Disruption of the Gene Encoding the Mitogen-regulated Translational Modulator PHAS-I in Mice*

(Received for publication, April 16, 1997, and in revised form, September 3, 1997)

Perry J. Blackshear‡, Deborah J. Stumpo§, Ester Carballo, and John C. Lawrence, Jr.¶

From the Departments of Medicine and Biochemistry, Division of Endocrinology, Metabolism and Nutrition, The Howard Hughes Medical Institute Laboratories and the Section of Diabetes and Metabolism, Duke University Medical Center, Durham, North Carolina, 27710 and the Departments of Pharmacology and Medicine, University of Virginia, Charlottesville, Virginia 22908

PHAS-I is the prototype of a group of eIF4E-binding proteins that can regulate mRNA translation in response to hormones and growth factors. To investigate the importance of PHAS-I in the physiology of the intact animal, we disrupted the PHAS-I gene in mice. Tissues and cells derived from the knockout mice contained no detectable PHAS-I protein. A related protein, PHAS-II, and eIF4E were readily detectable in tissues from these animals, but neither appeared to be changed in a compensatory manner. Mice lacking PHAS-I appeared normal at birth. However, male knockout mice weighed approximately 10% less than controls at all ages, whereas female weights were similar to those of controls. Both males and females were fertile. Tissues from adult animals appeared to be normal by routine histological staining techniques, as were routine blood cell counts and chemistries. Fibroblasts derived from PHAS-I-deficient mouse embryos exhibited normal rates of growth and overall protein synthesis, responded normally to serum stimulation of ornithine decarboxylase activity and cell growth, and rapamycin inhibition of cell growth. Under these experimental conditions, PHAS-I is apparently not required for the normal development and reproductive behavior of female mice, but is required for normal body weight in male mice; the mechanisms responsible for this phenotype remain to be determined.

PHAS-I1 is the prototype of a family of eIF4E-binding proteins that have been implicated in the stimulatory actions of insulin and growth factors on mRNA translation (1, 2). PHAS-I (3), also known as eIF4E-binding protein 1 (4), is now known to be present in many tissues and cell types, but it was first observed in 32P-labeled rat adipocytes where it was characterized as a heat- and acid-stable species that was phosphorylated in response to insulin (5). Subsequently, PHAS-I was shown to be phosphorylated in 3T3-L1 adipocytes in response to several mitogenic stimuli including epidermal growth factor, platelet-derived growth factor, serum, and phorbol 12-myristate 13-acetate (6, 7). Nonphosphorylated PHAS-I binds with high affinity to eIF4E (4, 8). Phosphorylation of PHAS-I on the appropriate site(s) in response to insulin or growth factors promotes dissociation of the PHAS-I-eIF4E complex (4, 8) releasing eIF4E to participate in mRNA translation initiation.

eIF4E is the protein that binds to the mRNA-cap, m7GpppN (where N = any nucleotide), which is found at the 5′ end of almost all eukaryotic mRNAs (9). eIF4E facilitates initiation of translation, which results in increased protein synthesis, because initiation is usually the rate-limiting phase of translation (10–12). Messages that contain a high degree of secondary structure in their 5′-untranslated regions are particularly dependent on eIF4E for translation (10–12). This dependence is probably explained by the role of eIF4E in generating eIF4F, a complex containing eIF4E, eIF4G, and eIF4A. eIF4G, also known as eIF4γ or p220, binds both eIF4A and eIF4E. eIF4A is an ATP-dependent helicase whose activity is markedly enhanced when eIF4A is incorporated into the eIF4F complex. As the concentrations of eIF4E are thought to be lower than those of the other two subunits, the availability of eIF4E is believed to determine the amount of eIF4F (10–12). Thus increasing eIF4E increases eIF4F, which melts secondary structure in mRNA through the helicase activity of the 4A subunit. This action facilitates translation initiation by allowing more rapid binding and/or scanning by the 40 S ribosome.

When bound to PHAS-I, eIF4E retains its ability to bind to the cap site (4, 8), but its binding to eIF4G is abolished (13, 14). Thus, PHAS-I blocks formation of the eIF4F complex and inhibits eIF4E-dependent translation both in vitro and in vivo (4). Release of eIF4E as a result of PHAS-I phosphorylation appears to be an important mediator of the actions of insulin and growth factors on protein synthesis (1, 2). There is also reason to suspect that by regulating availability of eIF4E, PHAS-I may be involved in the control of cellular proliferation. For example, overexpression of eIF4E has been shown to increase the rate of growth and cause aberrant morphology of HeLa cells (15, 16), whereas microinjection of eIF4E into rodent fibroblasts increased rates of proliferation and resulted in a transformed phenotype (17). Furthermore, stable overexpression of eIF4E in 3T3 fibroblasts increased growth and caused malignant transformation of the cells (18). Recently, the over-

* This work was supported in part by National Institutes of Health Grants DK 50628 and DK 28302 (to J. C. L., Jr.). 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.

† Investigator of the Howard Hughes Medical Institute during the performance of these studies. Present address: Office of Clinical Research and Laboratory of Signal Transduction, National Institute of Environmental Health Sciences, P.O. Box 12233, Research Triangle Park, NC 27709. To whom correspondence should be addressed. Tel.: 919-541-4899; Fax: 919-541-4571; E-mail: black009@niehs.nih.gov.

‡ Present address: Office of Clinical Research and Laboratory of Signal Transduction, National Institute of Environmental Health Sciences, P.O. Box 12233, Research Triangle Park, NC 27709.

§ Present address: Office of Clinical Research and Laboratory of Signal Transduction, National Institute of Environmental Health Sciences, P.O. Box 12233, Research Triangle Park, NC 27709.

¶ Supported by a grant from the Spanish Ministry of Education and Science/Fulbright Scholarship. Present address: Office of Clinical Research and Laboratory of Signal Transduction, National Institute of Environmental Health Sciences, P.O. Box 12233, Research Triangle Park, NC 27709.

1 PHAS-I, phosphorylated heat- and acid-stable protein regulated by insulin; PHAS-II, phosphorylated heat- and acid-stable protein phosphorylated by insulin II; eIF, eukaryotic initiation factor; kb, kilobase(s); bp, base pair(s); PCR, polymerase chain reaction; MEF, mouse embryo fibroblasts; E, embryonic day; ODC, ornithine decarboxylase; FCS, fetal calf serum.
expression of PHAS-I in eIF4E-transformed 3T3 cells was found to decrease both the rate of cell growth and the ability of the cells to grow in soft agar (19).

The present experiments were performed to investigate the importance of PHAS-I in the physiology of the intact animal through targeted disruption of the PHAS-I gene. These knockout mice were used to evaluate the importance of PHAS-I in intact animals, and to investigate the specific effects of PHAS-I removal on rates of cell proliferation and protein synthesis in cultured cells derived from these animals.

MATERIALS AND METHODS

Construction of Homologous Recombination Vector—A phage clone (GC1) containing the mouse PHAS-I gene was cloned from a mouse genomic PCC4 library (Stratagene, La Jolla, CA) by standard techniques using the mouse cdNA (20) as a probe. An 8-kb NotI/BglI fragment of the original 14-kb GC1 clone was subcloned into the NotI and blunt XhoI site of pPNT (21). A 1.5-kb 3′-fragment of GC1 was generated by the polymerase chain reaction (PCR) and subcloned into the XbaI/BamHI site of pPNT. pPNT has the Neo gene under the PGK-1 promoter just downstream of the XbaI site and the th gene under control of the PGK-1 promoter just downstream of the BamHI site. The final targeting vector, pPNT/GC1, has Neo replacing exon 2 (Fig. 1). The targeting vector was linearized with HindIII and electroporated into embryonic stem cells (E14TG2a) by standard techniques (22). Selection of positive embryonic stem cell clones and generation of knockout mice was performed as described (22).

**Fig. 1. PHAS-I gene targeting vector.** As shown, exon 2 of the PHAS-I gene was replaced by a PGKneo (Neo) cassette and flanked by 8 kb of 5′ and 1.5 kb of 3′ PHAS-I genomic sequence (solid black lines). The thymidine kinase gene (tk; PGKtk) was included in the targeting vector as a marker for negative selection. Genomic DNA from embryonic stem cells was analyzed by PCR to detect homologous recombination. Primers for Neo (5′/Neo) and the PHAS-I gene (3′/GC1) were used to detect correctly targeted embryonic stem cells (2-kb PCR product). Tail biopsies from offspring of chimeric PHAS-I mice were genotyped by PCR. Two independent reactions were carried out to differentiate between +/− and −/− mice. Primers for exon 2 (5′/EX2 and 3′/EX2) were used to determine the presence or absence of the endogenous PHAS-I gene (185 nucleotides, PCR product); primers for Neo (5′/Neo) and PHAS-I (3′/GC1) were used to determine the presence or absence of the targeted homologously recombined PHAS-I gene.

**RESULTS**

**Characteristics of PHAS-I Knockout Mice**—Interbreeding of male and female mice that were +/− for the PHAS-I gene...
resulted in litters of normal size with the expected Mendelian frequency of genotypes. Of 218 total offspring tested, 56 (26%) were $+/+$, 113 (52%) were $+/-$, and 49 (22%) were $-/-$, indicating that there was no significant intrauterine mortality.

Both male and female PHAS-I-deficient mice appeared normal at birth. However, the male $-/-$ mice weighed on average 10% less than their $+/+$ and $+/-$ littermates at all time points after 2 weeks; the differences between the mean weights of the $-/-$ mice and the combined $+/+$ and $+/-$ littermates were statistically significant at all time points after two weeks (Fig. 2A). Similar differences were observed when the $-/-$ mice were compared with only the $+/+$ mice (data not shown). In contrast, the female $-/-$ mice weighed on average only 3% less than their control littermates until week 8, after which the $-/-$ mice were used for breeding; the mean weights of the $-/-$ mice were not significantly different at any time point (Fig. 2B).

Both male and female $-/-$ mice displayed normal fertility and interbreeding has resulted in litters of normal size (mean ± S.D. of 5.6 ± 2.6; $n = 18$ litters).

Blood glucose, triglyceride concentrations, complete blood counts, and white cell differential counts were indistinguishable between the $+/+$ and $-/-$ animals (not shown). The $-/-$ mice of both sexes appeared to be normal at autopsy; in addition, routine staining of paraffin-embedded sections of all organs and tissues revealed no apparent abnormalities at the level of hematoxylin and eosin staining and light microscopy (not shown). Particular attention was paid to tissues expressing the greatest amount of PHAS-I, especially white and brown adipose tissue, liver, and lactating mammary gland; these all appeared normal.

To confirm that the PHAS-I gene had been disrupted in the putative $-/-$ mice, mRNA and protein levels were measured in white adipose tissue, lactating mammary gland, and in cultured primary MEF derived from the wild-type mice and their knockout littermates (Figs. 3 and 4). In the fibroblasts, the wild-type mRNA of approximately 0.9 kb seen in the cells from the $+/+$ mice decreased in amount in the $+/-$ cells, and was accompanied by the formation of two novel species of approximately 1.5 and 0.7 kb. Experiments in which the same mRNA were probed with $5^{'}, 3'$ and Neo cDNA probes demonstrated that the 1.5-kb band represented a fusion mRNA species that contained the Neo sequences and was missing the 185 bp of deleted PHAS-I sequence, whereas the 0.7-kb band represented an mRNA species containing $5'$ and $3'$ but not intervening PHAS-I sequences (data not shown). In the $-/-$ cells, the wild-type mRNA had disappeared completely, whereas the 1.5- and 0.7-kb mRNA species represented the entire hybridizing signal (Fig. 3A).

The complete absence of PHAS-I protein was demonstrated in parallel Western blots conducted on extracts from the same cells (Fig. 3B). The two immunoreactive bands representing the $M_r$ 22,000 and 20,000 forms of PHAS-I had decreased in amount by about 50% in the $+/-$ MEF and had completely disappeared from the $-/-$ cells. No evidence of a truncated protein was present in these blots; in addition, the design of the knockout construct ensured that the elF4E binding site in PHAS-I (20) was eliminated. In blots of the same cell extracts, PHAS-II protein was not detected using a separate specific antibody (23) even after long exposure of the autoradiographs (not shown).

Similar results were obtained in tissue samples from $+/+$ and $-/-$ female littermates that were lactating at 10–12 days postpartum (Fig. 4A). The normal doublet of PHAS-I protein could be readily detected in lactating mammary gland and white adipose tissue from the $+/+$ mouse but not in the analogous tissues from the $-/-$ mouse (Fig. 4A). PHAS-II could be detected readily in Western blots of the same tissues both as a minor band in the blots exposed to the anti-PHAS-I antibody (Fig. 4A) and using a specific antibody to PHAS-II (Fig. 4B). However, there was no compensatory increase in PHAS-II protein expression in these tissues in the PHAS-I knockout mice (Fig. 4A and B). Tissue homogenates from the same tissues were used to assess the amounts of elF4E using a specific antibody (20). There were no differences in the amount of this protein per unit of homogenate protein when extracts of mammary gland and adipose tissue were compared between the $+/+$...
and \(-/\sim\) mice (Fig. 4C). A blot processed in parallel using nonimmune serum was completely blank under the same conditions (Fig. 4D). We also tested a wide variety of tissues from male PHAS-I knockout mice by Western blotting for PHAS-I and PHAS-II. In all cases, PHAS-I was absent, and there was no evidence of a compensatory increase in levels of PHAS-II in any of these tissues (not shown).

Characteristics of MEF Derived from PHAS-I Knockout Mice—Because the phosphorylation and activity of PHAS-I are inhibited by treatment of 3T3-L1 adipocytes with rapamycin (20), we tested the ability of rapamycin to inhibit the growth of primary MEF based on previously described assays in cultured mouse fibroblasts (25). Rapamycin (20 ng/ml) markedly inhibited the growth of Swiss 3T3 fibroblasts (25). Cells were harvested from triplicate wells at 24-h intervals for 5 days; each point represents the average of cell counts from these three wells at each time point expressed as a percentage of the mean value obtained at day 5 in the control cells, which was designated 100%. A, cells from a \(+/+\) mouse (WT); B, cells from a \(-/\sim\) mouse (KO). The closed circles (○) represent cells treated with control conditions (MeSO4), and the open circles (○) represent cells treated with rapamycin.

We analyzed the induction of ODC in large numbers of passage 4 primary cultured embryonic fibroblasts of the \(+/+\), \(-/\sim\), and \(-/-\) genotypes analyzed at 72 h after plating. Serum was used because of its known ability to promote phosphorylation of PHAS-I in a manner similar to that of insulin (7) and because of the paucity of insulin receptors on primary mouse fibroblasts. There were no differences in cell numbers under these conditions (mean ± S.D. of cells \(10^4\) per 35-mm dish analyzed in triplicate): \(+/+\), 31 ± 14 (\(n = 10\)); \(-/-\), 26 ± 7 (\(n = 10\)); \(-/\sim\), 39 ± 12 (\(n = 10\)). The means from the \(+/+\) and \(-/\sim\) cells were not significantly different from each other using Student’s \(t\) test.

The rate of overall protein synthesis was measured in these same fibroblasts at the same passage in either the serum-deprived state or after 4 h of stimulation with 10% FCS using pulse labeling with \(^{35}\)S)methionine for the last 60 min of the stimulation. In both the serum-deprived state and serum-stimulated state, we could determine no effect of PHAS-I genotype on overall protein synthesis rates. For the serum-deprived cells, the protein synthesis rates were as follows (expressed as trichloroacetic acid precipitable cpm per mg of protein, assayed in triplicate wells from each of 10 primary cultures per genotype at passage 4 (mean ± S.D.)): \(+/+\), 13.9 ± 2.9; \(-/-\), 17.4 ± 6.9; \(-/\sim\), 11.3 ± 2.8. For the serum-stimulated cells, the results were: \(+/+\), 10.1 ± 2.1; \(-/-\), 10.4 ± 2.7; \(-/\sim\), 9.1 ± 1.7. The differences between the \(+/+\) and \(-/-\) means were not statistically significant in either group.

Finally, we analyzed the induction of ODC 3 h after stimulation of serum-deprived MEF with 10% FCS. The ODC mRNA contains a long 5’-untranslated region with considerable secondary structure, and its mitogen-stimulated translation has been used as a model of eIF4E activity (29–31). The 3 h time point was chosen because, in previous experiments in NIH-3T3 cells, near-peak induction of enzyme activity occurred at this time in response to FCS (28). In the present experiments, 10% FCS induced ODC approximately 3–4-fold over control, but there were no reproducible statistically significant differences between the basal enzyme activities or the magnitude of this induction in cells from the \(+/+\) and \(-/-\) genotypes (not shown).

**DISCUSSION**

The PHAS-I protein has been implicated as a mediator of mitogen-stimulated protein synthesis in its role as a phospho-
rylation-regulated eIF4E-binding protein (1, 2). PHAS-I is expressed in many cells and tissues with the highest levels in white and brown adipose tissue (23). PHAS-II or eIF4E-binding protein 2 is also expressed in many of the same cells and tissues that express PHAS-I, and PHAS-II appears to be a functional homologue of PHAS-I (3, 4, 23). Because PHAS-II can bind to eIF4E in normal tissues (4), it would be expected to continue this function in cells and tissues from PHAS-I knockout animals. Moreover, searches of expressed sequence tag data bases indicate that additional members of the PHAS family are expressed in cells. Because of the possible functional redundancy conferred by these PHAS family members, it was impossible to predict the phenotype of the PHAS-I knockout mouse. However, we hoped that the phenotype would shed some light on the physiological importance of this protein in the intact animal.

To date, the only consistent phenotype that we have observed in the PHAS-I knockout mice was that the male /−/ mice weighed on average 10% less than their control littermates at all ages between 4 and 24 weeks. These differences were statistically significant at all time points between 4 and 24 weeks and remained significant when the mean weights of the /−/ males were compared with those of the +/+ littermates only. The weights of the female /−/ mice were not significantly lower than those of their control littermates. Otherwise, the PHAS-I-deficient mice were phenotypically normal with normal external appearance, reproductive behavior, and tissue histology. These animals have been back-crossed to the C57BL/6J strain for only a single generation at present, and it is possible that the small amount of remaining 129 strain genome is diluting out a more dramatic phenotype that will become obvious after the 8 or 9 back-crosses generally required to remove the last remnants of the 129 genome. However, we suspect that even then such a phenotype is unlikely to be severe under the normal living conditions of laboratory mice. Instead, it seems likely that PHAS-I is not necessary for a relatively normal life in the mouse, perhaps because of the expression of functional homologues of PHAS-I. Such presumed compensation by functional homologues has been seen in other single knockouts of genes belonging to multigene families (32).

Several types of future studies can be used to address these possibilities. For example, 8 or 9 back-crosses into the C57BL/6J strain will be used in an attempt to remove the remaining 129 genome, a procedure that may make the low-weight phenotype more severe and/or extend it into females. In addition, crossing into other strains may magnify or alter this phenotype. If a more severe phenotype can be produced by these methods, then the mechanism of the decreased body weight can be investigated more thoroughly. For example, cancer analysis showing normal lean body mass but deficient adipose tissue stores would lead to an evaluation of triglyceride synthesis and breakdown rates in adipose tissue from the /−/ mice, the tissue in which PHAS-I is most highly expressed (23). Given the likely compensatory behavior of other members of the PHAS family, double or triple knockouts are likely to be more severely affected, as has been shown to be the case when multiple members of other gene families have been knocked out (32).

The facts that fibroblasts derived from PHAS-I deficient mice exhibited normal rates of serum-stimulated growth and overall protein synthesis were somewhat unexpected, since recent studies have shown that overexpression of PHAS-I results in slower rates of cell growth in 3T3 cells (19). These data have led Sonenberg and colleagues (19) to propose that PHAS-I and -II might behave as tumor suppressors, by virtue of their capacity to inhibit mitogen-stimulated protein synthesis. Future studies will be necessary to fully characterize the tumorigenic capacity of the fibroblasts derived from the PHAS-I knockout mice; it will also be of interest to follow these mice as they age, since this hypothesis would suggest that they may have a predilection for tumor development. Our oldest PHAS-I knockout mice are 13 months of age at the time of this writing, and no evidence of a tendency to excess tumor growth has been noted. This may be an instance in which the response to pathological rather than physiological stimuli may need to be evaluated, such as the response to transplanted tumors, expressed onco- genes, or transforming viruses.

Acknowledgments—We are very grateful to Betsy Kennington, Jane Tuttle, and Suhong Pang for expert technical assistance, to Dr. Beverly Koller and her colleagues at the University of North Carolina at Chapel Hill for generating the chimera mice, and to Dr. Daniel Schenkmann for help with the evaluation of the mouse histopathology.

REFERENCES

1. Lawrence, J. C., Jr., Faden, P., Haystead, T. A. J., and Lin, T.-A. (1997) in Advances in Enzyme Regulation (Weber, G., ed) pp. 239–267, Pergamon Press, NY.
2. Sonenberg, N. (1996) in Translational Control (Hershey, J. W. B., Mathews, M. B., and Sonenberg, N., eds) pp. 245–269, Cold Spring Harbor Press, Cold Spring Harbor, NY.
3. Lin, T. A., and Lawrence, J. C., Jr. (1996) Proc. Natl. Acad. Sci. U. S. A. 91, 3730–3734.
4. Pause, A., Belsham, G. J., Donze, O., Lin, T. A., Lawrence, J. C., Jr., and Sonenberg, N. (1994) Nature 371, 762–7670.
5. Denton, R. M., Browney, R. W., and Belsham, G. J. (1981) Diabetologia 21, 347–362.
6. Blackshear, P. J., Witters, L. A., Girard, P. R., Kuo, J. F., and Quamo, S. N. (1995) J. Biol. Chem. 260, 2634–2635.
7. Blackshear, P. J., Nemenoff, R. A., and Avruch, J. (1983) Biochem J. 214, 11–19.
8. Lin, T.-A., Kong, X., Haystead, T. A. J., Pause, A., Belsham, G., Sonenberg, N., and Lawrence, J. C., Jr. (1994) Science 266, 653–656.
9. Shatkin, A. J. (1987) Bioessays 7, 257–277.
10. Altman, M., and Traschel, H. (1993) Trends Biochem. Sci. 18, 429–432.
11. Rhoads, R. E. (1993) J. Biol. Chem. 268, 3017–3020.
12. Sonenberg, N. (1994) Biochimie (Paris) 76, 839–846.
13. Haghiñab, A., Mader, S., Pause, A., and Sonenberg, N. (1995) EMBO J. 14, 5701–5709.
14. Mader, S., Lee, H., Pause, A., and Sonenberg, N. (1995) Mol. Cell. Biol. 15, 4990–4997.
15. DeBenedetti, A., and Rhoads, R. E. (1990) Proc. Natl. Acad. Sci. U. S. A. 87, 8212–8216.
16. DeBenedetti, A., Joshi-Barve, S., Rinker-Schaeffer, C., and Rhoads, R. E. (1991) Mol. Cell. Biol. 11, 5435–5445.
17. Smith, M. R., Jaramillo, M., Liu, Y.-L., Dever, T. E., Merrick, W. C., Kung, H.-F., and Sonenberg, N. (1990) New Biol. 2, 448–454.
18. Lazaris-Karatzas, A., Montine, K. S., and Sonenberg, N. (1990) Nature 345, 544–547.
19. Rousseau, D., Gringras, A. C., Pause, A., and Sonenberg, N. (1996) Oncogene 13, 2415–2420.
20. Lin, T.-A., Kong, X., Saltiel, A. R., Blackshear, P. J., and Lawrence, J. C. (1995) J. Biol. Chem. 270, 18531–18538.
21. Tybulewicz, V. L. J., Crawford, C. E., Jackson, P. K., Bronson, R. T., and Mulligan, R. C. (1991) Cell 65, 1153–1163.
22. Stumpo, D. J., Bock, C. B., Tuttle, J. S., and Blackshear, P. J. (1995) Proc. Natl. Acad. Sci. U. S. A. 92, 944–948.
23. Lin, T. A., and Lawrence, J. C., Jr. (1996) J. Biol. Chem. 271, 30199–30204.
24. Taylor, G. A., Carbell, E., Lee, D. M., Lai, W. S., Thompson, M. J., Patel, D. D., Schenkmann, D. L., Gilkeson, G. S., Brummeier, H. E., Haynes, B. F., and Blackshear, P. J. (1996) Immunity 4, 445–454.
25. Chung, J., Kuo, C. J., Crabtree, G. R., and Blenis, J. (1992) Cell 69, 1227–1236.
26. Warner, L. M., Adams, L. M., and Sehgal, S. N. (1994) Arthritis Rheum. 37, 289–287.
27. Taylor, G. A., and Blackshear, P. J. (1995) J. Cell. Physiol. 162, 378–387.
28. Hovis, J. G., Stumpo, D. J., Halsey, D. L., and Blackshear, P. J. (1986) J. Biol. Chem. 261, 10380–10386.
29. Manzella, J. M., and Blackshear, P. J. (1990) J. Biol. Chem. 265, 11817–11822.
30. Manzella, J. M., Rheeukil, W., Rhoads, R. E., and Blackshear, P. J. (1991) J. Biol. Chem. 266, 2383–2389.
31. Thach, R. E. (1992) Cell 68, 177–180.
32. Champon, P. (1994) Semin. Cell Biol. 5, 115–125.