Protease Inhibitor 10 Inhibits Tumor Necrosis Factor α-induced Cell Death

EVIDENCE FOR THE FORMATION OF INTRACELLULAR HIGH Mₙ PROTEASE INHIBITOR 10-CONTAINING COMPLEXES

Protease inhibitor 10 (PI10) is a member of the ovalbumin family of serine protease inhibitors (ov-serpin) that is expressed at elevated levels in patients with acute myeloid leukemia and chronic myelomonocytic leukemia. Based upon the ability of the related serpin plasminogen activator inhibitor 2 (PAI-2) to protect cells against tumor necrosis factor α (TNFα)-induced cell death, this study was initiated to investigate the potential cytoprotective activity of PI10. Two different expression systems (i.e. plasmids encoding either PI10 alone or PI10 fused to the tag: enhanced green fluorescent protein, EGFP) were utilized to stably transfect an eukaryotic model cell system (i.e. HeLa cells) that neither expresses PAI-2 nor PI10. The level of PI10 expression in the stable transfectants was found to correlate with their resistance to TNFα-induced cell death. Immunoprecipitation/immunoblotting experiments demonstrated that PI10 is able to form SDS-stable complexes (i.e. Mₙ >100,000) with a cytosolic protein(s). Increased levels of the PI10-containing complexes can be detected by TNFα treatment by preventing intracellular degradative activities with the proteasome inhibitor N-carbenzoyl-tyrosine-leucine-leucine-norvalinal. PI10-containing complexes are dissociated with conditions known to separate classical protease-serpin complexes (i.e., 1.5 M ammonium hydroxide in the presence of SDS). These data support a role for the regulation of intracellular protease activities by ov-serpins.

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The abbreviations used are: PI10, protease inhibitor 10; TNFα, tumor necrosis factor α; ELISA, enzyme-linked immunosorbent assay; PBS, phosphate-buffered saline; PAI, plasminogen activator inhibitor; GFP, green fluorescent protein; EGFP, enhanced GFP, mAb, monoclonal antibody; CLLN, N-carbenzoyl-tyrosine-leucine-leucine-norvalinal; u-PA, urokinase-type plasminogen.

serpin raises the possibility that PI10 may play a key role in normal hematopoietic development, a process that requires the regulation of cell viability, growth, and differentiation.

The ability of diverse types of serine protease inhibitors to prevent cell death suggests that intracellular proteases other than cysteine aspartic-specific proteases can also participate in this process (for reviews, see Refs. 4 and 5). This concept is supported by the observation that the ov-serpin PAI-2 is able to confer resistance from the cytotoxic effects of tumor necrosis factor α (TNFα) in two cell lines (i.e. H11080 (6) and HeLa (7)). Unfortunately, high Mₙ SDS-stable complexes between PAI-2 and another molecule have not been observed during these cell death experiments, thus impeding further research on the pathway. Based upon (i) the high amino acid homology (48%) between PAI-2 and PI10 (1) and (ii) our observations that patients with acute myeloid leukemia and chronic myelomonocytic leukemia express high levels of PI10 (2), we hypothesized that this ov-serpin might regulate a similar cell death pathway and provide leukemic cells another survival mechanism. Because cells in the monocytic lineage are likely to be exposed to concentrated doses of cytokines, including TNFα, this study was designed to determine whether PI10 also exhibits cytoprotective activity against TNFα.

MATERIALS AND METHODS

Plasmid Constructions—The coding region for PI10 (1) and β-galactosidase (β-gal) (pNIDlacZ; Invitrogen, Carlsbad, NM) were, respectively, subcloned into a NheI- or a NheI-digested pCI-neo expression vector (Promega, Madison, WI). pEGFP/P10 and pEGFP/P10 expression constructs were prepared as described previously (3).

Immunosassays for PI10—Affinity-purified rabbit antibodies were prepared, biotinylated, and employed in immunoblotting analysis as described previously (2). To quantitate PI10 antigen, an ELISA was developed based upon standard two-site antibody based assays (8). Briefly, flat bottom microtiter plates were precoated overnight with affinity-purified rabbit anti-PI10 (10 µg/ml in PBS, 4 °C). The wells were blocked with blotto (5% skimmed milk powder in 10 ml Tris-HCl, pH 7.5, 2 h, 37 °C). Test samples and standard curves of purified PI10 were diluted in blotto containing 0.5% Triton X-100 and 5 ng/ml goat IgG (Sigma) prior to their incubation (2 h, 37 °C) in the antibody-coated wells. Bound PI10 was detected by sequential incubation with biotin-labeled rabbit anti-PI10 (5 µg/ml, 100 µl/well; 1 h), a streptavidin-alkaline phosphatase conjugate (Zymed Laboratories Inc., San Francisco, CA; 1,000 dilution; 30 min), and the substrate para-nitrophenyl phosphate. The resulting color change was measured at 405 nm or 10 min. The assay exhibited a linear range between 0.25 and 5 ng/ml. The specificity of assay for PI10 was confirmed by preabsorption of the biotinylated rabbit anti-PI10 using purified PI10 conjugated to Sepharose. No color development over background was observed using appropriate absorbed antibodies. Furthermore, the following purified serpins (PAI-1 (8), PAI-2 (American Diagnostica), PIb (9), and ovalbumin (Sigma)) were negative in the assay at ≤100 ng/ml.

Transfection and Fractionation of HeLa Cells—Transfection and selection of HeLa cells with mammalian expression vectors were performed as described previously (3). Washed cells were disrupted with a

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Dounce homogenizer, and nuclei were isolated as described previously (3). The cytosol-containing supernatant above the nuclear pellet was harvested and the clarified by centrifugation (15,000 x g, 30 min). Nuclear lysates and cytosol samples were separately subjected to immunoprecipitation (5) and quantitative immunoblotting (10) by modification of published protocols. Briefly, rabbit antisera to GFP (product 8366, CLONTECH) or non-immune rabbit serum (1 ug/ml, respectively) were incubated (1 h, 4 °C) with protein A-Sepharose beads. In indicated experiments, the antibodies were directly coupled to cyanogen-activated Sepharose using the manufacturer’s instructions. Washed beads were incubated (16 h, 4 °C) with either nuclear lysates or cytosol derived from the transfected cells. The beads were washed by centrifugation, eluted with boiling/reducing SDS-sample buffer, and subjected to SDS-PAGE/immunoblotting using a monoclonal antibody to GFP (#8362-1, 1:500 dilution, CLONTECH) as the primary detecting antibody. Bound antibodies were detected by incubation with peroxidase-labeled anti-mouse IgG followed by the enhanced chemiluminescence system (Amersham Pharmacia Biotech). Quantitative immunoblotting was performed by modifying previously described procedures (10) to utilize a dose response of purified GFP (0.01–1 ng/lane; CLONTECH) that was included on each immunoblot. Quantification of immunoreactive bands was performed using a laser densitometer and Image Quant software (both from Molecular Dynamics) (10).

**Induction of High M, Forms of EGFP/PI10 by Treatment with Ammonium Hydroxide Treatment/SDS—EGFP/PI10-transfected HeLa cytosol was immunoprecipitated with rabbit anti-GFP. The immunoprecipitates were solubilized by incubation (2 h, 37 °C) with 2% SDS. The protein A-Sepharose beads were removed by centrifugation and the solubilized proteins incubated (20 min, 22 °C) with an equal volume of either 3 mM ammonium hydroxide, pH 12, or PBS. Samples were neutralized by dialysis against PBS containing 0.2% SDS and subjected to SDS-PAGE/immunoblotting with mAb anti-GFP.

**RESULTS AND DISCUSSION**

Transfection of HeLa Cells with Vectors Encoding PI10 Reduces TNFα-induced Cytotoxicity—Based upon the experimental approach suggested by Dickinson et al. (7), the eukaryotic model cell line “HeLa” was selected because these cells neither express PAI-2 (7) nor PI10 (2) and thus permit experimental analysis in the absence of endogenously produced PAI-2 or PI10. Because PI10 is present both as a cytoplasmic and a nuclear molecule, we utilized direct immunoblotting of total cell lysates in conjunction with an ELISA for PI10 as a means to identify clones producing PI10. The inset to Fig. 1A demonstrates a representative immunoblotting profile obtained with a pCI/β-gal-transfected (lane 1) and a pCI/PI10-transfected (lane 2) clone. PI10 production was identified by the specific presence of a Mr 42,000 protein using rabbit anti-PI10 (lane 2 versus lane 1); however, the binding of normal rabbit IgG (lane 3) to several high Mr, electrophoresed proteins hindered the initial identification of other Mr, species of PI10.

Because studies in the laboratories of both Baglioni (6) and Antalis (7) utilized a crystal violet staining assay to correlate the expression of PAI-2 with a resistance to TNFα-induced cell death, a similar protocol was employed to extend this type of analysis to PI10. Fig. 1A shows a representative experiment demonstrating that four clones of pCI/PI10-transfected HeLa cells are more resistant to TNFα-induced cytotoxicity than three clones of pCI/β-gal-transfected HeLa cells. Our laboratory (3) has described previously the identification of clones of HeLa cells expressing either the fluorescent tag EGFP or the fusion construct EGFP/PI10. Analysis of their sensitivity to TNFα revealed that the clones expressing EGFP/PI10 were also more resistant to TNFα-induced cytotoxicity than those cells expressing EGFP alone (Fig. 1B). Plotting the log of the concentration of TNFα required for 50% cell death for the PI10- and EGFP/PI10-expressing clones against the log of the PI10 concentration expressed by a particular clone revealed a linear relationship (Fig. 1B inset). The PI10- and EGFP/PI10-transfected cells were also analyzed for their resistance to TNFα utilizing a tetrazolium salt-based assay that measures mitochondrial dehydrogenase as a marker for cell viability (7), and results similar to those obtained with the crystal violet staining assay were obtained (data not shown). These data indicate that PI10 expression reduces a cell’s sensitivity to TNFα/cyclohexami-
mide-induced cell death and the concentrations of PI10 required for this effect are comparable with those previously observed using cells transfected with PAI-2 (7).

Multiple Cytosolic Species of PI10 Exist in Transfected HeLa Cells—To investigate potential mechanisms by which PI10 might protect cells against TNFα-induced cytotoxicity, it was necessary to optimize an assay that detects trace levels of PI10 associated with another protein. Previous studies in our laboratory with EGFP/PI10 (3) have revealed that the EGFP moiety is not only a practical fluorophore for monitoring the targeting of PI10 into the nucleus but also a useful tag for combined immunoprecipitation/immunoblotting experiments. Because our present TNFα-mediated cytotoxicity experiments with PI10 extend published observations obtained with cells transfected with the ov-serpin PAI-2 (6, 7), a molecule that is primarily localized to the cytoplasm (6, 7), we attempted to utilize the EGFP tag to immunologically enrich and characterize the forms of EGFP/PI10 both in the nucleus and in cytosol prepared from TNFα/cycloheximide- and CLLN-treated EGFP/PI10-transfected HeLa clone C2E2 was immunoprecipitated with either rabbit anti-GFP (lane 2), rabbit anti-PI10 (lane 3), or normal rabbit IgG (lane 4), and the immunoprecipitates were subjected to SDS-PAGE/immunoblotting with mAb anti-GFP. Lane 1 contains 1 ng of EGFP.

**FIG. 2** Immunoblotting analysis of EGFP in cells transfected with EGFP fusion constructs. A, nuclear extracts (lanes 1, 2, 5, and 6; 200 μg/lane) and cytosol (lanes 3, 4, 7, and 8; 200 μg/lane) from EGFP/PI10-transfected clone 2C2 (lanes 1–4) and EGFP-transfected clone D5 (lanes 5–8) were subjected to immunoprecipitation with rabbit anti-GFP (lanes 1, 3, 5, and 7) or normal rabbit IgG (lanes 2, 4, 6, and 8). Immunoprecipitated material was subjected to SDS-PAGE and immunoblotting using mAb anti-GFP. Lane 9 contains 2.5 ng of purified GFP and a 15-min exposure is shown. B, cytosol samples (200 μg/lane) prepared from HeLa cells transfected with EGFP/PI10 (lane 2, clone 2C2; lane 3, clone C2E2; lane 4, clone C2H8; lane 5, clone C2I; lane 6, clone C4; lane 7, clone B2) were subjected to immunoprecipitation/immunoblotting for GFP. Lane 1 contains 1 ng of purified GFP, and a 15-min exposure is shown. C, HeLa cells were incubated in media supplemented with LipofectAMINE and either no vector (lane 1), pEGFP (lane 2), pEGFP/PAI-2 (lane 3), or pEGFP/PI10 (lane 4). Two days later, cytosol (200 μg/lane) was prepared and subjected to immunoprecipitation with rabbit anti-GFP followed by SDS-PAGE/immunoblotting as described previously. Lane 5 contains 2.5 ng of GFP and a 15-min exposure is shown.

**FIG. 3** Characteristics of high $M_r$ forms of EGFP/PI10 during TNFα/cycloheximide-induced cell death. A, EGFP/PI10-transfected HeLa clone 2C2 (lanes 2–5) and EGFP-transfected HeLa clone D5 (lanes 6–9) were incubated in growth medium in the absence (lanes 2, 4, 6, and 8) or presence (lane 3, 5, 7, and 9) of the proteasome inhibitor CLRN (50 μM). After 30 min, the cells were treated with cycloheximide in the absence (lanes 2, 3, 6, and 7) or presence of TNF (10 ng/ml; lanes 4, 5, 8, and 9) for 4 h. Cells were harvested, washed, homogenized, and clarified by centrifugation. Cytosol (100 μg/lane) was immunoprecipitated with rabbit anti-EGFP, subjected to SDS-PAGE, followed by immunoblotting with mAb anti-GFP. Lane 1 contains 1 ng of EGFP. B, cytosol (200 μg/lane) prepared from TNFα/cycloheximide- and CLRN-treated EGFP/PI10-transfected HeLa clone C2E2 was immunoprecipitated with rabbit anti-EGFP and treated with 2% SDS in the presence of either PBS (lane 2) or 1.5 M ammonium hydroxide (lane 3) for 20 min at 22°C. Samples were neutralized by dialysis against PBS containing 0.2% SDS and subjected to SDS-PAGE/immunoblotting with mAb anti-GFP. Lane 1 contains 1 ng of EGFP. C, cytosol (150 μg/lane) prepared from TNFα/cycloheximide- and CLRN-treated EGFP/PI10-transfected HeLa clone C2H8 was immunoprecipitated with either rabbit anti-GFP (lane 2), rabbit anti-PI10 (lane 3), or normal rabbit IgG (lane 4), and the immunoprecipitates were subjected to SDS-PAGE/immunoblotting with mAb anti-GFP. Lane 1 contains 1 ng of EGFP.

combined immunoprecipitation/immunoblotting experiments. Because our present TNFα-mediated cytotoxicity experiments with PI10 extend published observations obtained with cells transfected with the ov-serpin PAI-2 (6, 7), a molecule that is primarily localized to the cytoplasm (6, 7), we attempted to utilize the EGFP tag to immunologically enrich and characterize the forms of PI10 both in the nucleus and in cytosol prepared from the transfected cells. As reported previously (3), rabbit anti-GFP is able to immunoprecipitate a $M_r$ 80,000 form of EGFP/PI10 from the nuclei isolated from EGFP/PI10-transfected HeLa cells (Fig. 2A, lane 1). Bands at $M_r$ 55,000 are also present in samples immunoprecipitated with either rabbit anti-GFP (lanes 1, 3, 5, and 7) or rabbit IgG (lanes 2, 4, 6, and 8) and are caused by the binding of the immunoblotting detection reagents to the electrophoresed rabbit IgG-heavy chains (3). Prolonged exposures are shown in Fig. 2 to permit the detection of trace species of EGFP/PI10 (e.g. $M_r$ 30,000 bands in Fig. 2A, lane 1). These small molecular species may represent cleavage...
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TABLE I

| Clone         | M, 210,000 EGFP-immunoreactive material | ng/mg cytosolic protein |
|---------------|----------------------------------------|-------------------------|
|               | Cycloheximide alone | Cycloheximide + CLLN | Cycloheximide + TNFα | Cycloheximide + TNFα + CLLN |
| GFP/PI10 C2C  | 0.76 ± 0.17         | 2.28 ± 0.34           | 0.98 ± 0.38          | 7.21 ± 0.92                  |
| GFP/PI10 C2E2 | 1.92 ± 0.31         | 3.97 ± 0.55           | 2.23 ± 0.47          | 13.48 ± 1.73                 |
| GFP/PI10 C2H8 | 0.28 ± 0.09         | 0.85 ± 0.21           | 0.31 ± 0.11          | 2.57 ± 0.33                  |

Quantitative immunoblotting analysis of the high Mr forms of EGFP/PI10 during TNFα/cycloheximide-induced cell death

GFP/PI10-transfected HeLa clones were treated with the indicated reagents. Cytosol was prepared, subjected to immunoprecipitation, SDS-PAGE, and quantitative immunoblotting as described under “Materials and Methods.”

The product of EGFP/PI10. In comparison, analysis of cytosol preparations revealed that EGFP/PI10 was not only present as a Mr 80,000 species but also as a series of high Mr bands that co-migrated with the 210-kDa marker (Fig. 2A, immunoprecipitation with anti-GFP in lane 3 versus normal IgG in lane 4). These high Mr species of EGFP were not present in the nuclei or cytosol isolated from cells transfected with EGFP alone (Fig. 2A, lanes 5–8), thus indicating that these species of EGFP were dependent upon the presence of PI10 in the fusion construct. To further delineate the spectrum of EGFP/PI10 species present within the transfectants, we covalently coupled rabbit anti-EGFP to Sepharose beads and utilized these reagents to immunoprecipitate cytosol of several HeLa clones. Fig. 2B shows data from six EGFP/PI10-transfected HeLa clones (lanes 2–7) that demonstrates (i) the major species of EGFP/PI10 in the clones migrates at Mr 80,000, (ii) Mr 210,000 species of EGFP/PI10 are present in all 6 cytosolic samples, and (iii) degradative species of EGFP/PI10 vary between clones.

Because polymers of PAI-2 can be detected by electrophoretically separating cell extracts under nondenaturing conditions (11), the possibility existed that the high Mr species of PI10 in the fusion construct may be covalently associating with the high Mr species of EGFP. To further delineate the spectrum of high Mr forms of EGFP/PI10 during TNFα/cycloheximide-induced cell death results in a 7–9-fold increase in the Mr 210,000 EGFP/PI10 cytosolic species. High Mr species of EGFP was not detected in the cytosol of EGFP-transfected cells under any condition, including treatment with TNFα/cycloheximide ± CLLN (Fig. 3A, lanes 6–9).

Although the high Mr forms of EGFP/PI10 suggest the activation of a cytosolic protease(s) and its subsequent formation of an SDS-stable complex with PI10, the related ov-serpin PAI-2 has been observed to form SDS-stable interactions with proteins utilizing amino acids other than the reactive site residue. More specifically, PAI-2 can be cross-linked to vitronectin and other structural proteins utilizing a series of glutamine residues in the loop connecting helix C and D (16). Therefore, we decided to further characterize the high Mr forms of EGFP/PI10 by examining their sensitivity to conditions known to dissociate complexes between several classical serpins and their respective proteases. For example, 1.5 M ammonium hydroxide in the presence of SDS is able to dissociate complexes formed between either anti-thrombin (17), α2-anti-plasmin (18), or PAI-1 (19) and their target proteases. Fig. 3B shows a representative experiment in which EGFP/PI10-transfected HeLa clone C2E2 were treated with TNFα/cycloheximide in the presence of the proteasome inhibitor CLLN to induce a spectrum of high Mr species of cytosolic EGFP/PI10. These forms were immunoprecipitated with anti-GFP, and the SDS-solubilized material was treated with either PBS (Fig. 3B, lane 2) or ammonium hydroxide (lane 3). Following neutralization and SDS-PAGE/immunoblotting with MAB anti-GFP, immunoreactive species of EGFP/PI10 above Mr 100,000 were detected only in samples treated with PBS/SDS (lane 2). In contrast, EGFP/PI10-immunoreactive species greater than Mr 100,000 were not detected in samples treated with ammonium hydroxide/SDS (lane 3). The high Mr forms of EGFP present in the immunoprecipitates of EGFP/PI10-transfected HeLa clone 2C2 and clone C2H8 (Fig. 2B, lanes 2 and 4, respectively) were also dissociated by ammonium hydroxide/SDS treatment (data not shown). These observations implicate the existence of a complex between EGFP/PI10 and one or more proteases; however, the predominant use of immunologic reagents against GFP led us to document the presence of PI10 in these high Mr species. Therefore, rabbit anti-PI10 was coupled to Sepharose beads and used in conjunction with Sepharose-anti-GFP and Sepharose-IgG normal beads to immunoprecipitate cytosol of CLLN- and TNFα/cycloheximide-treated HeLa clone C2H8. Fig. 3C
shows a representative experiment demonstrating that both Sepharose anti-GFP (lane 2) and Sepharose anti-PI10 (lane 3) are able to immunoprecipitate Mr 80,000 EGFP/PI10, as well as high Mr forms of the construct. Similar immunoprecipitation/immunoblotting results were obtained using the cytosol of clones C2C and C2E2 (data not shown).

These studies indicate that the transfer of the gene encoding PI10 into HeLa cells results in recombinant protein expression that correlates with both the formation of high Mr PI10-containing complexes and a reduced cytotoxicity to TNFα. Thus, cytoprotective activity against TNFα is not restricted to PAI-2, which raises the potential of a common function for other ov-serpins containing arginine as the P1 reactive site residue. Our observed increase in the Mr 210,000 PI10-containing complexes during TNFα/cycloheximide-induced cytotoxicity suggests that a serine protease is activated during this process and consistent with the concept that multiple proteases play a role in the death of cells (4, 5). The inability of numerous researchers to detect protease-PAI-2 complexes within either normal or transfected cells (11–13) would suggest that the target proteins for PAI-2 and PI10 are different; however, an alternative explanation can be proposed based upon the unique ability of certain serpins to react against the pro- or single-chain form of proteases. A relevant example is the ability of PAI-1, but not PAI-2, to generate high Mr SDS-stable complexes with single-chain u-PA in comparison to the rapid inactivation of two-chain u-PA by both PAI-1 and PAI-2 (20). Is it possible that an analogous situation exists concerning the reactivity of PI10 and PAI-2 for various single- and two-chain proteases within HeLa cells? The ability of PI10 to form SDS-stable intermediates with a set of cytoplasmic proteins in both resting and TNFα-stimulated cells provides researchers a useful probe for both identifying these molecules and clarifying their role in the cell death process.

REFERENCES

1. Riewald, M., and Schleef, R. R. (1995) J. Biol. Chem. 270, 26754–26757
2. Riewald, M., Chuang, T. L., Neubauer, A., and Schleef, R. R. (1998) Blood 91, 1256–1262
3. Chuang, T. L., and Schleef, R. R. (1999) J. Biol. Chem. 274, 11194–11198
4. Kumar, S., and Harvey, N. L. (1995) FEBS Lett. 375, 169–173
5. Patel, T., Gores, G. J., and Kaufmann, S. H. (1996) FASEB J. 10, 587–597
6. Kumar, S., and Baglioni, C. (1991) J. Biol. Chem. 266, 20960–20964
7. Dickinson, J. L., Bates, E. J., Ferrante, A., and Antalis, T. M. (1995) J. Biol. Chem. 270, 27894–27904
8. Schleef, R. R., Higgins, D. L., Pillemier, E., and Levitt, J. J. (1989) J. Clin. Invest. 83, 1747–1752
9. Riewald, M., and Schleef, R. R. (1996) J. Biol. Chem. 271, 14526–14532
10. Riewald, M., Morgenstern, K. A., and Schleef, R. R. (1996) J. Biol. Chem. 271, 7160–7167
11. Ny, T., and Mikus, P. (1997) Adv. Exp. Med. Biol. 425, 123–130
12. Bachmann, P. (1995) Thromb. Haemostasis 74, 175–179
13. Kruthof, E. K. O., Baker, M. S., and Bunn, C. L. (1995) Blood 86, 4007–4024
14. Korntizer, D., and Ciechanover, A. (2000) J. Cell Phy. 182, 1–11
15. Haas, M., Page, S., Page, M., Neumann, F.-J., Marx, N., Adam, M., Veigler-Heitbrock, H. W. N. D., and Brand, K. (1998) J. Leukoc. Biol. 63, 395–404
16. Jensen, P. H., Schuler, E., Woodrow, G., Richardson, M., Goss, N., Hjrup, P., Petersen, T. E., and Rasmussen, L. K. (1994) J. Biol. Chem. 269, 15394–15398
17. Danielsen, A., and Bjork, I. (1982) Biochem. J. 207, 21–28
18. Wiman, B., and Collen, D. (1979) J. Biol. Chem. 254, 9291–9297
19. Philips, M., Juul, A. G., and Thorsen, M. (1984) Biochem. Biophys. Acta 802, 99–110
20. Manchanda, N., and Schwartz, B. S. (1995) J. Biol. Chem. 270, 20032–20035
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