Purification, Molecular Cloning, and Characterization of TRP32, a Novel Thioredoxin-related Mammalian Protein of 32 kDa*

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We purified a protein of 32 kDa from human thymoma HPB-ALL cells that was co-purified with a catalytic fragment of MST (mammalian STE-20-like), a kinase of the STE20 family, which is proteolytically activated by caspase in apoptosis (Lee, K.-K., Murakawa, M., Nishida, E., Tsubuki, S., Kawashima, S., Sakamaki, K., and Yonehara, S. (1998) Oncogene 16, in press). Molecular cloning of the gene encoding this 32-kDa protein (TRP32) reveals that it is a novel protein of 289 amino acid residues and contains an NH2-terminal thioredoxin domain with a conserved thioredoxin active site. The human and mouse TRP32 proteins have 99% homology, and the thioredoxin domains are completely identical. The thioredoxin domain of TRP32 has thioredoxin-like reducing activity, which can reduce the interchain disulfide bridges of insulin in vitro. However, the thioredoxin domain of TRP32 is more sensitive to oxidation than human thioredoxin. Northern blot analysis showed that TRP32 is expressed in all human tissues. Expression of TRP32 was also confirmed in all mammalian cell lines tested by Western blot analysis using anti-TRP32 monoclonal antibody. Subcellular fractionation and immunostaining analysis showed TRP32 is cytoplasmic protein. These findings suggest that TRP32 is a novel cytoplasmic regulator of the redox state in higher eukaryotes.

Thioredoxin, a small protein of approximately 100 amino acid residues, is ubiquitously present and is evolutionarily conserved from prokaryotes to higher eukaryotes, plants, and animals (1–3). It is characterized by an amino acid sequence of an active site, -Cys-Gly-Pro-Cys-, conserved throughout evolution. The active site of thioredoxin is localized in a protrusion of its three-dimensional structure (4), and the two cysteine residues provide the sulfhydryl groups involved in the thioredoxin-dependent reducing activity. Thioredoxin exists in either a reduced form or an oxidized form. Oxidized thioredoxin, Trx-S2, where the two cysteine residues are linked by an intramolecular disulfide bond is reduced by flavoenzyme thioredoxin reductase and NADPH (2). Reduced thioredoxin, Trx-(SH)2, contains two thiol groups and can efficiently catalyze the reduction of many exposed disulfides. Thus, thioredoxin participates in various redox reactions via the reversible oxidation and reduction of the two cysteine residues in the active center.

In Escherichia coli, thioredoxin was first identified as an electron donor for ribonucleotide reductase, the enzyme that reduces ribonucleotides to deoxyribonucleotides for DNA synthesis and repair (5). E. coli thioredoxin can also function as a hydrogen donor for 3':phosphoadenosine 5':phosphosulfate reductase in the sulfate assimilation pathway as well as methionine sulfoxide reductase (6, 7). Apart from these functions, E. coli thioredoxin functions as the essential subunit of T7 DNA polymerase and in the maturation of filamentous bacteriophages M13 and f1 (8–10). In eukaryotic cells, thioredoxin has been implicated in a wide variety of biochemical and biological functions. It can function as a hydrogen donor, similar to the prokaryotic thioredoxin (2). In addition, thioredoxin can facilitate refolding of disulfide-containing proteins (11) and modulate the activity of some transcription factors such as NF-kB and AP-1 (12, 13). Thioredoxin is an efficient antioxidant, which can reduce hydrogen peroxide (14), scavenge free radicals (15), and protect cells against oxidative stress (16). Another role of thioredoxin is the growth stimulation of human T cells. Adult T cell leukemia-derived factor, which augments the expression of interleukin-2 receptor, was found to be identical to human thioredoxin (17). Furthermore, thioredoxin reportedly inhibits the expression of human immunodeficiency virus in macrophages (18).

A number of eukaryotic proteins are known to contain domains evolutionarily related to thioredoxin, and most of them appear to belong to the protein-disulfide isomerase (PDI) family, endoplasmic reticulum (ER) enzymes that catalyze the rearrangement of disulfide bonds in various proteins (19–21). All members of the PDI family, which contain two or three Trx domains per one PDI molecule (22), are primarily retained within the ER lumen by the recognition system for their carboxyl-terminal tetrapeptide motif, (K/H)DEL. DsbA, the bacterial functional equivalent of PDI, also contains a Trx domain and acts as a thiol-disulfide interchange protein that allows disulfide bond formation in some periplasmic proteins (23).

Here we report on the biochemical purification and cloning of a novel protein, designated TRP32, which is a 32-kDa protein with an N-terminal Trx domain. TRP32 is ubiquitously expressed in human tissues and mammalian cell lines and localized in the cytoplasm.

* This work was supported in part by research grants from the Ministry of Education, Science and Culture of Japan. 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.

The nucleotide sequence(s) reported in this paper has been submitted to the GenBankTM/EBI Data Bank with accession number(s) AF052659 and AF052660 (human and mouse TRP32, respectively).

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1 The abbreviations used are: PDI, protein-disulfide isomerase; Trx, thioredoxin; ER, endoplasmic reticulum; FCS, fetal calf serum; PBS, phosphate-buffered saline; DTT, dithiothreitol; PAGE, polyacrylamide gel electrophoresis; PCR, polymerase chain reaction; GST, glutathione S-transferase; kb, kilobase pair(s); MST, mammalian STE-20-like.
**Cloning of a Novel Thioredoxin-related Mammalian Protein**

**EXPERIMENTAL PROCEDURES**

### Purification of a 32-kDa Protein—Human thymoma-derived cell line, HB-PI-ALL was cultured to 5 × 10⁶ cells/ml in 20 liters of RPMI supplemented with 10% bovine fetal calf serum (FCS), 20 μM HEPE (pH 7.3), 50 μM β-mercaptoethanol, 50 units/ml penicillin, and 50 μg/ml streptomycin. Cells were concentrated to a density of 2.5 × 10⁶ cells/ml and stimulated with 1 μg/ml anti-Fas monoclonal antibody (CH-11) (24) for 2 h. After washing once with cold PBS, cells were harvested and frozen in liquid nitrogen. Cell pellets were thawed on ice and suspended with 90 ml of 20 mM Tris-HCl (pH 7.5) containing 10% glycerol, 50 mM NaF, 10 mM β-glycerol phosphate, 2 mM EDTA, 1 mM DTT, 1 mM vanadate, 1 mM phenylmethylsulfonyl fluoride, and 5 μl/ml aprotinin (buffer IA), and cells were homogenized in a Dounce homogenizer with 10 strokes.

After centrifugation for 1 h at 100,000 × g at 4°C, the supernatant was loaded onto Sephacryl FF (20 ml, Amersham Pharmacia Biotech). The column was eluted with a 250-ml gradient of 10–500 mM potassium phosphate (buffer QA) (25). Northern Blot Analysis—Adult and fetal human multiple-tissue Northern blots (CLONTECH) were hybridized in accordance with manufacturer's instructions. A probe of 723 base pairs encoding the N-terminal 34 amino acid residues of TRP32 was generated by PCR, random-radiolabeled with [α-32P]dCTP, and spin column-purified (Amersham Pharmacia Biotech).

### Purification of Bacterially Expressed Proteins—The GST fusion proteins and His-tagged proteins were expressed in E. coli strain XL1blue. The expression of recombinant proteins was induced with 0.1 mM isopropyl-β-D-galactopyranoside for 5 h at 37°C at a cell density of A₅₆₂₀ 1.0. GST fusion proteins were bound to glutathione-Sepharose beads and washed once with glutathione (10 mM) buffer (26). The His-tagged proteins were loaded onto a His-Trap column (Amersham Pharmacia Biotech) and eluted with a 5–500 mM imidazole gradient.

### Western Blot Analysis—Cellular total proteins (30 μg) were separated by SDS-PAGE and transferred to polyvinylidene difluoride membrane (Millipore). The membrane was blocked in TBST (20 mM Tris-HCl (pH 7.5) containing 10% FCS until confluent, and then the hybridoma cells were moved to ASP104 medium without FCS. Cells were transfected with lipofectAMINE (Life Technologies, Inc.) in accordance with the manufacturer's instructions.

### Insulin Dismutase Reduction Assay—E. coli thioredoxin (MBI Fermentas), His-tagged human thioredoxin, and His-tagged TRP32 were compared for the reducing activity of insulin dismutase bonds as described previously (29) with slight modifications. A reduced form of the proteins was prepared by incubating with 2 mM DTT, and oxidized proteins were prepared by incubation with 10 mM Na₂SeO₃ at 4°C overnight. Then excess amounts of DTT or Na₂SeO₃ were removed by gel chromatography on a NAP-5 column (Amersham Pharmacia Biotech) with ice-cold nitrogen equilibrated 50% methanol and 100 mg/ml Na₂CO₃. Lymphocytes from the human HPB-ALL cells were maintained in Dulbecco’s modified Eagle’s medium supplemented with 10% heat-inactivated FCS and hypoxanthine/aminopterin/thymidine for the selection of fused cells. Two cloning procedures by serial dilution were carried out after the selection, and finally all of the wells containing single clones became positive for anti-TRP32 antibody production. Cloned hybridoma cell lines were grown in ASP104 medium with 10% FCS until confluent, and then the hybridoma cells were moved to ASP104 medium without FCS. Culture supernatant without FCS was loaded onto protein G-Sepharose column and eluted with 50 mM glycine-HCl (pH 2.3). The eluate was immediately neutralized with 1 M Tris-HCl (pH 9.0) and dialyzed against 20 mM Tris-HCl (pH 7.5).

### Northern Blot Analysis—Northern blots (CLONTECH) were hybridized in accordance with manufacturer's instructions. A probe of 723 base pairs encoding the N-terminal 34 amino acid residues of TRP32 was generated by PCR, random-radiolabeled with [α-32P]dCTP, and spin column-purified (Amersham Pharmacia Biotech).
tibody (Cappel), was used at a 1:500 dilution for 45 min. Cells were washed with PBS, incubated with Hoechst 33342 (5 μg/ml), and washed again. Slides were analyzed using a fluorescence microscope.

RESULTS

cDNA Cloning of TRP32—Previously, we reported that a histone kinase with a molecular mass of 34 kDa is activated in Fas-mediated apoptosis, and this kinase was identified as catalytic fragment of MST, a STE20-related serine/threonine kinase (30). In the course of the purification of this kinase, we purified a protein of 32 kDa that was co-eluted with a catalytic fragment of MST to the final chromatographic purification step (see below). To clarify the molecular identity of this protein, we sequenced the polypeptides that were derived from proteolytic digestion with lysylendopeptidase. Eight peptide sequences were revealed (Fig. 1), and since a public data base search showed no homology to any known proteins, we tried cDNA cloning of this protein, designated TRP32. Degenerate oligonucleotides corresponding to peptides 1 and 2 (Fig. 1) were used for reverse transcriptase PCR using mRNA from HPB-ALL cells as a template. One cDNA fragment of 290 nucleotides was obtained and sequenced. The data base search showed that this cDNA fragment encoded a novel protein with a Trx-like domain. Using the amplified cDNA fragments as probes, we screened the human HPB-ALL cDNA library. A clone of 1.3 kb was isolated and contained a perfect Kozak consensus sequence (AGGATGG) and a stop codon upstream from the putative start codon at the 5′ side (Fig. 1). The putative polyadenylation site and poly(A) tail were also identified at the 3′ side. The 1.3-kb cDNA contained an open reading frame of 289 amino acid residues with a predicted molecular mass of 32 kDa that coincided well with the molecular weight of the purified TRP32 estimated by SDS-PAGE (see below). All eight peptide sequences obtained from the purified protein were confirmed in the open reading frame of TRP32. We also cloned a mouse
homologue of human TRP32. Mouse TRP32 showed 99% homology to human TRP32, and only three amino acids were substituted.

Similarity of TRP32 to Other Thioredoxin Domain-containing Proteins—Data base searches using the BLAST program showed that the predicted amino acid sequence of the TRP32 gene was a novel protein and that the N-terminal 100 amino acid residues shared significant similarities with thioredoxin, an evolutionarily conserved ubiquitous protein that participates in various redox reactions (Fig. 2A). The N-terminal domain of TRP32 has 43% identity and 56% similarity to human thioredoxin and has 26–29% identity and 40–43% similarity to the Trx domains of other Trx-related proteins (Fig. 2B). All thioredoxin molecules from prokaryotes to higher eukaryotes have the conserved active site sequence Cys-Gly-Pro-Cys, and in particular, two active site cysteines are preserved in all proteins containing the functional Trx domain. The Trx-like domain of TRP32 also has the active site sequence Cys-Gly-Pro-Cys. Taken together, the N-terminal 100 residues of TRP32 are closely related to thioredoxin in the primary structure.

The C-terminal 190 amino acids of TRP32, however, had no similarity to any proteins in the public data bases. The C-terminal domain was rich in acidic amino acids, and the calculated pI was 4.3, similar to the theoretical and experimentally determined pI of TRP32 (see below), although it was more acidic than the N-terminal Trx-like domain (pI 7.1). The C-terminal region did not contain any predicted peptide sequences for subcellular localization or extended hydrophobic regions for secretion or membrane spanning.

Expression of mRNA and Protein—Northern blot analysis using poly(A) RNA from multiple human tissues demonstrated that the TRP32 transcript was ubiquitously expressed (Fig. 3). A specific 1.4-kb mRNA was expressed at the highest levels in the heart and skeletal muscle. We prepared anti-TRP32 monoclonal antibodies and performed immunoblot analysis using one clone, LE4, that recognized the C-terminal 190 amino acids. LE4 recognized a protein of 32 kDa in all human (HPB-ALL, Jurkat, 293, KB), mouse (NIH3T3, Balb/c 3T3), and monkey (COS-7) cells examined (Fig. 4). A band with the same molecular weight was also detected by LE4 in the highly purified TRP32 fraction. In addition, recombinant HA-tagged TRP32 (HA-TRP32), with an 11-amino acid extension at the N terminus, could be detected as a slower migrating band on Western blots with LE4. These results show that LE4 specifically recognizes TRP32. Taken together, both the TRP32 transcript and protein are ubiquitously expressed in human tissues and various mammalian cell lines.

Analysis of Purified TRP32—Final chromatographic fractions from the Superdex 75 gel filtration were analyzed by SDS-PAGE. A doublet with a molecular mass of 32 kDa, which was identified as TRP32 by peptide sequencing, was eluted at the expected elution volume from its molecular weight (Fig. 5A). The anti-TRP32 antibody, LE4, also specifically recognized doublet bands of 32 kDa, proving the LE4-reactive proteins to be TRP32 (Fig. 5B). The purified TRP32 might be modified or cleaved during chromatographic procedures because TRP32 was detected as a single band in the total cell lysates (Fig. 4). Only a single band of TRP32 was also observed in apoptosis-induced HPB-ALL cells. The elution pattern of TRP32 by gel filtration chromatography was nearly identical to that of the kinase-active catalytic fragment of MST (Fig. 5, C and D), confirming the co-purification of these two proteins. TRP32 and the catalytic fragment of MST were superimposable even in the two-dimensional electrophoresis analysis (30) with a molecular mass of 32–34 kDa and isoelectric point (pI) of 5.2 (Fig. 5E). The pI of purified TRP32 was slightly higher than the theoretical value of 4.6.

Reductase Activity of TRP32—To investigate the Trx-like
reducing activity of TRP32, we expressed recombinant TRP32 and human thioredoxin as His-tagged forms (His-TRP32 and His-Trx) in E. coli. A truncated TRP32 (His-TRP32N) consisting of N-terminal Trx-like 107 amino acid residues was also prepared. In addition, Cys→Ser mutants, His-TRP32(C34S,C37S) and His-TRP32N(C34S,C37S), in which the two cysteines (Cys34 and Cys37) homologous to those in the thioredoxin active site were mutated to serines, were generated from His-TRP32 and His-TRP32N, respectively. The expressed recombinant proteins were purified by His-Trap column chromatography. His-TRP32N showed reducing activity for the insulin disulfide bonds with kinetics slower than that of His-Trx (Fig. 6A) but faster than that of purified E. coli thioredoxin. His-TRP32N(C34S,C37S) completely failed to reduce insulin, indicating that the N-terminal Trx-like domain of TRP32 is functionally related to thioredoxin. However, full-length His-TRP32 failed to reduce insulin, suggesting that the C-terminal region may regulate the activity of the N-terminal Trx domain or that bacterially expressed His-TRP32 may not be folded to its native structure. We tested the effect of the C-terminal region on the N-terminal reducing activity by mixing His-TRP32C (residue 107–289). However, His-TRP32C did not inhibit the reducing activity of His-TRP32N even in the presence of a 5-fold molar excess. In addition, direct interaction between the N-terminal and the C-terminal domains of TRP32 could not be detected either in vitro or in vivo.2 We studied the effect of oxidation on the reducing activity of the His-TRP32N (Fig. 6B). The reducing activity of oxidized His-TRP32N had not recovered after 2 h of incubation with DTT, although His-Trx rapidly recovered the reducing activity within 5 min in the presence of DTT, suggesting that reduction to reductase-active His-TRP32N by DTT is a time-consuming and rate-limiting process. These results imply that the Trx domain of TRP32 is functionally related to thioredoxin but not identical.

Subcellular Distribution of TRP32—To investigate the subcellular distribution of endogenous TRP32, we prepared subcellular fractions from HPB-ALL cells. The endogenous TRP32 was detected in the cytoplasmic fraction.2 The cytoplasmic distribution of TRP32 did not change when HPB-ALL cells underwent apoptosis by treatment with the anti-Fas monoclonal antibody, CH-11.

NIH3T3 cells were transiently transfected with HA-TRP32, and the localization of expressed protein was visualized using the anti-HA monoclonal antibody. HA-TRP32 was localized predominantly in the cytoplasm, confirming the result of the subcellular fractionation.2

**DISCUSSION**

We have purified a protein with a molecular mass of 32 kDa, which was co-purified with the catalytic fragment of protein kinase MST. cDNA cloning of the gene encoding this protein, TRP32 (thioredoxin-related protein of 32 kDa), shows that TRP32 is a novel protein containing one Trx domain. Human and mouse TRP32 proteins are 99% identical, and in particular, the Trx domains are completely homologous. We also identified a Drosophila expressed sequence tag (GenBank™ accession number AA264961) whose predicted N-terminal 100 residues are similar to the Trx domain of TRP32 (48% identity). This clone may be a Drosophila homologue of TRP32 because the adjacent region to the Trx domain (100–150 residues) shows high homology to the C-terminal domain of TRP32, which has no similarity to any proteins in the public data bases. However, we could not identify any putative TRP32 homologue in E. coli or yeast genome sequences. Thus, TRP32 may be evolutionarily conserved in multicellular eukaryotes.

TRP32 seems to be closely related to thioredoxin. The Trx
domain of TRP32 is most similar to thioredoxin in mammalian Trx-related proteins. TRP32 is widely expressed in human tissues, and expression of TRP32 protein has been confirmed in various mammalian cell lines (Figs. 3 and 4). The recombinant Trx domain of TRP32 (His-TRP32N) has reducing activity comparable with human thioredoxin (Fig. 6). TRP32 is localized in the cytoplasm. TRP32 might have acquired specialized functions in multicellular eukaryotes during evolution from thioredoxin, which is conserved from prokaryotes to animals.

Despite the high similarity of the two proteins, however, some functional and structural differences were observed. The Trx domain of TRP32 is more sensitive to oxidation than thioredoxin, and full-length TRP32 has no reducing activity (Fig. 6). Mammalian thioredoxin contains other conserved cysteine residues in addition to the two cysteine residues in the active site. The human thioredoxin contains three such extra cysteine residues (Fig. 2A). These noncatalytic cysteine residues, Cys62, Cys69, and Cys73, in human thioredoxin can reportedly undergo oxidation, which leads to inactivation and dimerization of thioredoxin (31). In particular, Cys73, located on the surface close to the active site, is reportedly linked to the redox regulation of human thioredoxin (32, 33). In contrast, the Trx domain of TRP32 has only one extra cysteine residue, Cys64, corresponding to the Cys62 of human thioredoxin. Cys73 of human thioredoxin is replaced by Ala in TRP32, which is also changed to Gly74 in E. coli thioredoxin. Thus, the reducing activity of TRP32 may be differently regulated from mammalian thioredoxin, and TRP32 is functionally different from thioredoxin, since the C-terminal region of TRP32 may regulate the reducing activity (Fig. 6). The C-terminal region of human TRP32 does not have homology to other proteins but is conserved in mouse and probably Drosophila, suggesting its functional importance. The role of the C-terminal region of TRP32 should be elucidated.

The function of TRP32 may be also different from that of the PDI family because TRP32 has no homology to PDI except in the Trx domain. Furthermore, the Trx domain of TRP32 is significantly different from PDI. TRP32 has one Trx domain, but two or three Trx domains exist in the PDI family. In addition, TRP32 and mammalian thioredoxins have the conserved active site sequence, Cys-Gly-Pro-Cys, in which the third Pro is replaced by His in the PDI family (Fig. 2B). Pro→His-replaced thioredoxin reportedly shows increased reduction potential and can complement the PDI null mutant of Saccharomyces cerevisiae (34), implying that the Trx domain of TRP32 is biochemically and biologically different from that of PDI. Different localization of two proteins, PDI in ER and TRP32 in cytoplasm, also suggests different functions in the different environments.

Recently, two new mammalian Trx-related proteins have been reported (Table I). Trx2 (35), which consists of 166 amino acid residues with the active site sequences found in mammalian thioredoxin, has higher homology with the E. coli thioredoxin than with the known mammalian proteins. Trx2 has a mitochondrial translocation signal, and the mature protein is localized in mitochondria. Kurooka et al. (36) reported another Trx-related nuclear protein, nucleoredoxin. Nucleoredoxin has one Trx domain in the central region with a modified active site sequence, -Cys-Pro-Pro-Cys, but it can reduce insulin disulfide in vitro. The second Pro residue in the active site sequence is quite rare, and no other proteins with Pro in this residue have been reported. Nucleoredoxin is localized in the nucleus when overexpressed in NIH3T3 cells. TRP32 may be also functionally different from these two proteins. First, these proteins have no

![Reductase activity of Trx-like domain of TRP32](image)

**FIG. 6. Reductase activity of Trx-like domain of TRP32.** Thioredoxin and TRP32 were assayed for their ability to reduce the disulfide bonds of insulin. A, reducing activity was measured using reduced forms of recombinant His-tagged proteins. X, Trx (E. coli); ●, His-Trx (human); ○, His-TRP32; △, His-TRP32(C34S,C37S); ■, His-TRP32N; □, His-TRP32N(C34S,C37S). B, reduced and oxidized recombinant proteins, prepared as described under “Experimental Procedures,” were compared for their reducing activity. ○, oxidized His-Trx; △, oxidized His-TRP32N; □, oxidized His-TRP32N(C34S,C37S); ●, reduced His-Trx; ■, reduced TRP32N; ■, reduced TRP32N(C34S,C37S). Absorbance at 690 nm was measured at 5-min intervals with mixing. Similar results were obtained from two independent experiments.

| Trx-related protein | Molecular mass (kDa) | Localization | Evolutionary conservation | Functions |
|--------------------|---------------------|--------------|--------------------------|-----------|
| Trx                | 12                  | Cytoplasm    | All species              | Reduction of protein disulfide. Regulation of transcription factors, cell growth. |
| Trx2               | 12                  | Mitochondria | Rat                      | Unknown   |
| PDI                | 55                  | ER           | Yeast to human           | Folding catalyst. Disulfide formation and rearrangement by thiol/disulfide exchange. |
| Nucleoredoxin      | 48                  | Nucleus      | Mouse                    | Unknown   |
| TRP32              | 32                  | Cytoplasm    | Human, mouse             | Unknown   |
homologous regions except the Trx domain and show a low homology only at the sequences surrounding the active site of the Trx domain. Second, their subcellular localizations are completely different. Trx2 and nucleoredoxin are mitochondrial and nuclear protein, respectively, whereas TRP32 is a cytoplasmic protein. The restricted localization of these proteins including PDI may reflect their specific roles in different subcellular compartments.

One possible role of TRP32 may be the redox regulation of cytoplasmic proteins including transcription factors. The DNA binding activity of some transcription factors, including AP-1, c-Jun, and nuclear protein, respectively, whereas TRP32 is a cytoplasmic protein. The restricted localization of these proteins including PDI may reflect their specific roles in different subcellular compartments.

Another possible role of TRP32 may be the regulation of apoptosis. Reactive oxygen intermediates are implicated in apoptosis (32). Some process of apoptosis might be regulated by the cellular redox state, and TRP32 may regulate apoptosis through the control of the redox state. TRP32 was co-purified with the catalytic fragment of MST, which is proteolytically activated by caspase in apoptosis. The possibility cannot be excluded that TRP32 controls apoptosis by regulating the activity of MST and/or vice versa, although our preliminary experiment does not show co-immunoprecipitation of TRP32 and the catalytic fragment of MST. A study of TRP32 upon apoptosis is currently under way. Our cloning of a novel human thioredoxin-related protein may contribute to elucidating the redox state in cells, and the function of TRP32 should be further clarified.

Acknowledgments—We thank Shin-ichi Tsukumo for construction of the pME18S-HA vector. cDNA of human thioredoxin was generously provided by Dr. Junji Yodoi.

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