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# EGF-Stimulation Activates the Nuclear Localization Signal of SHP-1

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**Abstract** Protein tyrosine phosphatase SHP-1 plays a critical role in the regulation of a variety of intracellular signaling pathways. SHP-1 is predominantly expressed in the cells of hematopoietic origin, and is recognized as a negative regulator of lymphocyte development and activation. SHP-1 consists of two Src homology 2 (SH2) domains and one protein tyrosine phosphatase (PTP) domain followed by a highly basic C-terminal tail containing tyrosyl phosphorylation sites. It is unclear how the C-terminal tail regulates SHP-1 function. We report the examination of the subcellular localization of a variety of truncated or mutated SHP-1 proteins fused with enhanced green fluorescent protein (EGFP) protein at either the N-terminal or the C-terminal end in different cell lines. Our data demonstrate that a nuclear localization signal (NLS) is located in the C-terminal tail of SHP-1 and the signal is primarily defined by three amino-acid residues (KRK) at the C-terminus. This signal is generally blocked in the native protein and can be exposed by fusing EGFP at the appropriate position or by domain truncation. We have also revealed that this NLS of SHP-1 is triggered by epidermal growth factor (EGF) stimulation and mediates translocation of SHP-1 from the cytosol to the nucleus in COS7 cell lines. These results not only demonstrate the importance of the C-terminal tail of SHP-1 in the regulation of nuclear localization, but also provide insights into its role in SHP-1-involved signal transduction pathways. J. Cell. Biochem. 94: 944–953, 2005. 

**Key words:** protein tyrosine phosphatase SHP-1; nuclear localization signal; epidermal growth factor; subcellular translocation

Protein tyrosine phosphorylation governs enzymatic activation, protein relocalization, and formation of the signaling protein complexes in many signaling pathways. Formation of these complexes is critical in the control of normal cell behavior such as cell growth, cell differentiation, and apoptosis [Neel, 1993; Tonks and Neel, 1996, 2001; Neel and Tonks, 1997; Tsui et al., 2002]. Protein tyrosine phosphorylation is regulated by two types of enzymes: protein tyrosine kinases (PTKs) that catalyze the phosphorylation, and protein tyrosine phosphatases (PTPs) that are responsible for dephosphorylation [Van Vactor et al., 1998; Neel et al., 2003]. Abnormal tyrosine phosphorylation levels may cause cell disorders including various cancers [Wu et al., 2003a,b]. PTPs play critical roles in regulating intracellular processes in response to extracellular signals and stimuli including antigens, cytokines, growth factors, and hormones, as well as intracellular signals such as DNA damage [Yi et al., 1993; Keilhack et al., 1998, 2000; Yoshida et al., 1999].

SHPs, including SHP-1, SHP-2, Csw from *Drosophila* and PTP-2 from *Caenorhabditis elegan*s are a family of Src homology 2 (SH2) domain-containing non-receptor like PTPs. SHP-1 and SHP-2 have highly homologous primary structures (overall 55% identity), and both are composed of two tandem SH2 domains and one PTP catalytic domain, followed by a C-terminal tail. Biochemical and structural analyses have contributed significantly to elucidation of the critical role of SH2 domains for SHP-1 and SHP-2 [Tenev et al., 1997; Barford and Neel, 1998; Hof et al., 1998; Tsui et al., 2002; Yang et al., 2003]. Crystallographic studies on derivatives lacking the C-terminal tail revealed a similar auto-inhibition conformation for these two enzymes [Hof et al., 1998; Yang et al., 2003].

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However, SHP-1 and SHP-2 differ greatly in cellular distribution and biological function. SHP-1 is predominantly expressed in hematopoietic cells and epithelial cells, and behaves as a negative regulator in the B- and T-cell activation signaling pathways. In contrast, SHP-2 is expressed ubiquitously in cells with various origins and acts primarily as a positive regulator [Plutzky et al., 1992]. Interestingly, the C-terminal tail region (~60 residues) of these two enzymes shows lower sequence homology (~15% identity) than the overall homology (Fig. 1a). Therefore, the difference in the C-terminal tails of SHPs, could be responsible, at least in part, for the differences between them in function and subcellular localization. However, the structure and function of the C-terminal tail remains unknown, primarily due to the lack of full-length SHP structures.

There are two tyrosyl phosphorylation sites (Tyr536 and Tyr564) on the C-terminal tail of SHP-1. Recent studies revealed the importance of these tyrosine phosphorylation sites, supporting the idea that the C-terminal tail of SHP-1 functions in the regulation of its activity and substrate recognition [Pei et al., 1994; Zhang et al., 2003]. The C-terminal tail of SHP-1 is highly basic; 30% of the residues are lysines or arginines (Fig. 1a). This region, identified by two groups, contains a nuclear localization signal (NLS) that controlled the subcellular localization of SHP-1 [Craggs and Kellie, 2001; Yang et al., 2002]. One group reported that the C-terminal tail contained an NLS that targets SHP-1, in a cell-type dependent-fashion, to the cytosol of hematopoietic cells but to the nuclei of non-hematopoietic cells. This NLS of SHP-1 was identified as the last three amino acids (KRK) at the C-terminus [Craggs and Kellie, 2001]. In contrast, another group reported that SHP-1 was located in the cytosol and could be in part translocated into nuclei in response to cytokine activation, regardless of cell type. Also, in the latter case, a bipartite NLS was suggested to exist in the C-terminal tail region [Yang et al., 2002]. Thus different, even contradictory, subcellular localization patterns of SHP-1 were observed within different cell lines under different physiological conditions, implying there is complexity in the function of the C-terminal tail of SHP-1.

In order to define more carefully about the role of the C-terminal NLS on subcellular localization of SHP-1, we have examined the localization of different forms of SHP-1 in a variety of cell lines. Our data confirm the presence of an NLS in the C-terminal tail of SHP-1 and define it as the triple amino-acid cluster KRK at the C-terminus. We have also demonstrated that variation in how SHP-1 is fused to enhanced green fluorescent protein (EGFP) can explain the contradictory reports on SHP-1's subcellular distribution. Our results further support the important role of the KRK

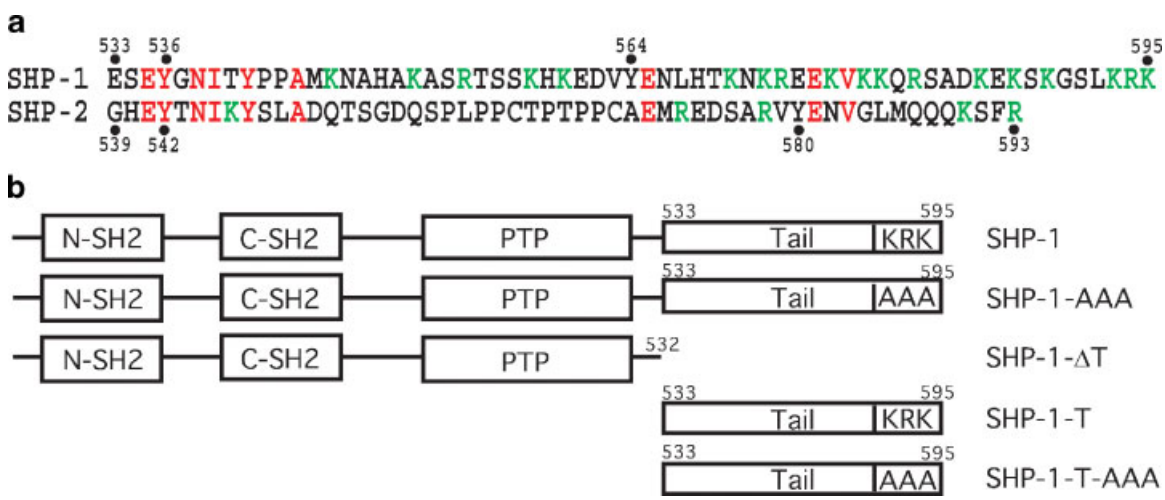


**Fig. 1.** C-terminal tail of SHPs and scheme of the SHP-1 constructs. **a**: Sequence alignment of the C-terminal tails of SHP-1 and SHP-2. Identical residues are red, lysine and arginine residues are green, and tyrosine phosphorylation sites are labeled. **b**: SHP-1 constructs designed for nuclear localization studies, full-length SHP-1 (SHP-1), SHP-1 with the three residues at C-terminus mutated to AAA (SHP-1-AAA), SHP-1 C-terminal tail (SHP-1-T), SHP-1-T with last three residues mutated into AAA (SHP-1-T-AAA), and C-terminal truncated SHP-1 (SHP-1-ΔT). [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

cluster on SHP-1 nuclear translocation and suggest the blocking of the NLS, unless translocation is activated. We have also shown that the blocked NLS was released with EGF stimulation, resulting in the translocation of the SHP-1 protein into nuclei in COS7 cell lines.

## MATERIALS AND METHODS

### Preparation of EGFP Fused Constructs

The coding sequences of full-length SHP-1 (SHP-1), C-terminal truncated SHP-1 (residues 1-532, SHP-1-ΔT), SHP-1 with KRK at C-terminus mutated to AAA (SHP-1-AAA), C-terminal tail of SHP-1 (residues 533-595, SHP-1-T), and C-terminal tail with the KRK cluster mutated to AAA (SHP-1-T-AAA) were generated by polymerase chain reaction (PCR) with Platinum *Pfx* polymerase as described previously (Fig. 1b) [Liang et al., 1997]. These coding sequences were cloned into pEGFP-N1 and pEGFP-C2 vectors (Clontech) with the cleavage of *Xhol*I and *Hind*III sites at the 5′ end and 3′ end of the corresponding PCR products, to obtain the N- and C-terminally EGFP-fused constructs, respectively. All cloned plasmids were confirmed by DNA sequencing.

### Cell Culture

All cell lines were obtained from American Type Culture Collection and maintained at 37°C in a humidified 5% $CO_2$ incubator. HEK293, COS7, A431, and HT 29 were grown in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS, Life Technologies, Inc.), 100 units/ml penicillin, and 100 μg of streptomycin (Life Technologies, Inc.) HepG2 cells were maintained in minimum essential medium (Eagle) with 2 mM L-glutamine and Eagle's BSS, and 10% FBS, whereas H9 and Raji cells were grown in RPMI 1640 with 10% FBS and 2 mM L-glutamine.

### EGF-Stimulation and Extraction of Cell Fractions

COS7 cells were transiently transfected with pEGFP-SHP-1 using lipofectamine (Invitrogen) for 6 h, and grown in complete medium for 24 h. For further treatment, cells were serum-starved for 3 h, and treated with epidermal growth factor (EGF, 100 ng/ml) (Calbiochem) for 1, 3, and 5 min. To prepare cytoplasmic and nuclear fractions, EGFP-SHP-1 transfected COS-7 cells were lysed in buffer A (10 mM Tris-HCl, pH 7.8, 1.5 mM $MgCl_2$, 10 mM KCl, 1 mM benamidine, 0.1 mM phenylmethylsulfonyl fluoride, 20 μg/ml leupeptin, 1 mM pepstain A, 1 μg/ml aprotinin, and 1 mM DTT) for 15 min on ice, then added 10% NP-40, mixed for 10 s and centrifuged at 8,000 rpm for 1 min, to separate the nuclear pellet from the cytoplasmic supernatant. The nuclear pellet was re-suspended in the buffer C (20 mM HEPES, pH 7.9, 400 mM NaCl, 1 mM EDTA, 1 mM EGTA, 0.1 mM phenylmethylsulfonyl fluoride, and 1 mM DTT) for 45 min on ice, and centrifuged at 14,000 rpm for 10 min. The cytoplasmic and nuclear fractions were analyzed by SDS–PAGE and transferred to polyvinylidene difluoride (PVDF) membranes. The membranes were blocked in TBST buffer (10 mM Tris-HCl, pH 8.0, 150 mM NaCl, and 0.1% Tween-20) with 5% non-fat dry milk for 1 h at room temperature. Then they were incubated with anti-SHP-1 rabbit polyclonal antibody [Liang et al., 1997], followed by incubation with AP-conjugated secondary antibodies and detection with enhanced chemiluminescence (ECL) kit. The amounts of histone H1 and tubulin (Santa Cruz) were identified in the corresponding samples by Western analysis and were used as nuclear and cytosolic markers, respectively.

### Immunocytochemistry and Fluorescence Microscopy

Cells were grown on glass coverslips and transfected with the corresponding plasmid DNA by lipofectinamine reagent. Thereafter, cells were grown in complete medium for 24 h. For lymphoma cells (H9), transfected cells were spun onto glass coverslips at 500 rpm for 5 min using a Cytospin 3 rotor. For further treatment, COS7 and A431 cells were serum-starved for 3 h, and then treated with EGF (100 ng/ml) for 5 min. Cells were fixed with 4% formaldehyde in PBS for 20 min. After extensive washing with PBS, cells were mounted with Vectashield mounting medium (Vector). To visualize endogenous SHP-1, A431, HT29, HepG2, and Raji cells were fixed with 4% paraformaldehyde in PBS for 20 min and permeabilized with PBS containing 1% FBS and 0.5% TritonX-100, then incubated with anti-SHP-1 rabbit polyclonal antibody for 2 h at room temperature. The cells were washed three times for 10 min each in PBS before fluorescein isothiocyanate anti-rabbit antibody (1:1,000) (Biosource) was applied for 30 min. After washing three times with the

same buffer, the coverslips were mounted with the same mounting medium. All mounted cells were examined using a MRC1024 confocal microscope (Bio-Rad). Images were captured with Adobe Photoshop.

## RESULTS

### NLS on the C-Terminal Tail of SHP-1

An NLS on the C-terminal tail of SHP-1 has been reported before. However, the NLS affected nuclear localization differently in two independent studies. Craggs' group, using a site-directed mutagenesis approach, suggested that the NLS was primarily determined by a KRK cluster located at the C-terminus of SHP-1 [Craggs and Kellie, 2001], whereas Yi's group, through examination of the localization of a series of constructs of different lengths, identified that the NLS functions in an apparently bipartite fashion, which is composed of two separate basic clusters on the C-terminal tail [Yang et al., 2002]. To further examine the NLS, the SHP-derivatives SHP-1-AAA, SHP-1-T, SHP-1-T-AAA, and SHP-1-ΔT; and the entire SHP-1 coding region were (Fig. 1b) fused with EGFP at the C-terminal end and transfected into a human embryonic kidney cell line (HEK293) and a human T lymphoma cell line (H9). The subcellular distribution of SHP-1 and its derivatives was determined (Fig. 2).

SHP-1 (Fig. 2a), as well as SHP-1-T (Fig. 2c), was localized within the nuclei of all HEK293 cells examined. However, the C-terminal tail truncated SHP-1 (SHP-1-ΔT), and the constructs with the KRK cluster mutated to AAA (SHP-1-AAA and SHP-1-T-AAA) were either localized mainly in the cytoplasm of HEK293 cells (SHP-1-AAA, Fig. 2b and SHP-1-ΔT, Fig. 2f) or distributed like EGFP (Fig. 2e) throughout the cells (SHP-1-T-AAA, Fig. 2d). Consistent with the observations from Craggs' group [Craggs and Kellie, 2001], our results confirmed that the NLS of SHP-1 was located at the C-terminal tail and that the KRK cluster at the C-terminus was required for nuclear localization. In contrast, in hematopoietic cells, all the SHP-1 constructs had a clear cytosolic localization (H9 cells, Fig. 2g–k; data for K562 and Romas cells is not shown). These findings demonstrate that the subcellular localization of SHP-1 is cell-type dependent, and SHP-1 is localized in the cytoplasm of hematopoietic cells and in the nuclei of non-hematopoietic cells.

### Subcellular Localization of SHP-1 Depends on the Position of Fused EGFP

As described above, subcellular localization of SHP-1 has been studied previously. Both groups demonstrated the cytosolic distribution of EGFP-fused SHP-1 in hematopoietic cells. However, in non-hematopoietic cells, the results from two groups were contradictory: one demonstrated nuclear distribution and the other demonstrated cytosolic distribution of SHP-1. The major differences between two groups were that EGFP was fused at the N-terminus and the C-terminus of SHP-1, respec-

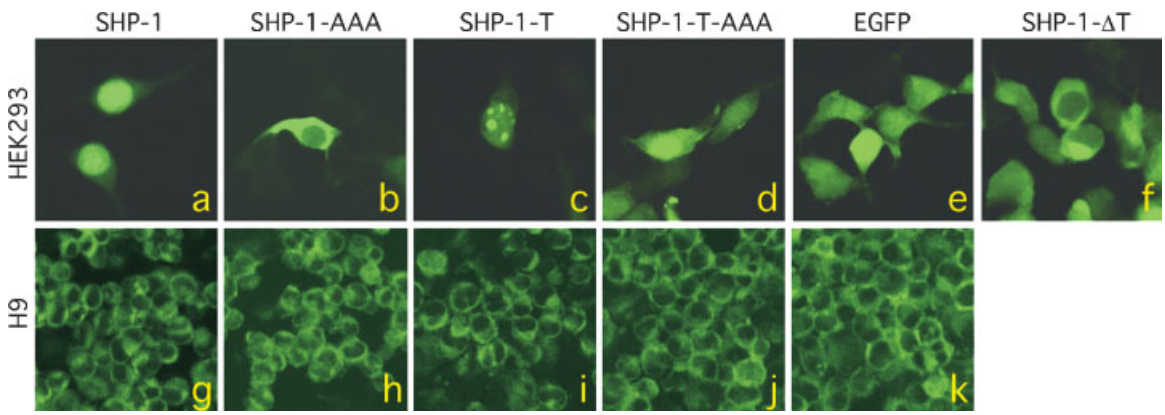


**Fig. 2.** Subcellular localization of SHP-1 from different constructs in representative hematopoietic and non-hematopoietic cells. The localization of C-terminally EGFP-tagged full-length SHP-1, SHP-1-AAA, SHP-1-T, SHP-1-T-AAA and SHP-1-ΔT was compared in HEK293 cells (upper row, non-hematopoietic) and H9 cells (bottom row, hematopoietic). In HEK293 cells, SHP-1 (**a**) and SHP-1-T (**c**) showed a nuclear distribution, SHP-1-AAA (**b**) and SHP-1-ΔT (**f**) showed a clear cytosolic distribution, and SHP-1-T-AAA (**d**) showed a similar distribution to EGFP only (**e**). In contrast, SHP-1 (**g**), SHP-1-AAA (**h**), SHP-1-T (**i**) and SHP-1-T-AAA (**j**) showed cytosolic distribution similar to that of EGFP (**k**) in H9 cells. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

tively, and the studies were carried in different cell lines. Those differences might be the primary reasons for the conflicting results. To test this hypothesis, both N-terminally and C-terminally EGFP-fused SHP-1 (EGFP-SHP-1 and SHP-1-EGFP) were cloned and transiently transfected into a variety of cell lines (Fig. 3). Surprisingly, EGFP-SHP-1 and SHP-1-EGFP had similar distributions in cancer cell lines but had much different localization in normal cell lines. In cancer cell lines (A431 and HT29 cell lines), both EGFP-SHP-1 and SHP-1-EGFP were localized in the cytoplasm (Fig. 3c–d & g–h). The observed cytosolic distribution of SHP-1 in A431 cell lines is consistent with the previously reported perinuclear distribution of SHP-1 in A431 cells [Tenev et al., 2000]. However, in normal cell lines such as HEK293 cells and monkey embryonic kidney (COS7) cells, SHP-1-EGFP was localized in the nuclei (Fig. 3e–f), whereas EGFP-SHP-1 was predominantly localized in the cytoplasm (Fig. 3a–b). This clear difference on SHP-1's distribution within normal cell lines can explain the contradictory results from the Craggs (C-terminally EGFP-fused SHP-1) and Yi (N-terminally EGFP-fused SHP-1) groups [Craggs and Kellie, 2001; Yang et al., 2002]. The results suggest that the distribution of EGFP-fused SHP-1 in normal cells depend on whether EGFP is fused to the N- or C-terminus.

To further examine the potential dependence of NLS activity on the position of the fused EGFP protein, derivatives of the SHP-1 C-terminal tail construct (SHP-1-T) were made carrying EGFP fused to either the N-terminus EGFP-SHP-1-T or the C-terminus (SHP-1-T-EGFP) (see Fig. 1b). These constructs were transiently transfected into HEK293 and COS7 cell lines. Interestingly, both EGFP-SHP-1-T (Fig. 4a–d) and SHP-1-T-EGFP (Fig. 4e–h) showed nuclear localization in these cell lines, implying that when only the C-terminal tail is expressed the NLS is unaffected by the position of EGFP.

One possible explanation for the observed difference is that the NLS (KRK) at the C-terminal tail of SHP-1 is blocked in the native SHP-1 structure, possibly through binding to or being buried in an acidic cavity on the surface of SHP-1 protein itself, or on the surface of other SHP-1 binding proteins. Therefore, when EGFP was fused to the N-terminus of SHP-1, the NLS at the C-terminus was still blocked and buried,

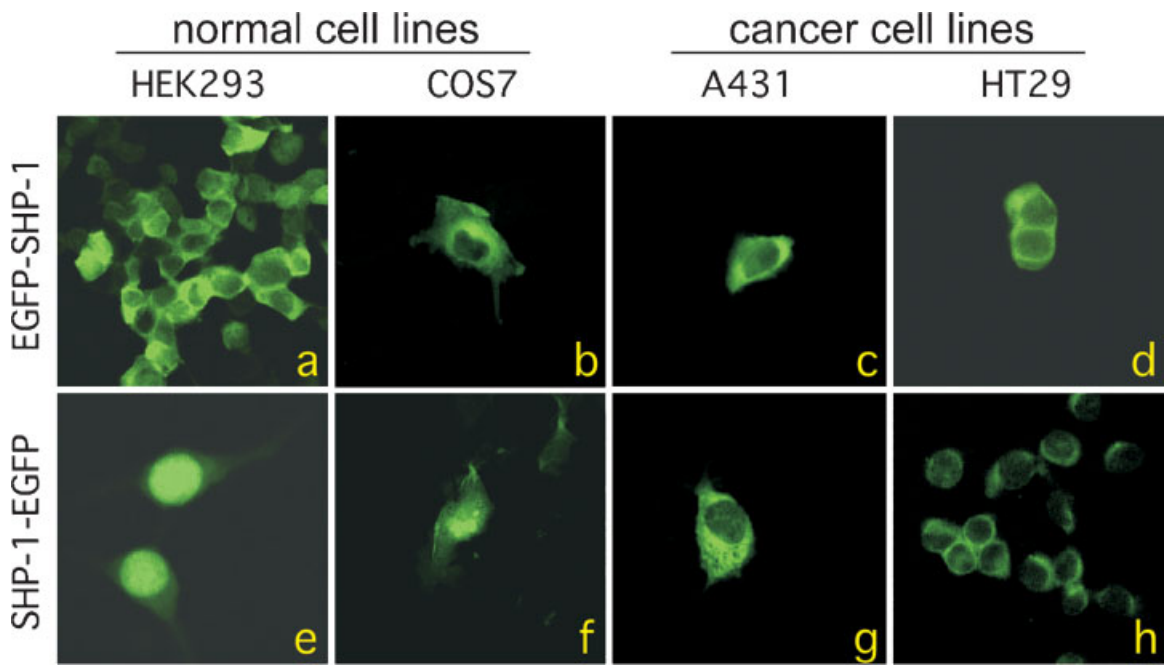


**Fig. 3.** Subcellular localization of SHP-1 with EGFP-tag at different positions. The localization of EGFP-SHP-1 (upper, EGFP tagged at N-terminus of SHP-1) and SHP-1-EGFP (lower, EFGF tagged at C-terminus of SHP-1) was compared in normal cell lines (HEK293 and COS7) and cancer cell lines (A431 and HT29). EGFP-SHP-1 showed a cytosolic localization in all these cell lines (**a**–**d**), whereas SHP-1-EGFT showed nuclear localization in normal cell lines (**e**, **f**), but cytosolic distribution in cancer cell lines (**g**, **h**). [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

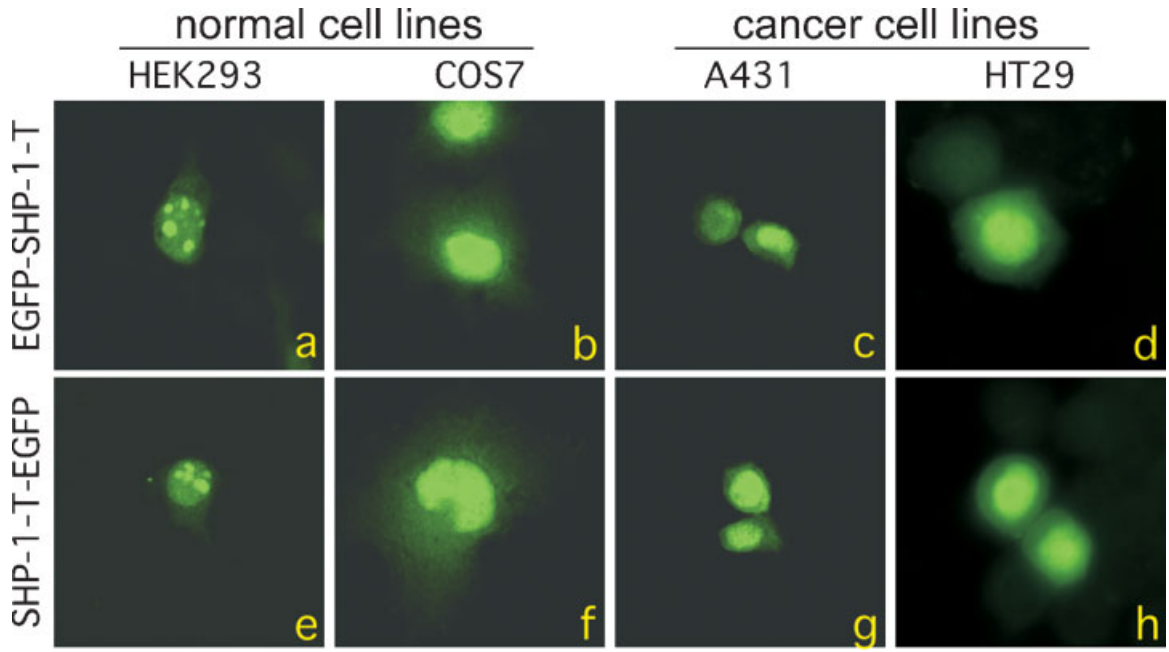


**Fig. 4.** Subcellular localization of SHP-1-T with EGFP-tag at the N- or C-terminus. The localization of EGFP-SHP-1-T (upper, EGFP tagged at N-terminus of SHP-1) and SHP-1-T-EGFP (lower, EGFP tagged at C-terminus of SHP-1) was compared in HEK293, COS7, A431, and HT29 cell lines. In all these cell lines, SHP-1-T showed a nuclear localization. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

therefore, the fused protein has the cytosolic distribution. However, when EGFP protein was fused at the C-terminus of SHP-1, the NLS would be exposed somehow due to the presence of EGFP nearby, therefore, the fused protein has the nuclear distribution. When the C-terminal tail is expressed by itself, the NLS is exposed, resulting in nuclear distribution, regardless of the position of the fused EGFP. Therefore, our data, together with the previously published results suggest that the NLS at the C-terminus of SHP-1 is buried in the basal state of the native SHP-1 protein. Perhaps activation signals, such as growth factor stimulated activation, could expose the tail of SHP-1 and target the SHP-1 into the nuclei for its physiological function.

### Translocation of SHP-1 in Response to EGF Stimulation

SHP-1 has been implicated in the EGF-stimulated signal transduction pathway. SHP-1 is a positive regulator of EGF-induced activation of signal transducers and activators of transcription (STAT) [Keilhack et al., 1998; Tenev et al., 2000]. SHP-1 can also affect the EGF-induced activation of the mitogen-activated protein kinase pathway [Keilhack et al., 1998]. In addition, overexpression of SHP-1 significantly decreased the tyrosine phosphorylation level of EGF receptor, and vise versa, EGF induced the phosphorylation and recruitment of SHP-1. Furthermore, the inducible overexpression of SHP-1 attenuated the EGF-dependent tyrosine phosphorylation and the EGF-dependent activation of STAT1/3 DNA-binding activity in the A431 cell line [Tenev et al., 2000]. These data suggest the possible interference of SHP-1 with the EGF-receptor signaling pathway. Therefore, it is of interest to see whether or not the NLS of SHP-1 can be activated by EGF-stimulation.

To address this question, the construct with the N-terminally fused EGFP (EGFP-SHP-1), whose NLS was blocked under the basal condition, was used for localization studies in A431 and COS7 cell lines (Fig. 5). In the absence of EGF-stimulation, EGFP-SHP-1 was localized in the cytosol of both A431 and COS7 cell lines, as expected from previous results (above). However, 5 min after EGF-stimulation, different localization was observed in between A431 cell lines and COS7 cell lines. In COS7 cell lines, SHP-1 was significantly relocalized from cyto-

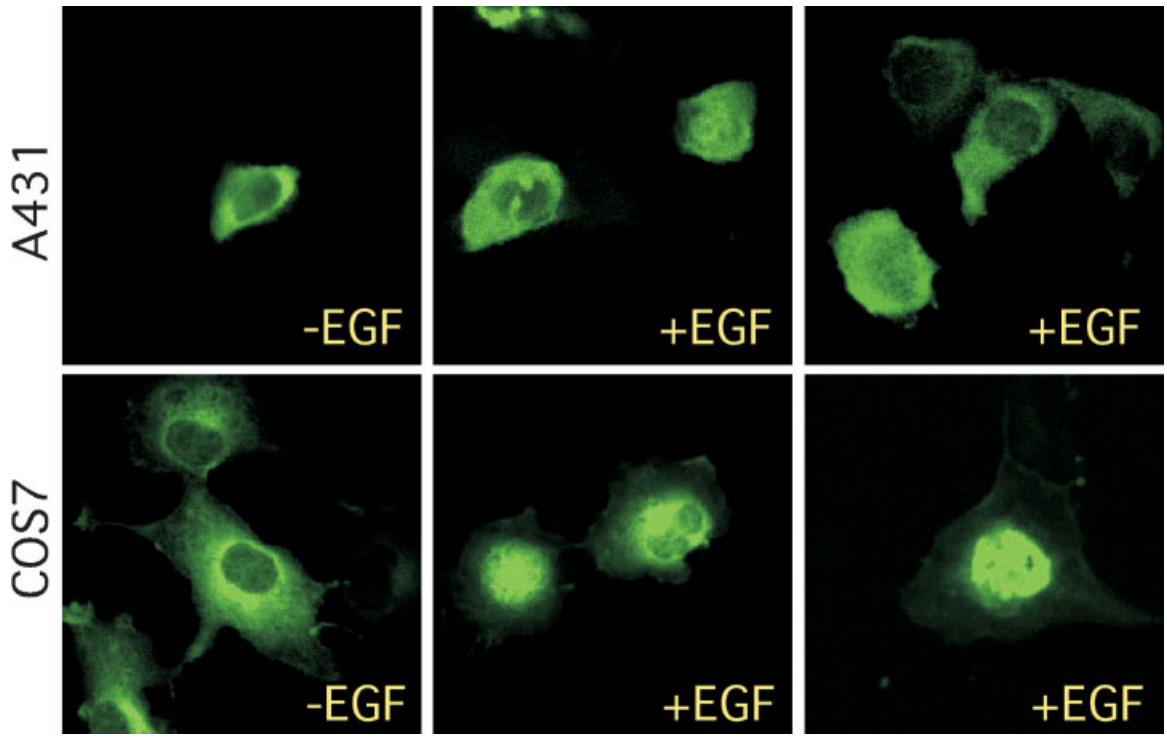


**Fig. 5.** Translocation of SHP-1 in response to EGF-stimulation in A431 (upper) and COS7 (lower) cell lines. The subcellular localization of SHP-1 is shown prior to EGF-stimulation (−EGF) and after EGF-stimulation (+EGF). [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

plasm to nucleus in most cells. In contrast, in A431 cell lines, EGFP-SHP-1 was still localized predominantly in the cytoplasm. This result is consistent with the perinuclear distribution of SHP-1 in A431 cells reported previously [Tenev et al., 2000].

To further confirm the EGF-stimulated translocation of SHP-1 in COS7 cells, the amount of SHP-1 protein in the cytosolic and nuclear fractions of COS7 cells after EGF-stimulation were examined using Western blot analysis (Fig. 6). The cytosolic and nuclear fractions of

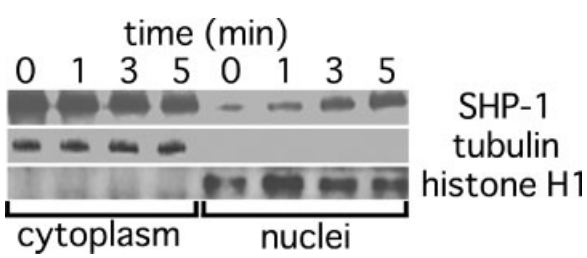


**Fig. 6.** Western blotting of SHP-1 with and without EGF-stimulation in COS7 cells. Tubulin and histone H1 were used as markers for cytoplasm (left) and nucleus (right), respectively. Western blotting of SHP-1 from COS7 cells with stimulation time at 0, 1, 3, and 5 min are shown.

the COS7 cells stimulated with EGF for different times were separated as described previously [Yang et al., 2002]. The amount of SHP-1 proteins in nuclear and cytosolic fractions was examined using the corresponding antibody. Tubulin and histone H1 were used as the markers for cytosolic and nuclear fractions, respectively. Absence of tubulin in the nuclear fraction and absence of histone H1 in the cytosolic fraction demonstrated the success of this separation. In agreement with the results obtained by immunofluorescence, Western blotting analysis clearly revealed that SHP-1 was almost absent from the nuclei of COS7 cell lines (Fig 6, lane 5) prior to EGF-stimulation. In response to the EGF-stimulation, some SHP-1 protein was translocated into the nuclei, and this translocation was continued for 5 min. The amount of SHP-1 protein reached a plateau more than 5 min after the EGF-stimulation in COS7 cells (data not shown). However, the same experiment for A431 cells did not demonstrate EGF-stimulated translocation of SHP-1 protein (data not shown), which is consistent with the immunostaining results (Fig. 5). We conclude that the nuclear translocation of SHP-1 in

response to EGF-stimulation occurs, and this translocation is dependent on cell types.

### Subcellular Localization of Endogenous SHP-1

Endogenous SHP-1 is expressed predominantly in the cytosol of hematopoietic cell, as reported by several different groups [Plutzky et al., 1992; Yi et al., 1992; Ram and Waxman, 1997; Craggs and Kellie, 2001]. However, endogenous SHP-1 is localized in the nuclei of cancer cells of non-hematopoetic origin, such as Hela cervical adenocarcinoma, A549 lung carcinoma and MCF-7 mammary adenocarcinoma cell lines [Craggs and Kellie, 2001]. This is incongruent with our finding of the EGFP-fused SHP-1 primarily in the cytoplasm of cancer cell lines (Fig. 3c–d, g–h) and led us to examine the localization of endogenous SHP-1 in different carcinoma cell lines with the immunocytochemical method. As reported before, immunostaining with SHP-1-specific antibody reveals a clear cytosolic distribution of endogenous SHP-1 in hematopoietic cell lines (Raji) (Fig. 7). However, in the human epithelial carcinoma cells (A431), human colonic adenocarcinoma cells (HT29), and human hepatocellular carcinoma cells (HepG2), endogenous SHP-1 is localized mainly in cytoplasm (Fig. 7), with a perinuclear but not nuclear distribution. Our observation, though is different from Cragg's group [Craggs and Kellie, 2001], is consistent with previous observations in A431 and COS7 cell lines [Tenev et al., 2000].

## DISCUSSION

The C-terminal tail of SHP-1 is indispensable for the regulation of SHP-1-regulated signal transduction pathways. This highly basic tail containing a pair of tyrosyl phosphorylation sites has been shown to regulate the enzymatic activity and nuclear localization of SHP-1 [Pei et al., 1994; Craggs and Kellie, 2001; Yang et al., 2002; Zhang et al., 2003]. Our studies here have focused on the role of this C-terminal tail in nuclear localization.

With different constructs of EGFP-fused SHP-1, we have confirmed the published reports that an NLS is located at the C-terminal tail of SHP-1, and this signal is defined by the KRK at the C-terminus [Craggs and Kellie, 2001; Yang et al., 2002]. In addition, we have demonstrated that endogenous SHP-1, regardless of cell type, has a cytosolic distribution (Fig. 7). However, the EGFP-fused SHP-1 shows varied subcellular localizations, depending on the position of the fused-EGFP and/or on the cell type. Our results suggest that the NLS is blocked in the native state, and the signal could be triggered by exposing the NLS onto the surface. Fusing EGFP at the C-terminus of SHP-1 or expressing the C-terminal tail of SHP-1 alone activated the NLS, which in turn led to the nuclear localization of SHP-1 (Fig. 2). However, fusing EGFP at the N-terminus of SHP-1, which still hides the NLS in the native protein, keeps the SHP-1 in the cytoplasm (Fig. 2). The data suggest that the contradictory observations of the subcellular localization of the EGFP-fused SHP-1 were due to the different location of the fused EGFP relative to the C-terminus.

SHP-1 is involved in the EGF-activated signaling pathway and positively regulates the EGF-induced STAT activation [Keilhack et al., 1998; Tenev et al., 2000]. SHP-1 binds to the EGF receptor via its two SH2 domains and

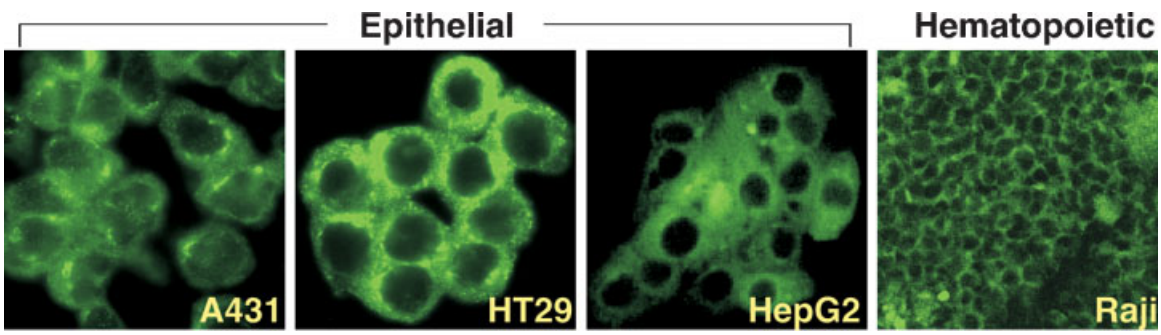


**Fig. 7.** Subcellular localization of endogenous SHP-1. Endogenous SHP-1 shows a cytosolic distribution in all three epithelial cells (A431, HT29, and HepG2) and in a hematopoietic cell line (Raji). [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

dephosphorylates the EGF receptors [Tenev et al., 1997]. Our results from both immunofluorescence and Western blot analysis have identified that EGF-stimulation can activate the NLS of SHP-1, which led to the translocation of SHP-1 from cytoplasm to nucleus in COS7 cell lines but not in A431 cell lines (Fig. 5).

It is noteworthy that the translocation of SHP-1 from cytoplasm to nuclei, in response to the stimulation of EGF or growth hormone (GH) is also dependent on cell type. In response to stimulation, translocation of SHP-1 takes place in normal cell lines such as COS7 cells, similar to the observed translocation of SHP-1 in CWSV-1 cells (rat liver cells) [Gebert et al., 1997; Ram and Waxman, 1997], but not in carcinoma cell lines (A431 cells) [Tenev et al., 2000]. In A431 cells, SHP-1 has a perinuclear but not nuclear distribution, and the induced overexpression of SHP-1 leads to the attenuation of EGF receptor auto-phosphorylation and of EGF-induced DNA binding of STAT1 and STAT3 [Tenev et al., 2000]. In CWSV-1 cells, GH also induced the nuclear translocation of SHP-1 protein, which plays a role in the deactivation of nuclear STAT5b following the termination of a plasma GH pulse in rat liver cells in vivo. The response time for the translocation after the EGF- or GH-stimulation is short [Ram and Waxman, 1997; Tenev et al., 2000], suggesting that SHP-1 is tightly involved in the signaling pathways. Further evidence for the translocation of SHP-1 in response to stimulation of cytokines has been obtained with IL-4 or IL-7 within the mouse fibroblast NIH3T3 cell lines [Yang et al., 2002]. However, in comparison with the EGF- or GH-stimulation, the response time to IL-4 or IL-7 stimulation was much longer ($\sim$6 h) [Yang et al., 2002].

EGF-stimulation can recruit SHP-1 to bind to the phosphorylated EGF receptor. SHP-1 then leads to dephosphorylation of the EGF receptor in normal cell lines such as COS7 [Yi et al., 1992]. The binding of EGF receptor to SHP-1 may result in a conformational change of SHP-1 that leads to the activation of the NLS at the C-terminus of SHP-1 that is blocked unless stimulated (by EGF, in this case). However, in several tested carcinoma cell lines, different forms of SHP-1, either endogenous SHP-1 or different EGFP fused SHP-1, were observed in the cytoplasm. In those cell lines, EGF stimulation did not target the SHP-1 into nuclei (Fig. 5). The data suggested that either the signal pathway regulating the nuclear translocation of SHP-1 was blocked, or the SHP-1 protein was kept in cytoplasm or excluded from the nuclei in the tested cancer cell lines. We have shown that EGFP-fused with the SHP-1 tail (not the full length SHP-1), regardless of the orientation, has nuclear distribution (Fig. 4), which suggests that the signal pathway for nuclear translocation is normal in the test cancer cells. Nevertheless, binding level of SHP-1 to EGF receptor may be dependent on cell type, and SHP-1 activity on signaling cascades is varied similarly, depending on the levels and activation state of critical signaling proteins [Tenev et al., 1997; You and Zhao, 1997; Keilhack et al., 1998]. In addition, SH2 domains are also thought to direct the subcellular localization of SHP-1, for example, through binding to receptors [Eck et al., 1996; Tenev et al., 1997, 2000]. Thus, examination of nuclear localization of SHP-1 in more cell lines will help us better understand how SHP-1 activity is regulated by its C-terminal tail.

In conclusion, we have shown that an NLS is located at the C-terminal tail of SHP-1, and is primarily defined by the three amino-acid residues KRK at the C-terminus. This NLS peptide is blocked in the basal state, and can be activated by EGF stimulation, which results in the translocation of SHP-1 from cytosol to nucleus in normal cell lines.

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