The Tetratricopeptide Repeat Domain of Protein Phosphatase 5 Mediates Binding to Glucocorticoid Receptor Heterocomplexes and Acts as a Dominant Negative Mutant*

(Received for publication, April 11, 1996, and in revised form, August 16, 1996)

Mei-Shya Chen, Adam M. Silverstein, William B. Pratt, and Michael Chinkers

From the Vollum Institute and Department of Cell and Developmental Biology, Oregon Health Sciences University, Portland, Oregon 97201-3098 and Department of Pharmacology, University of Michigan Medical School, Ann Arbor, Michigan 48109

We previously identified a protein-serine phosphatase designated PP5, based on the binding of its tetratricopeptide repeat (TPR) domain to the atrial natriuretic peptide receptor (Chinkers, M. (1994) Proc. Natl. Acad. Sci. U. S. A. 91, 11075–11079). We have now identified another protein complex to which PP5 is targeted through its TPR domain. A 90-kDa protein, identified as heat shock protein 90 (hsp90) by immunoblotting, specifically co-immunoprecipitated from COS-7 cell lysates with the FLAG-tagged TPR domain of PP5. hsp90 also co-immunoprecipitated with full-length FLAG-tagged PP5 overexpressed in COS-7 cells and with endogenous PP5 from untransfected COS-7 cells or rat brain. During gel filtration, PP5 and hsp90 comigrated in a high molecular weight complex. Since glucocorticoid receptors (GR) exist as large heterocomplexes containing hsp90 bound to TPR proteins, we hypothesized that PP5 might be associated with these complexes. Consistent with this hypothesis, PP5 specifically co-immunoprecipitated with GR from mouse L cell lysates. To test the functional importance of this TPR-mediated association in living cells, we used a dominant negative PP5 mutant consisting only of its TPR domain. The mutant inhibited GR-mediated transactivation by approximately 70% in transfected CV-1 cells. This is the first evidence that the TPR proteins in steroid receptor heterocomplexes may be required for signaling in vivo.

Steroid receptors acquire the ability to bind hormone only after they are assembled into a heterocomplex containing several other proteins (1, 2). The first component of the receptor heterocomplexes to be identified was heat shock protein 90 (hsp90), which is important for the assembly of functional receptor heterocomplexes (1, 2). Glucocorticoid receptor (DME, Dulbecco’s modified Eagle’s medium; PAGE, polyacrylamide gel electrophoresis; CAT, chloramphenicol acetyltransferase; TES, N-tris(hydroxymethyl)methyl-2-aminoethanesulfonic acid).

This work was supported by National Institutes of Health Grants HL 47063 and HD 30236 (to M. C.) and CA 28010 (to W. B. P). 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.

§ To whom correspondence should be addressed. Tel.: 503-494-4669; Fax: 503-494-4353; E-mail: chinkers@ohsu.edu.

This paper is available on line at http://www-jbc.stanford.edu/jbc/
Dominant Negative Effect of TPR Domain of PP5 on GR

glucocorticoid receptor (GR) heterocomplexes. We further show that a truncated PP5 containing only the TPR domain acts as a dominant negative mutant, blocking GR signaling. To our knowledge, this is the first evidence that steroid receptor-associated TPR proteins are required for hormonally induced transcriptional activation in living cells.

EXPERIMENTAL PROCEDURES

Tissue Culture—COS-7 cells and CV-1 cells were maintained in DME containing 10% fetal bovine serum. L929 mouse fibroblasts (L cells) were grown in monolayer culture in DME containing 10% heat-inactivated calf serum. Sf9 cells were maintained in Grace’s medium supplemented with lactalbumin hydrolysate, Yeastolate (Life Technologies, Inc.), and 10% fetal bovine serum.

Engineering of FLAG-tagged PP5 and FLAG-tagged TPR Domain of PP5 for Expression in Mammalian and Insect Cells—The 5’ end of rat PP5 was amplified by polymerase chain reaction using a 5’ primer that encoded an EcoRI site, an initiation codon, the FLAG epitope (DYKDDDDK), the first five amino acids of PP5, and a 3’ primer complementary to nucleotides 815–832 of our rat PP5 cDNA clone (20). The polymerase chain reaction product was subcloned and sequenced from its 5’ end to the Nhel site at position 392 of the original cDNA, to ensure the absence of mutations. The 5’ end of the FLAG-tagged PP5 was then excised as a Nhel fragment and blunt ended to correspond with the corresponding 5’ end of the original cDNA clone. The resulting construct was cloned into the EcoRI sites of the pcMV6 vector (for expression in mammalian cells) or the pVL1393 vector (for baculovirus construction).

During the course of these studies, it became clear that the sequence at the 5’ end of our original rat cDNA clone (20) was derived from a chimeric cDNA, and that the sequence published by Becker et al. (18) corresponded to the authentic 5’-coding sequences of PP5 (23). Our original cDNA clone encoded the sequence QGY immediately after the initiation codon, whereas the correct sequence is AEGERTECAEPRDEPP.

We used oligonucleotide-directed mutagenesis to correct the initiation codon, whereas the correct sequence is AEGERTECAEPRDEPP. We used oligonucleotide-directed mutagenesis to correct the initiation codon, whereas the correct sequence is AEGERTECAEPRDEPP.

Purification of Epitope-tagged PP5 from Sf9 Cells—A 100-ml suspension culture of Sf9 cells was infected with a recombinant baculovirus (18). The recombinant baculovirus, lacking a truncated PP5 containing only the TPR domain, was then used for the mammalian cell expression studies described below.

The FLAG-tagged PP5 construct in Bluescript SK (+) (with the correct amino terminus) was truncated at a unique HindIII site to remove sequences encoding the carboxyl portion of PP5. Blunt-ending with Klenow fragment and insertion of a linker encoding an Nhel site and a stop codon resulted in a cDNA encoding the FLAG-tagged TPR domain terminating at Leu181. Few non-TPR residues were present in the FLAG-tagged construct; the TPR domain begins at Ala28 and ends at Leu181 (36) (using the naming of residues described by Becker et al. (18)). The cDNA fragment encoding the FLAG-tagged TPR domain was excised as an EcoRI/Sall fragment and cloned into the corresponding sites of the pcMV6 vector (24) for mammalian cell expression.

Preparation of a Recombinant Baculovirus Expressing Epitope-tagged PP5—A baculovirus for expression of FLAG-tagged PP5 was prepared by homologous recombination of pVL1393-FLAG-PP5 with BaculGold linearized baculovirus DNA (Pharmingen) as described previously (25). Plaque-purified secondary virus stocks were used for infections.

Purification of Epitope-tagged PP5 from Sf9 Cells—A 100-ml suspension culture of Sf9 cells was infected with a recombinant baculovirus expressing FLAG-tagged PP5 at a multiplicity of infection of 3, then incubated for 3 days at 27°C. Cells were collected by centrifugation, washed once with 20 ml Hepes, pH 7.4, 150 mM NaCl, and sonicated in 5 ml of 20 mM Hepes, pH 7.4, 50 mM NaCl, 1 mM dithiothreitol, 20 mM benzamidone, 10 μg/ml each aprotinin, leupeptin, and pepstatin. The homogenate was centrifuged at 12,000 × g for 30 min at 4°C, and the supernatant was then centrifuged at 125,000 × g for 30 min at 4°C. The supernatant from the second centrifugation was applied to a 1-ml MonoQ column at 4°C at a flow rate of 1 ml/min. Proteins were fractionated by elution with a 20-ml gradient of 50–500 mM NaCl in 20 mM Hepes, 1 mM dithiothreitol. Peak fractions containing FLAG-tagged PP5, as determined by immunoblotting with a monoclonal antibody to the FLAG epitope (25), were pooled and further purified by immunofusion to increase their purity. The pool was incubated with 1 ml of M2-agarose beads (IBI) for 1 h at 4°C, then transferred to a column and washed three times with 5 ml of Hepes-buffered saline containing 1 mM dithiothreitol. Epitope-tagged PP5 was then eluted by five repeated applications of 1 ml of Hepes-buffered saline, 1 mM dithiothreitol containing 200 μg/ml FLAG peptide (DYKDDDDK) for 15 min each. Fractions were analyzed by immunoblotting using the M2 antibody, and peak fractions were pooled. The FLAG peptide was then removed by ultrafiltration. Approximately 200 μg of purified protein were typically recovered from a 100-ml culture.

Preparation of Rabbit Antisera against PP5—After preparation of preimmune serum, female New Zealand White rabbits were injected subcutaneously with 25–50 μg of purified FLAG-tagged PP5. The antigen was mixed with Freund’s complete adjuvant for the initial injection and with Freund’s incomplete adjuvant for booster injections given at 30-day intervals. Blood was collected and serum prepared 10 days postinjection.

Transfection—COS-7 cells in 10-cm plates were transfected with 10 μg of pCMV6-FLAG-PP5, pCMV6-FLAG-TPR, or pCMV6 vector for control plates, using a DEAE-dextran procedure (26). CV-1 cells in 6-cm plates were transfected with 0.5 μg of pSVL-GR (27), 2 μg of the reporter plasmid PRE-PBL7 (28), 0.5 μg of pBSV-luciferase (29) (to normalize for transfection efficiency), and 5 μg of plasmid containing indicated amounts of pCMV6-FLAG-TPR (with the remainder of the 5 μg made up of control pCMV6 vector), using a calcium phosphate procedure (30). The GR and CAT reporter plasmids were obtained from Dr. Stoney Simons (NIH). One day after transfection, CV-1 cells were rinsed with phosphate-buffered saline and incubated for 24 h in fresh growth medium, in the absence or presence of 100 nM dexamethasone.

Metabolic Labeling and Immunoprecipitation—COS-7 cells were incubated for 16 h in medium containing 9% methionine and cysteine-free DME and 1% FCS, in the absence or presence of 100 nM dexamethasone.

Immunoprecipitation and Immunoblotting—COS-7 cells were incubated for 18 h in medium containing 100 μCi/ml [35S]methionine/cysteine (EXPRE35S35S protein labeling mix, DuPont NEN). Cells were then placed on ice, washed once with cold phosphate-buffered saline and once with 10 ml Hepes, pH 7.4, 1 mM EDTA, 20 mM sodium molybdate, 10 mM MgCl2 (buffer A). Cells were then scraped into buffer A containing 2 mM phenylmethylsulfonyl fluoride and 10 μg each aprotinin, leupeptin, and pepstatin, and lysed by passage 30 times through a 25-gauge needle. The lysates were then subjected to centrifugation at 15,000 × g for 5 min at 4°C, and the supernatant from this centrifugation was clarified by centrifugation at 100,000 × g for 1 h at 4°C. Samples were normalized for total radioactivity, the clarified lysates were preclariied by incubating for 1.5 h at 4°C with 20 μl of goat anti-mouse-IgG beads (Sigma, for immunoprecipitation with monoclonal antibody M2 (IBI) directed against the FLAG epitope) or protein A-agarose beads (Pierce, for immunoprecipitations using anti-PP5 serum). After removing the beads by centrifugation, the preclariied extracts were incubated for 1.5 h at 4°C with either 20 μl of goat anti-mouse-IgG beads or 20 μl of protein A-agarose beads to which the IgG from 5 μl of preimmune or anti-PP5 serum had been preadsorbed. Beads were then washed five times with 10 ml Hepes, pH 7.4, 1 mM EDTA, 20 mM sodium molybdate, 50 mM KCl, 10% glycerol, and immune complexes were released by heating in SDS sample buffer.

Samples were then analyzed by SDS-PAGE as indicated in the figure legends, followed by fluorography using sodium salicylate (31). Molecular weight markers were detected by staining with Coomassie Blue or (for immunoblots) by using Rainbow molecular weight markers (Amer sham Corp.).

Immunoprecipitation and Immunoblotting—Antiserum to human PP5 was prepared from unlabelled COS-7 cells using the methods described above, normalizing for protein before performing immunoprecipitations, and fractionated by SDS-PAGE using Rainbow molecular weight markers (Amer sham Corp.) as standards. For analyzing the association of hsp90 with PP5 in rat brain, half of a rat brain was homogenized in 6 ml of ice-cold buffer A containing 10 μg/ml each aprotinin and leupeptin, and 20 mM benzamidine, using three 15-s bursts with a Potter homogenizer. The homogenate was centrifuged at 12,000 × g for 10 min at 4°C. The supernatant from that centrifugation was further centrifuged at 100,000 × g for 1 h at 4°C, and 1 ml aliquots of the preclariied supernatant were then subjected to immunoprecipitation with preimmune or anti-PP5 serum as described above, except using 40 μl of protein A-agarose beads and 10 μl of serum. Following SDS-PAGE of the washed immune complexes and blotting to nitrocellulose filters, immunoblotting to detect hsp90 was performed as described previously (25) using a 1:1000 dilution of monoclonal anti-
body AC-16 (Sigma H1775) followed by incubation with peroxidase-conjugated secondary antibody and chemiluminescent detection. For immunoprecipitation of glucocorticoid receptors from L cells, cells were harvested by scraping into Earle’s balanced salt solution followed by a wash in the same buffer and centrifugation at 500 × g. The washed cells were suspended in 1.5 volumes of 10 mM Hapes, 1 mM EDTA, 20 mM sodium molybdate, pH 7.4, and lysed by Deounce homogenization. Homogenates were centrifuged for 1 h at 100,000 × g, and the supernatant fluid was used for immunoprecipitation. The BuGR2 monoclonal antibody against glucocorticoid receptors (Affinity Bioreagents, Golden, CO) or nonimmune mouse IgG was prebound to protein A-Sepharose pellets by incubating 40 μl of a 20% slurry of protein A-Sepharose for 1 h at 4°C with 150 μl of TEG buffer (10 mM TES, 50 mM NaCl, 4 mM EDTA, 10% (w/v) glycerol, pH 7.6), and 6 μg of antibody, followed by centrifugation and washing with TEG. Glucocorticoid receptors were immunoadsorbed from 400 μl aliquots of L cell cytosol by rotation for 2 h at 4°C with 8 μl of protein A-Sepharose prebound with BuGR2 or nonimmune IgG followed by three washes with 1 ml of TEGM buffer (TEG plus 20 mM sodium molybdate). Immune complexes were fractionated by SDS-PAGE on a 12% gel, transferred to Immobilon-P membranes, and probed with 1 μg/ml BuGR2 antibody for the glucocorticoid receptor, 1 μg/ml of the monoclonal antibody AC88 for hsp90 (StressGen), or 1,000-fold dilutions of anti-PP5 serum (above), and an anti-PP5 antibody to hsp90. Immune pellets and the immunoadsorbed cytosols were assayed by Western blotting for hsp90 and PP5.

To immunoadsorption of hsp90 from L cell cytosol (see Fig. 4B), cytosol was diluted 10-fold with 10 mM Hapes, 1 mM EDTA, 10% glycerol, and 100-μl aliquots were immunoadsorbed to 10-μl pellets of Actigel-ALD precomplexed with either nonimmune IgM or with 3G3 anti-hsp90 monoclonal IgM (Affinity Bioreagents). Immune pellets were washed three times in 1 ml of TEGM buffer, and both immune pellets and the immunoadsorbed cytosols were assayed by Western blotting for hsp90 and PP5.

Glucocorticoid-induced Transcriptional Activation—Transfected CV-1 cells, incubated in the absence or presence of dexamethasone as described above, were washed twice with phosphate-buffered saline and collected by centrifugation at 4°C for 3 min at 7,500 × g. Cells were resuspended in 100 μl of 0.1 M potassium phosphate, pH 7.8, 1 mM dithiothreitol, and lysed by three cycles of freezing in an ethanol/dry ice bath and thawing in a 37°C water bath. After clarifying the lysate by centrifugation at 4°C for 5 min at 18,500 × g, 85 μl of the supernatant were saved and diluted for luciferase (29) and CAT (33) assays. To inactivate endogenous acetylating enzymes, cell extracts were heated at 65°C for 15 min before performing CAT assays. To correct for variations in transformation efficiency, CAT activity was normalized to total protein concentration. Background CAT activity from cells incubated in the absence of dexamethasone was subtracted from total activity in dexamethasone-stimulated cells to determine dexamethasone-induced activity.

RESULTS

hsp90 Co-immunoprecipitates with the Overexpressed TPR Domain of PP5—We have shown an interaction between the TPR domain of PP5 and the protein kinase-like domain of the ANP receptor in the yeast two-hybrid system (20). In an attempt to identify additional proteins interacting with the TPR domain of PP5, we overexpressed the FLAG-tagged TPR domain of PP5 in COS-7 cells and performed co-immunoprecipitation experiments. A prominent 90-kDa protein co-immunoprecipitated with the FLAG-tagged TPR domain of PP5 from

FIG. 1. hsp90 co-immunoprecipitates with FLAG-tagged PP5 or with the FLAG-tagged TPR domain of PP5. A, COS-7 cells were transfected with control plasmid (lane 1), with a plasmid encoding the FLAG-tagged amino-terminal TPR domain of PP5 (lane 2) or with a plasmid encoding full-length FLAG-tagged PP5 (lane 3). Cells were then labeled with [35S]methionine/cysteine, and cell lysates were subjected to immunoprecipitation with a monoclonal antibody to the FLAG epitope. Immunoprecipitated proteins were analyzed by SDS-PAGE followed by fluorography. B, immunoblotting of immunoprecipitates prepared as in A but from unlabeled cell extracts, with a monoclonal antibody to hsp90. Lane 1, control plasmid; lane 2, FLAG-tagged TPR domain; lane 3, FLAG-tagged PP5.

hsp90 Co-immunoprecipitates with Overexpressed Full-length PP5—Under the same conditions, hsp90 also associated with full-length PP5 (Fig. 1). FLAG-tagged PP5, overexpressed in COS-7 cells, was immunoprecipitated with a monoclonal antibody to the FLAG epitope, either from [35S]methionine-labeled cells (Fig. 1A, lane 2) or unlabeled cells (Fig. 1B). As for the isolated TPR domain, a prominent 90-kDa protein that reacts with a monoclonal antibody to hsp90 co-immunoprecipitated with FLAG-tagged full-length PP5 (Fig. 1, lanes 3). Thus, the association of the TPR domain of PP5 with hsp90 is not an artifact of removing the phosphatase domain.

Association of Endogenous hsp90 with Endogenous PP5—We next tested whether this association was merely an artifact of overexpressing PP5, or whether endogenous PP5 associated with hsp90. This required production of an antiserum to PP5. Recombinant-murine TPR-tagged at its amino terminus with the FLAG epitope, was expressed in the baculovirus system. The protein was purified to homogeneity by MonoQ chromatography followed by immunoaffinity chromatography on a matrix
containing a monoclonal antibody to the FLAG epitope. The purified protein (Fig. 2) was used to immunize rabbits, resulting in specific, high titer anti-PP5 sera. We performed co-immunoprecipitation experiments to test whether endogenous PP5 associated with hsp90 in untransfected COS-7 cells or in rat brain (Fig. 3). We found that hsp90 co-immunoprecipitated with endogenous PP5 from extracts of COS-7 cells (Fig. 3, A and B) or rat brain (Fig. 3B). Neither PP5 nor hsp90 was immunoprecipitated by preimmune serum (Fig. 3, A and B). Thus, endogenous PP5 forms a complex with hsp90 in both cultured cells and in a normal tissue.

**PP5 and hsp90 Are Present in a High Molecular Weight Complex**—We used another method, gel filtration chromatography, to confirm the association between PP5 and hsp90. Rat brain extracts were fractionated on a Superose 6 column calibrated with various molecular weight markers, and fractions were analyzed by SDS-PAGE and immunoblotting (Fig. 4, A). As shown previously, hsp90, FKBP52, and CyP-40 were components of steroid receptor heterocomplexes (1, 2). We tested whether PP5 was associated with GR heterocomplexes in a co-immunoprecipitation experiment (Fig. 5). Low salt extracts from L cells, prepared in the presence of sodium molybdate to stabilize receptor complexes (34), were subjected to immunoprecipitation using a monoclonal antibody to GR (lane 3) or a nonimmune control antibody (lane 2), followed by immunoblotting. Blots were probed with antibodies specific for the receptor itself, hsp90, PP5, FKBP52, or CyP-40. For comparison, an aliquot of the cytosol before immunoprecipitation was also subjected to SDS-PAGE and immunoblotting (Fig. 5, lane 1). As shown previously, hsp90, FKBP52, and CyP-40 co-immunoprecipitated with GR. In addition, PP5 was a prominent component of the receptor complexes.

**The Expressed TPR Domain of PP5 Acts as a Dominant Negative Mutant**—The above experiments demonstrated an association between PP5 and GR heterocomplexes, but did not address the possible function of this association. In order to test the functional importance of PP5 in GR heterocomplexes, we
The fact that PP5 associates with progesterone receptor FKBP51, FKBP52, or CyP-40, and an acidic protein designated in STI1, while mature complexes contain the TPR proteins. Immature receptor heterocomplexes contain the TPR bind via TPR domain to hsp90, as well as additional accessory proteins. These steroid receptor-associated TPR proteins are important in controlling the activity of these receptors, further studies will be required to test whether this is the case. At least some of the TPR proteins appear to compete for binding to the same site on hsp90. It is possible, therefore, that the TPR domain of PP5 inhibits signaling in vivo by displacing one or more of these other proteins rather than by displacing endogenous PP5 alone.

In summary, we have shown that PP5, a recently discovered protein-serine phosphatase, forms a complex with hsp90 via a TPR domain. This complex is in turn associated with GR heterocomplexes. The observation that the TPR domain of PP5 acts as a dominant negative mutant to block GR signaling strongly suggests a role in vivo for PP5, or perhaps more generally for the TPR-containing proteins that bind to the same site in hsp90 complexes. This is the first direct evidence that these steroid receptor-associated TPR proteins are important for signaling.

Acknowledgments—We thank Elizabeth M. Wilson for technical assistance, and June Shiigi and Heidi Davison for preparing illustrations.Used a deletion mutant of PP5 containing the TPR domain, but lacking the phosphatase catalytic domain. The expressed TPR domain of PP5 should act as a dominant negative mutant by displacing endogenous PP5 from hsp90 in GR heterocomplexes. The TPR domain of PP5 was co-expressed with GR and a CAT reporter gene in CV-1 cells. We then examined the ability of dexamethasone to stimulate transcription of the reporter gene in the absence and presence of the PP5 mutant.

As shown in Fig. 6, the TPR domain of PP5 inhibited transactivation in a concentration-dependent manner, with dexamethasone-induced transcription inhibited by approximately 70% at the highest concentration of the TPR plasmid tested. Similar results were obtained in six separate experiments. This result strongly suggests a functional role for PP5 or other TPR proteins in GR signaling. At this time, we cannot exclude the possibility that the effect on transactivation is due to displacement of other TPR proteins, in addition to PP5, from GR heterocomplexes. To our knowledge, this is the first evidence for a functional role of TPR domains per se in GR signaling in vivo.

**DISCUSSION**

TPR domains are emerging as important mediators of protein-protein interactions and subcellular targeting (10). We show here that the recently identified protein-serine phosphatase, PP5, is able to bind to hsp90 through its TPR domain and thereby associate with GR heterocomplexes. Steroid receptor heterocomplexes, in addition to a single steroid receptor and a dimer of hsp90, were previously known to contain proteins that bind via TPR domains to hsp90, as well as additional accessory proteins. Immature receptor heterocomplexes contain the TPR protein p60, the mammalian homologue of the yeast TPR protein STI1, while mature complexes contain the TPR proteins FKBP51, FKBP52, or CyP-40, and an acidic protein designated p23 (5). The fact that PP5 associates with progesterone receptor heterocomplexes in co-immunoprecipitation experiments suggests that the association of PP5 with steroid receptors may be a general phenomenon.

---

2 M.-S. Chen and M. Chinkers, unpublished data.
Dominant Negative Effect of TPR Domain of PP5 on GR

25. Wilson, E. M., and Chinkers, M. (1995) Biochemistry 34, 4696–4701
26. Cullen, B. R. (1987) Methods Enzymol. 152, 684–704
27. Miesfeld, R., Rusconi, S., Godowski, P. J., Maler, B. A., Okret, S., Wikstrom, A. C., Gustafsson, J. A., and Yamamoto, K. R. (1986) Cell 46, 389–399
28. Tsai, S. Y., Tsai, M. J., and O'Malley, B. W. (1989) Cell 57, 443–448
29. de Wet, J. R., Wood, K. V., DeLuca, M., Helinski, D. R., and Subramani, S. (1987) Mol. Cell. Biol. 7, 725–737
30. Chen, C., and Okayama, H. (1987) Mol. Cell. Biol. 7, 2745–2752
31. Chamberlain, J. P. (1979) Anal. Biochem. 98, 132–135
32. Ruff, V. A., Yem, A. W., Munns, P. L., Adams, L. D., Reardon, I. M., Deibel, M. R., Jr., and Leach, K. L. (1992) J. Biol. Chem. 267, 21285–21288
33. Seed, B., and Sheen, J.-Y. (1988) Gene (Amst.) 67, 271–277
34. Pratt, W. B., Gehring, U., and Tisf, D. O. (1996) in Stress-Inducible Cellular Responses (Feige, U., Morimoto, R. I., Yahara, I., and Polla, B. S., eds), pp. 79–95, Birkhäuser/Springer, Heidelberg
35. Orti, E., Bodwell, J. E., and Munck, A. (1992) Endocr. Rev. 13, 105–128