. A., Mondal, K., Ralph, P. & Baldwin, A. J. (1992) Cell 65, 1281–1289
11. Tewari, M., Dobrzenski, P., Mohn, K. L., Cressman, D. E., Hsu, J. C., Bravo, R. & Taub, R. (1992) Mol. Cell. Biol. 12, 2898–2908
12. de Martin, R., Vanhove, B., Cheng, Q., Hofer, E., Csizmadia, V., Winkler, H. & Bach, F. H. (1993) EMBO J. 12, 2773–2779
13. Hatada, E. N., Nieters, A., Wulczyn, F. G., Naumann, M., Meyer, R., Nadhora, G., McKeel, T. W. & Scheiderer, C. (1992) Proc. Natl. Acad. Sci. USA 89, 2489–2493
14. Bhargava, A. K., Li, Z. & Weissman, S. M. (1993) Proc. Natl. Acad. Sci. USA 90, 10260–10264
15. Bauerle, P. A. (1991) Biochim. Biophys. Acta 1072, 63–80
16. Blank, V., Kourilsky, P. & Israel, A. (1992) Trends Biochem. Sci. 17, 135–140
17. Grilli, M., Chiu, J. J. & Lenardo, M. J. (1993) Int. Rev. Cytol. 143, 1–62
18. Bauerle, P. A. & Henkel, T. (1994) Annu. Rev. Immunol. 12, 142–170
19. Nolan, G. P. & Baltimore, D. (1992) Curr. Opin. Genet. Dev. 2, 211–220
20. Al-Ubaidi, M. R., Pittler, S. J., Champagne, M. S., Triantafyllos, J. T., McGinnis, J. F. & Baehr, W. (1990) J. Biol. Chem. 265, 20563–20569
21. Beelman, C. A. & Parker, R. (1995) Cell 81, 179–183
22. Huarte, J., Stutz, A., O’Connell, M. L., Gubler, P., Belin, D., Darrow, A. L., Strickland, S. & Vassalli, J. D. (1992) Cell 69, 1021–1030