THE JOURNAL OF BIOLOGICAL CHEMISTRY
Vol. 276, No. 26, Issue of June 29, pp. 23719–23725, 2001
Printed in U.S.A.

A Functional Nuclear Localization Sequence in the C-terminal Domain of SHP-1*

Received for publication, March 30, 2001
Published, JBC Papers in Press, April 25, 2001, DOI 10.1074/jbc.M102846200

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The Src homology 2 domain-containing protein tyrosine phosphatases SHP-1 and SHP-2 play an important role in many intracellular signaling pathways. Both SHP-1 and SHP-2 have been shown to interact with a diverse range of cytosolic and membrane-bound signaling proteins. Generally, SHP-1 and SHP-2 perform opposing roles in signaling processes; SHP-1 acts as a negative regulator of transduction in hemopoietic cells, whereas SHP-2 acts as a positive regulator. Intriguingly, SHP-1 has been proposed to play a positive regulating role in nonhemopoietic cells, although the mechanisms for this are not understood. Here we show that green fluorescent protein-tagged SHP-1 is unexpectedly localized within the nucleus of transfected HEK293 cells. In contrast, the highly related SHP-2 protein is more abundant within the cytoplasm of transfected cells. In accordance with this, endogenous SHP-1 is localized within the nucleus of several other nonhemopoietic cell types, whereas SHP-2 is distributed throughout the cytoplasm. In contrast, SHP-1 is confined to the cytoplasm of hemopoietic cells, with very little nuclear SHP-1 evident. Using chimeric SHP proteins and mutagenesis studies, the nuclear localization signal of SHP-1 was identified within the C-terminal domain of SHP-1 and found to consist of a short cluster of basic amino acids (KRK). Although the KRK motif resembles half of a bipartite nuclear localization signal, it appears to function independently and is absolutely required for nuclear import. Our findings show that SHP-1 and SHP-2 are distinctly localized within nonhemopoietic cells, with the localization of SHP-1 differing dramatically between nonhemopoietic and hemopoietic cell lineages. This implies that SHP-1 nuclear import is a tightly regulated process and indicates that SHP-1 may possess novel nuclear targets.

Tyrosine phosphorylation plays a central role in the transduction of signals from the cell surface to the nucleus. Protein-tyrosine phosphatases (PTPs)[1] act both as positive and negative regulators of signal transduction (1). Numerous PTPs have been identified so far, including the extensively studied SHP-1 (previously known as PTP1C, SHPTP-1, SHP, and HCP) and SHP-2 (previously termed SHPTP-2, Syp, PTP2C, and PTP1D) intracellular proteins (1–3). SHP-1 is expressed at high levels in hemopoietic cells and moderately in many other cell types, especially malignant epithelial cells (4–6), whereas SHP-2 is more widely distributed. Both proteins are structurally very similar, comprising two tandem Src homology 2 domains at the N terminus, a single central catalytic domain, and a C-terminal domain (7, 8). The Src homology 2 domains recruit SHP-1 and SHP-2 to tyrosine-phosphorylated molecules, enabling dephosphorylation to be performed by the catalytic domain (9). Considering the conserved nature of SHP-1 and SHP-2, it is perplexing that when integrated into signaling pathways, both proteins perform opposing roles. Generally, SHP-1 acts as a negative regulator of signal transduction, terminating signals from a diverse range of signaling molecules, including the epidermal growth factor receptor (10), interleukin 3 receptor (11), c-kit (12), colony-stimulating factor 1 receptor (13), B- and T-cell antigen receptors (14, 15), and the receptor-associated Janus-activated kinases (16–18). In contrast, SHP-2 plays a positive role in many signaling systems and can act as an adapter protein, linking tyrosine kinases and Grb2 to activate the mitogen-activated protein kinase pathway (19, 20).

Recent studies show that the role of SHP-1 depends on the cell type (21, 22). SHP-1 has a positive effect on mitogenic signaling in several nonhemopoietic cell types, which concurs with its overexpression in certain tumor cells. Overexpression of a catalytically inactive mutant of SHP-1 in HEK293 cells strongly suppresses mitogen-activated pathways and results in decreased cell growth, DNA synthesis, and the transcription of early response genes (21). Furthermore, transfection of HeLa cells with inactive SHP-1 reduces the signal transducer and activator of transcription (STAT)-DNA binding induced by interferon γ and epidermal growth factor (22). As yet, the molecular basis for the apparent opposite effects of SHP-1 in different cell systems has yet to be defined, although the phosphatase domain appears to be critical.

It is widely assumed that SHP-1 and SHP-2 are cytoplasmic proteins and have similar intracellular distributions. In this paper we demonstrate a novel nuclear localization for SHP-1 in nonhemopoietic cells, whereas SHP-2 is distributed throughout the cytoplasm. We also show that the nuclear import of SHP-1 is dependent on a short region of basic amino acid residues in the C-terminal domain that resembles half of a bipartite nuclear localization sequence. In addition, we show that SHP-1 localization differs between nonhemopoietic and hemopoietic cells, with the SHP-1 protein being present almost entirely within the cytoplasm of hemopoietic cell lines. These results have implications regarding the function of SHP-1 in nonhemopoietic cells.

EXPERIMENTAL PROCEDURES

Cell Culture and Transfection—All cell lines were obtained from the American Type Culture Collection and maintained at 37 °C in a humidified 5% CO_2 incubator. HEK293, HeLa, and A549 cells were grown in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum, 2 mM L-glutamine, 100 units/ml penicillin, and 100 μg of

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[1] The abbreviations used are: PTP, protein-tyrosine phosphatase; GFP, green fluorescent protein; PBS, phosphate-buffered saline; NGS, normal goat serum; NLS, nuclear localization signal; STAT, signal transducer and activator of transcription.

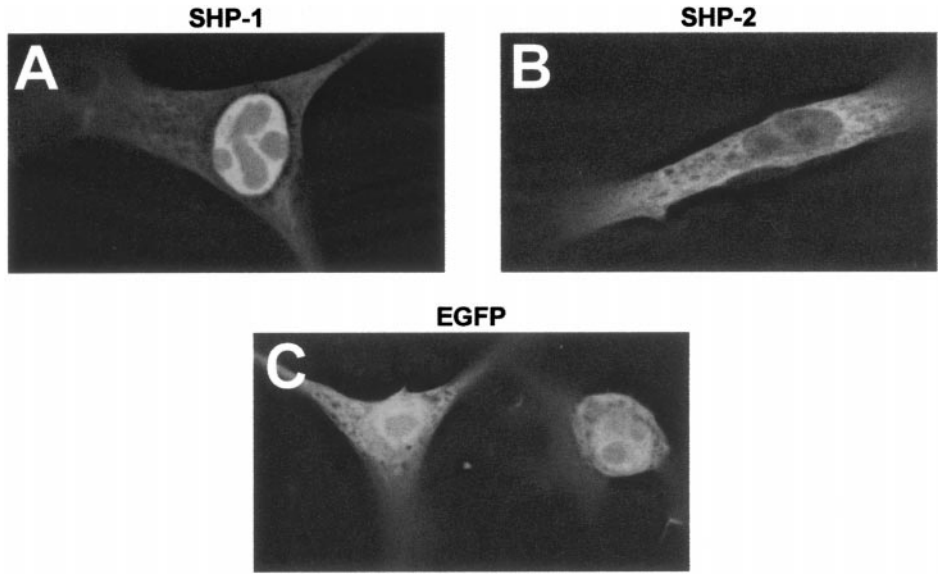

FIG. 1. **Intracellular distributions of SHP-1 and SHP-2 in transfected HEK 293 cells.** The fluorescence localization of GFP-tagged SHP-1 (*A*), GFP-tagged SHP-2 (*B*), and GFP (*C*) was compared in transiently transfected HEK293 cells. GFP-tagged proteins were expressed for 48 h, and their distribution was examined by fluorescence microscopy. GFP-tagged SHP-1 is present primarily in the nucleus of transfected cells, whereas GFP-tagged SHP-2 is distributed mainly within the cytoplasm. GFP alone diffuses throughout the cell. *EGFP*, enhanced GFP.

streptomycin (Life Technologies, Inc.). MCF-7 cells were maintained in the same growth medium supplemented with 10 μg/ml bovine insulin (Sigma). HL-60, U-937, and Jurkat cells were grown in RPMI 1640 medium (Life Technologies) supplemented with 10% fetal bovine serum, 2 mM L-glutamine, 100 units/ml penicillin, and 100 μg of streptomycin. The day before transfection, 5×10^4 HEK293 cells were seeded per well of a 24-well plate. The medium was exchanged for complete medium lacking antibiotics, and the cells were transfected with 1 μg of plasmid DNA and 1 μl of LipofectAMINE 2000 in OptiMEM (Life Technologies) for 6 h according to the manufacturer's instructions. After a change to complete medium, the cells were grown for a further 24 h before processing for microscopy.

Construction of green fluorescent protein-tagged SHP-1 and SHP-2—Green fluorescent protein (GFP)-tagged SHP-1 and SHP-2 fusion constructs were generated using the strategy of overlapping recombination polymerase chain reaction (23). Platinum *Pfx* polymerase (Life Technologies) was used for all polymerase chain reactions. The SHP-1 coding sequence was amplified using a forward SHP-1 primer containing an *Xho*I site and a Kozak sequence (5′-ccctcgagcccacatggtgaggtggtttcaccg-3′) and a downstream joining primer (5′-cctcgcccttgctcaccatcttcctcttgagggaaccc-3′). GFP (enhanced GFP; CLONTECH) was amplified using a forward joining primer (5′-gggttccctcaagaggaagatggtgagcaagggcgagg-3′) and a reverse GFP primer containing an *Xho*I site (5′-ccctcgagttacttgtacagctcgtccat-3′). The two resulting products were mixed, and a secondary polymerase chain reaction was performed using the forward SHP-1 and reverse GFP primers. The product was ligated as an *Xho*I fragment into the expression vector MSCVpuro (CLONTECH). GFP-tagged SHP-2 was constructed in a similar manner using a forward SHP-2 primer (5′-ccctcgagccaccatgacatcgcggagatggtt-3′), joining primers (5′-cctcgcccttgctcaccattctgaaacttttctgctgttg-3′ and 5′-caacagcagaaaagtttcagaatggtgagcaaggagggcgagg-3′), and a reverse GFP primer.

Construction of SHP-1/SHP-2 Chimeras—The method of overlapping recombination polymerase chain reaction was used to generate cDNAs encoding chimeric SHP-1/SHP-2 proteins from GFP-tagged SHP-1 and SHP-2 templates. SHP-2/1A, SHP-2/1B, and SHP-2/1C were constructed using the forward SHP-2 primer and reverse GFP primer with joining primers (SHP-2/1A, 5′-gtgcagatcctacctctgaaaggtggtaccatggccacatgtctg-3′ and 5′-catacatgtggccatggtaccacctttcagaggtaggatctgcac-3′; SHP-2/1B, 5′-caaacttctctacagccgaaaagaggggcagcggccagagaacaag-3′ and 5′-cttgttctctggccgctgcccctctttcggctgtagagaagtttg-3′; and SHP-2/1C, 5′-ctatatggcggtccagcattatattgaaacaactaagaagaagctggaggtcctgcag-3′ and 5′-ctgcaggacctccagcttcttcttagttgtttcaatataatgctggaccgccatatag-3′). SHP-1/2 was constructed using the forward SHP-2 primer and reverse GFP primer with joining primers 5′-catcgcccagttcattgaaaccctacagcgcaggattgaagaag-3′ and cttcttcaatcctgcgctgtagggtttcaatgaactgggcgatg-3′. Each chimeric cDNA was cloned as an *Xho*I fragment into MSCVpuro.

Site-directed Mutagenesis of SHP-1 Basic Domains—The basic residues in the C-terminal domain of GFP-tagged SHP-1 were mutated to alanines using the QuikChange site-directed mutagenesis kit (Stratagene). Oligonucleotides used were BD1 (5′-cagttcattgaaaccactgcggcggcgctggaggtcctgcagtcg-3′ and 5′-cgactgcaggacctccagcgccgccgcagtggtttcaatgaactg-3′), BD2 (5′-gaacaagagggaggaggcagtggcggcgcagcggtcagcagacaag-3′ and 5′-cttgtctgctgaccgctgcgccgccactgcctcctccctcttgttc-3′), and BD3, (5′gagcaagggttccctcgcggcggcgatggtgagcaagggcg-3′ and 5′cgcccttgctcaccatcgccgccgcgagggaacccttgctc-3′), where underlined nucleotides indicate mismatches.

Immunocytochemistry and Fluorescence Microscopy—HeLa, A549, and MCF-7 cells were plated onto acid-washed glass coverslips at 50% confluence and incubated overnight. HL-60, U-937, and Jurkat cells were spun onto glass slides at 500 rpm for 5 min using a Cytospin 3 (Shandon). Cells were washed for 5 min in phosphate-buffered saline (PBS) and then fixed in 4% paraformaldehyde in PBS for 20 min. After a further 5-min wash in PBS, cells were incubated in PBS, 10% normal goat serum (NGS), and 0.2% Triton X-100 for 30 min. The cells were then incubated overnight at 4 °C with either anti-SHP1 or anti-SHP2 mouse monoclonal antibodies (Transduction Laboratories) diluted 1:500 (0.5 μg/ml) in PBS and 1% NGS. Cells were washed four times for 5 min in PBS, 10% NGS, and 0.2% Triton X-100 before Alexa 488-conjugated goat anti-mouse antibody (Molecular Probes) was applied at a 1:200 dilution in PBS and 1% NGS for 1 h. After four additional 5-min washes in PBS, 10% NGS, and 0.2% Triton X-100, the cells were immersed briefly in distilled water and mounted in S3023 fluorescent mounting medium (Dako). Transfected HEK293 cells were trypsinized from the tissue culture plate and plated onto eight-well multichamber slides (Lab-Tek, Nunc) at a density of 1000 cells per well. After 24 h, the cells were washed in PBS and fixed in 4% paraformaldehyde in PBS for 20 min. After a brief wash in PBS, the cells were mounted in fluorescent mounting medium and coverslipped. Alexa-488 and GFP fluorescence was viewed at 467 nM using an Axiovert 135 microscope (Zeiss) coupled to a monochrome light source (Photonics Polychrome II). A series of optical scans through the *z* axis of the cells were captured using a Hamamatsu digital camera, and image deconvolution was performed with Openlab software (Improvision). The digitized images were processed using Openlab software on a Power Macintosh G3.

RESULTS

SHP-1 Is Localized within the Nucleus of HEK293 Cells—A knowledge of the intracellular distribution of nontransmembranal PTPs might give an insight into their cellular function and regulation. Surprisingly, there is a paucity of data on the intracellular localization of SHP-1 and SHP-2. It is widely believed that both SHP-1 and SHP-2 are located within the cytoplasm, presumably because their interactions with tyrosine-phosphorylated, membrane-associated proteins are well documented. The localization of both SHP-1 and SHP-2 was investigated by transiently transfecting HEK293 cells with cDNAs encoding each wild-type SHP protein fused with a C-terminal GFP tag. Unexpectedly, SHP-1 was found to be localized predominantly within the nucleus of all transfected HEK293 cells (Fig. 1*A*). In contrast, SHP-2 was distributed throughout the cytoplasm of all transfected cells (Fig. 1*B*), with little nuclear fluorescence evident. GFP protein alone was dis-

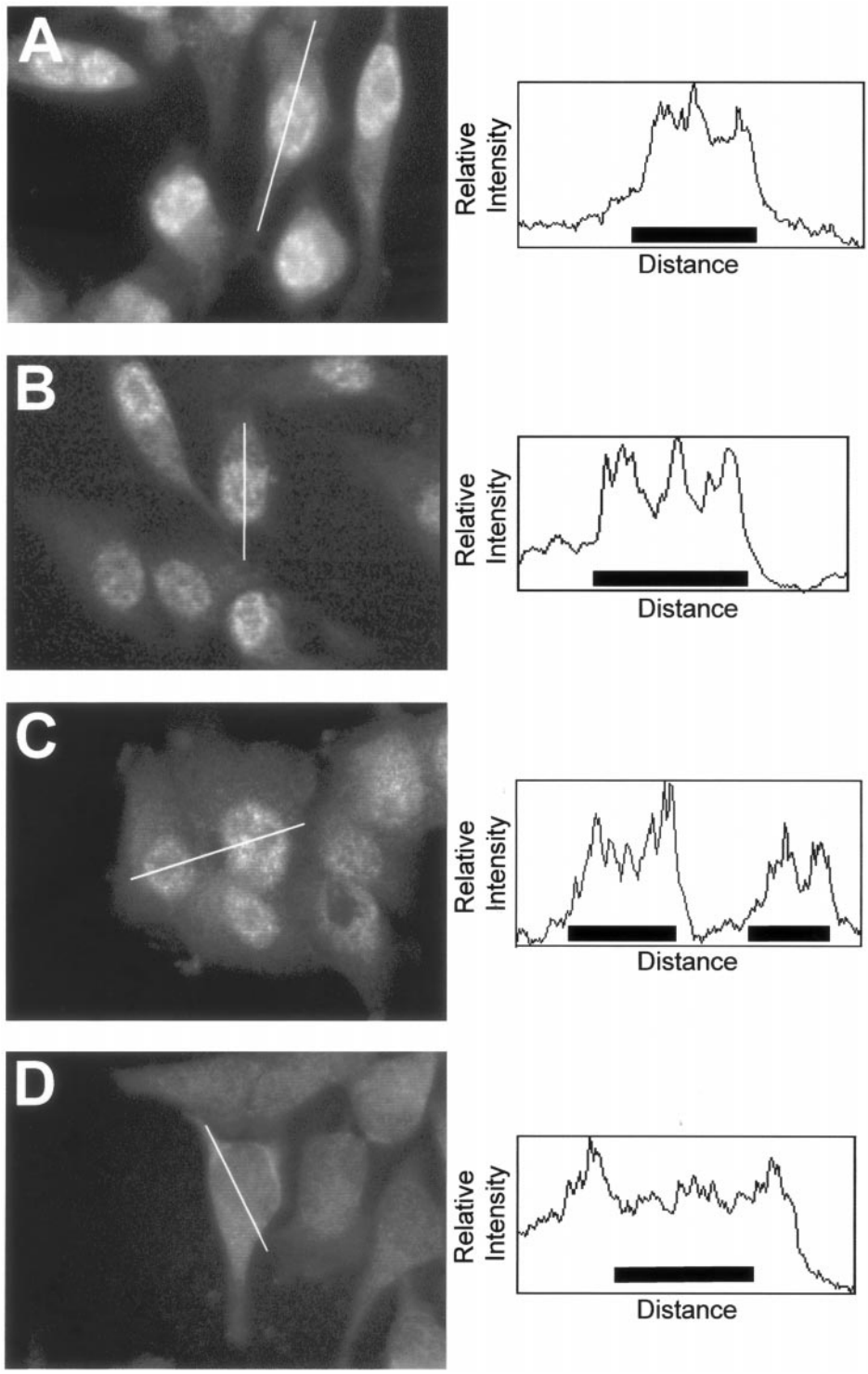

FIG. 2. **Localization of endogenous SHP proteins in nonhemopoietic cell lines.** Cells were immunostained using SHP-1- and SHP-2-specific monoclonal antibodies. In accordance with experiments performed using GFP-tagged proteins, SHP-1 is present predominantly within the nucleus of HeLa (*A*), MCF7 (*B*), and A549 (*C*) cells. Likewise, SHP-2 is present chiefly in the cytoplasm of cells with a lesser degree of nuclear staining (*D*). Shown is the change in relative fluorescence intensity across the *white lines*, with the *black bars* indicating the corresponding nuclear region.

tributed evenly throughout the cell (Fig. 1*C*). This nuclear *versus* cytoplasmic distribution of overexpressed GFP-tagged SHP-1 and SHP-2 appears to be consistent for a variety of nonhemopoietic cells that have been tested (HeLa, MCF7, A549, primary rat embryo fibroblast, and Chinese hamster ovary cells; data not shown).

Endogenous SHP-1 Exhibits Contrasting Localization in Nonhemopoietic and Hemopoietic Cell Lines—To exclude the possibility that the observed nuclear localization of SHP-1 was artifactual because of overexpression, the addition of GFP sequence, or both, the localization of endogenous SHP-1 was studied in several nonhemopoietic cell lines. Although SHP-1 is expressed predominantly in hemopoietic lineages, lower levels of expression have been observed in HeLa cervical adenocarcinoma, A549 lung carcinoma, and MCF-7 mammary adenocarcinoma cell lines (4, 6). Immunostaining using an SHP-1-specific antibody revealed that endogenous SHP-1 is localized within the nucleus of these three cell types (Fig. 2, *A–C*). This observation demonstrated that neither the overexpression of SHP-1 nor addition of GFP to the C-terminal end of SHP-1 was causing an alteration to its localization. Furthermore, detection of SHP-2 in HeLa cells using an SHP-2-specific antibody confirmed that SHP-2 is localized throughout the cytoplasm (Fig. 2*D*). These results show that each SHP protein has a distinct cellular distribution, with endogenous SHP-1 being localized within the nuclear compartment of native cells.

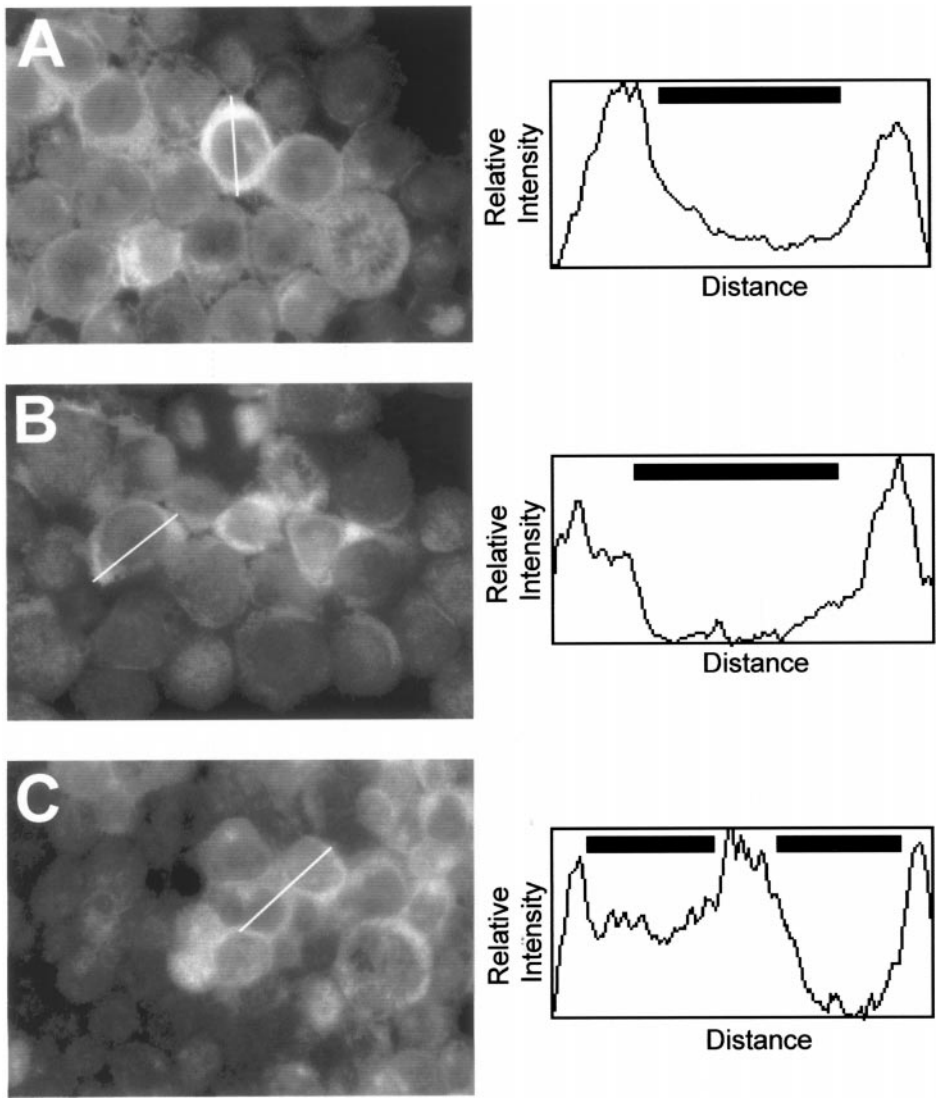

FIG. 3. **Localization of endogenous SHP-1 in hemopoietic cell lines.** The distribution of SHP-1 in three hemopoietic cell lines was determined by immunostaining using an antibody specific to the SHP-1 protein. SHP-1 is present almost entirely within the cytoplasm of U937 (*A*), HL-60 (*B*), and Jurkat (*C*) cells. Shown is the change in relative fluorescence intensity across the *white lines*, with the *black bars* indicating the corresponding nuclear region.

Next, we wished to address whether SHP-1 localization differs between nonhemopoietic and hemopoietic cells. Previously, SHP-1 has been found to be present in the cytoplasm of HL-60 promyeloblast cells (24) and primary human neutrophils (25). Detection of SHP-1 in HL-60, U-937 macrophage, and Jurkat T-cell leukemia cell lines by immunostaining revealed that endogenous SHP-1 is present within the cytoplasm and excluded from the nucleus (Fig. 3). Therefore, SHP-1 localization differs dramatically between the nonhemopoietic and hemopoietic cell lines tested.

The C-terminal Domain of SHP-1 Is Responsible for Nuclear Import—The maximum size of proteins allowed to passively translocate through the nuclear pore complex is ~40–50 kDa; larger proteins require an import signal (the nuclear localization signal (NLS)) to be targeted to the nucleus (26). Because the SHP-1-GFP fusion protein is ~93 kDa, active NLS-directed transport across the nuclear membrane must occur. SHP-1 and SHP-2 are closely related proteins and structurally very similar. They have nearly 55% overall sequence identity at the amino acid level, with the tandem Src homology 2 domains and catalytic domain exhibiting the highest level of similarity (Fig. 4*A*). To determine the region of SHP-1 responsible for nuclear import, chimeric cDNAs were constructed (Fig. 4*B*), each fused with a C-terminal GFP tag. When transiently expressed in HEK293 cells, the three chimeric cDNAs encoding varying lengths of SHP-2 with the C-terminal end of SHP-1 (SHP-2/1-A, SHP-2/1-B, and SHP-2/1-C) were found to be localized predominantly within the nuclei of transfected cells (Fig. 5, *A–C*) and indistinguishable from wild-type SHP-1. This revealed that the C-terminal domain of SHP-1 could replace the analogous region of SHP-2 and could allow the otherwise cytoplasmic SHP-2 molecule to efficiently enter the nucleus. Therefore, it was likely that the C-terminal domain of SHP-1 harbored the signal sequence responsible for nuclear translocation. This was demonstrated by expressing a cDNA encoding SHP-1 with the C-terminal domain replaced by the equivalent region of SHP-2 (SHP-1/2; Fig. 4*B*). The resulting protein was unable to enter the nucleus and accumulated within the cytosol of all transfected cells (Fig. 5*D*). Thus, the C-terminal domain of SHP-1 is necessary for the nuclear import of SHP-1. Furthermore, the C-terminal domain (amino acids 519–595) linked to GFP (SHP-1C) entered the nucleus of transfected HEK293 cells with high efficiency (Fig. 5*E*), indicating that the C-terminal domain was sufficient for nuclear import.

A Short Basic Region within the C-terminal Domain Is Responsible for SHP-1 Nuclear Import—Basic domains are well known to function as NLSs. Analysis of the C-terminal domains of SHP-1 and SHP-2 revealed that several basic regions (BD1, BD2, and BD3) are present within SHP-1 but not in SHP-2 (Fig. 6*A*). BD1 (TKKKL) is similar to the NLS of the SV40 T antigen (Fig. 6*B*). BD2 (KVKK) and BD3 (KRK) together re-

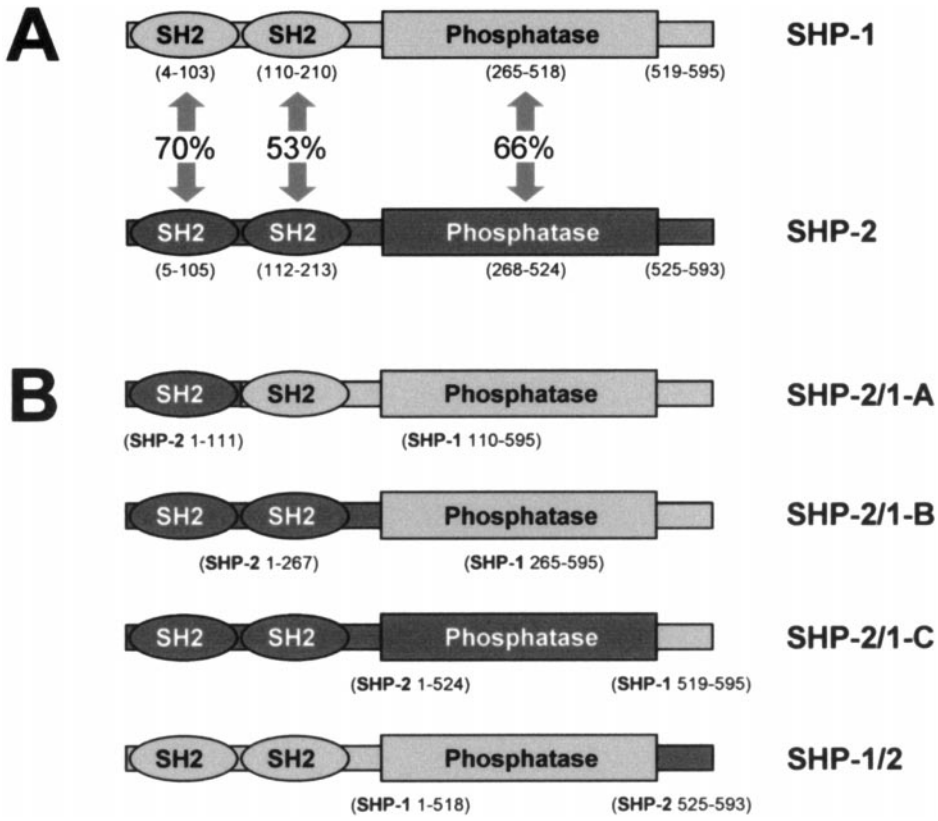

FIG. 4. **Structure of SHP-1, SHP-2, and chimeric proteins.** *A*, SHP-1 and SHP-2 structure, showing the conserved tandem Src homology 2 (*SH2*) domains, the single catalytic domain, and the degree of homology between them. *B*, structure of the SHP-1/SHP-2 chimeric molecules used to identify the C-terminal domain as the mediator of SHP-1 nuclear import.

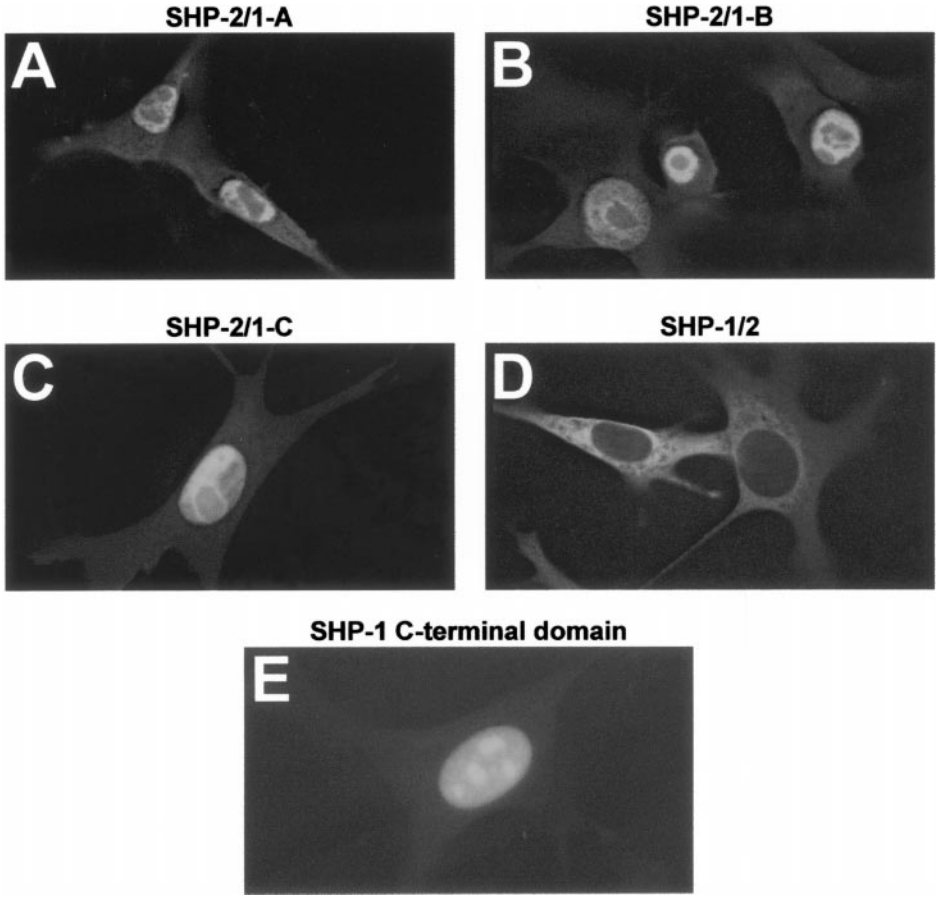

FIG. 5. **The C-terminal domain of SHP-1 confers nuclear import.** The fluorescence localization of GFP-tagged chimeras of SHP-1 and SHP-2 is shown. HEK293 cells were transiently transfected with cDNAs encoding chimeric proteins comprising varying lengths of SHP-1 and SHP-2 (see Fig. 4 and "Experimental Procedures"). Chimeric proteins containing regions of SHP-2 with the C-terminal domain of SHP-1 were imported into the nucleus (*A–C*). A protein consisting of SHP-1 with the C-terminal domain replaced by that of SHP-2 was unable to enter the nucleus and accumulated in the cytoplasm (*D*). The C-terminal domain of SHP-1 alone was also able to direct GFP into the nucleus with high efficiency (*E*).

semble the bipartite NLS of nucleoplasmin, consisting of two short stretches of basic amino acids separated by 10–22 residues (Fig. 6*C*). To establish whether these putative NLS domains were responsible for the nuclear import of SHP-1, each domain was removed by mutating the basic residues to alanines. Mutation of BD1 (SHP-1 K520A/K521A/K522A) resulted in a protein that still efficiently entered the nucleus (Fig. 7*A*). Likewise, mutation of BD2 (SHP-1 K576A/K578A/K579A) re-

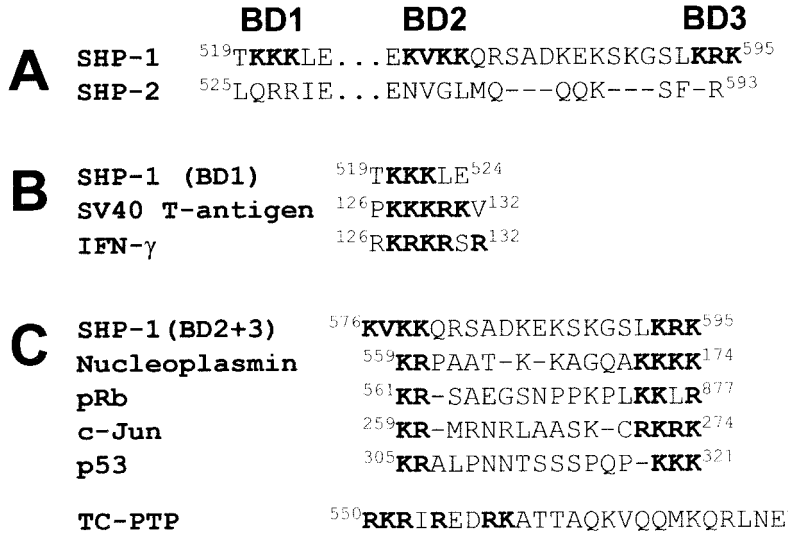

FIG. 6. **The basic domains of SHP-1.** *A*, comparison of the C-terminal segments of SHP-1 and SHP-2, showing the three basic domains (*BD1– BD3*) of SHP-1. *B*, comparison of BD1 with the NLS of SV40 large T-antigen and interferon γ (*IFN-γ*). *C*, BD2 and BD3 together resemble the bipartite NLS of nucleoplasmin and other proteins, including pRb, c-Jun, and p53. Also shown is the bipartite NLS of the protein-tyrosine phosphatase TC-PTP.

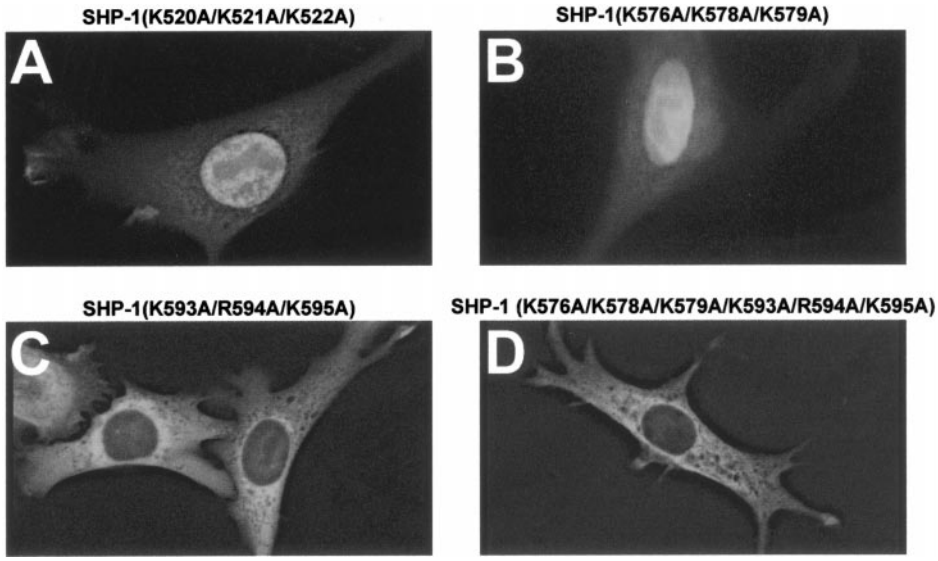

FIG. 7. **Mutation analysis of the C-terminal domain of SHP-1.** The fluorescence localization of GFP-tagged SHP-1 containing mutations of the three basic domains within the C-terminal domain is shown. Overexpressed SHP-1 lacking BD1 or BD2 remains nuclear (*A* and *B*), whereas mutation of BD3 prevents nuclear import (*C*). The SHP-1 protein containing mutations in both BD2 and BD3 is also excluded from the nucleus (*D*).

sulted in an SHP-1 protein that also entered the nucleus of transfected cells (Fig. 7*B*). In contrast, mutation of BD3 (SHP-1 K593A/R594A/K595A) almost completely prevented nuclear import, with the mutated SHP-1 protein accumulating within the cytosol (Fig. 7*C*). This distribution resembled that of the SHP-1/2 chimeric protein and indicated that the short KRK sequence constituting BD3 was responsible for nuclear import. Previously, it has been shown that mutations in either the basic cluster of the bipartite NLS of nucleoplasmin have only a partial effect on nuclear transport, with mutations in both clusters required to abolish nuclear transport. On the basis of these criteria, the BD2 and BD3 domains of SHP-1 do not form a bipartite NLS, even though the amino acid sequence resembles bipartite NLS from several nuclear proteins. Mutation of BD3 completely prevents the nuclear import of SHP-1 (Fig. 7*C*), whereas a protein lacking both BD2 and BD3 shows a similar distribution (Fig. 7*D*). Therefore, unusually, the short KRK motif present at the extreme C-terminal end of SHP-1 is absolutely required and appears solely responsible for nuclear import.

DISCUSSION

We have shown using GFP fusion proteins that SHP-1 is present predominantly within the nuclear compartment of HEK293 cells, an observation that extends to a variety of transfected nonhemopoietic cells. In contrast, a GFP fusion of the closely related protein SHP-2 is localized throughout the cytoplasm. Immunostaining of SHP-1 in several nonhemopoietic cell types reinforced the experiments performed with GFP-tagged proteins, demonstrating that the distribution of SHP-1 was not influenced by overexpression or the addition of a GFP tag. Endogenous SHP-1 clearly localized to the nucleus of three nonhemopoietic cell lines that are known to express readily detectable amounts of SHP-1. In contrast, endogenous SHP-2 was distributed throughout the cytoplasm in these cell types. Furthermore, the localization of SHP-1 differs dramatically between nonhemopoietic and hemopoietic cells. The HL-60, U-937, and Jurkat cells tested each exhibited SHP-1 staining that was confined within the cytoplasm. Therefore, we have revealed a novel nuclear import of the SHP-1 protein that appears to be restricted to nonhemopoietic cells.

The use of chimeric proteins showed that the C-terminal domain of SHP-1 harbors the signal required for the nuclear distribution of SHP-1. Site-directed mutagenesis revealed that a short KRK sequence at the extreme end of the C-terminal domain was necessary and sufficient for SHP-1 nuclear import. Mutation of this basic domain prevented the entry of SHP-1 into the nuclear compartment, resulting in the accumulation of SHP-1 in the cytoplasm. Generally, NLSs are either contiguous, as in the case of SV40 large T antigen, or bipartite, as illustrated by nucleoplasmin. In a bipartite NLS, neither basic

region can function independently to target proteins to the nucleus. In SHP-1, BD2 (KVKK) and BD3 (KRK) together resemble a bipartite NLS. However, mutation of BD3 appears to completely prevent nuclear import and implies that BD3 acts independently of BD2. Furthermore, mutation of BD2 imparts no observable change to the intracellular distribution of SHP-1. Therefore, the BD2 and BD3 motifs of SHP-1, although resembling a bipartite NLS, do not appear to function in this way. However, it would be extraordinary for a KRK sequence to direct nuclear import alone. It might be that BD2 and BD3 do function synergistically to direct the nuclear import of SHP-1, with BD3 being the more potent half of the partnership. This is not unprecedented; T-cell PTP (TC-PTP) is another example of a PTP that possesses a functional NLS (Fig. 6*C*), the bipartite nature of which remains controversial. In one investigation, the upstream region of the T-cell PTP NLS appears not to function in nuclear import (27). Yet in a later and more detailed study, both basic regions of the T-cell PTP NLS were shown to be required for efficient nuclear import but with each basic region functioning independently to a limited degree (28). Therefore, it remains undetermined whether the NLS of SHP-1 is truly bipartite, with further investigation necessary.

The biological importance of SHP-1 nuclear import is not defined. The clear difference in SHP-1 distribution between hemopoietic and nonhemopoietic cells implies that the trafficking of SHP-1 is under tight regulation in each cell type. In hemopoietic cells, SHP-1 must be exported from the nucleus or the C-terminal NLS must be silenced for SHP-1 to remain in the cytoplasm. This could be brought about by phosphorylation of amino acid residues within close proximity to the NLS or by masking of the NLS by protein-protein interaction. On the other hand, alternative splicing of SHP-1 may remove the region of the gene encoding the NLS. TC-PTP exists as two isoforms generated by differential splicing, one isoform directed to the nucleus and the other directed to the endoplasmic reticulum because of the addition of a stretch of hydrophobic residues (27). Several other PTPs exist in two forms targeted to distinct intracellular locations, including PTPϕ/GLEPP-1 (29) and DPTP61F (30). Interestingly, differential splicing of the SHP-1 gene has recently been reported, which produces the 70 kDa protein SHP-1L, which differs from SHP-1 in its C-terminal structure (31). The SHP-1L splice variant does not possess the BD3 domain shown to direct nuclear import. This raises the question of whether differential splicing of the SHP-1 gene could regulate SHP-1 localization, which in turn could regulate SHP-1 function by directing SHP-1 to target substrates at distinct cellular localities. However, the anti-SHP-1 antibody used in these studies was raised to the C-terminal domain of the protein and will only detect SHP-1 (not SHP-1L); thus the differential localization of endogenous SHP-1 proteins in hemopoietic and nonhemopoietic cells is more likely to be due to silencing of the NLS or enhanced nuclear export.

The import of SHP-1 into the nucleus of nonhemopoietic cells may serve as a mechanism by which SHP-1 function is regulated. SHP-1 might be localized within the nucleus to prevent interaction with cytoplasmic signaling molecules or might be directed toward unknown nuclear phosphoproteins. It has been reported that SHP-1 can act as a positive regulator of cell signaling in nonhemopoietic HEK293 and HeLa cells. It was speculated that this might be due to the selective dephosphorylation of tyrosine residues that have inhibitory signaling roles. Here we show that in these cell types, SHP-1 is present within the nucleus. Furthermore, we have demonstrated that SHP-1 is strikingly excluded from the nucleus of three hemopoietic cell lines, the first time that SHP-1 localization has been shown to be different within contrasting cell types. Consequently, it might be that SHP-1 performs its positive role by dephosphorylating nuclear tyrosine-phosphorylated proteins. Because SHP-1 is overexpressed in a variety of malignant nonhemopoietic cells, identifying targets of nuclear SHP-1 dephosphorylation is critical in understanding the role of SHP-1 in tumorigenesis. Relatively few nuclear tyrosine-phosphorylated proteins have been identified. However, potential targets for nuclear PTPs are the STAT proteins, which are activated by tyrosine phosphorylation and translocate to the nucleus (32). The tyrosine dephosphorylation event that switches off activated STAT-1 has been localized to the nucleus, but as yet the PTP(s) involved have not been identified (33, 34). This would provide a role for SHP-1 in directly regulating gene expression. Studies to identify novel nuclear targets of SHP-1 are under way.

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A Functional Nuclear Localization Sequence in the C-terminal Domain of SHP-1

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J. Biol. Chem. 2001, 276:23719-23725.
doi: 10.1074/jbc.M102846200 originally published online April 25, 2001
