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# Shc proteins are phosphorylated and regulated by the v-Src and v-Fps protein-tyrosine kinases

**(Src homology 2 domain/transformation/tyrosine phosphorylation)**

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ABSTRACT **The mammalian *shc* gene encodes two overlapping proteins of 46 and 52 kDa, each with a C-terminal Src homology 2 (SH2) domain and an N-terminal glycine/proline-rich sequence, that induce malignant transformation when overexpressed in mouse fibroblasts. $p46^{shc}$, $p52^{shc}$, and an additional 66-kDa *shc* gene product become highly tyrosine phosphorylated in Rat-2 cells transformed by the v-*src* or v-*fps* oncogene. Experiments using temperature-sensitive v-*src* and v-*fps* mutants indicate that Shc tyrosine phosphorylation is rapidly induced upon activation of the v-Src or v-Fps tyrosine kinases. These results suggest that Shc proteins may be directly phosphorylated by the v-Src and v-Fps oncoproteins *in vivo*. In cells transformed by v-*src* or v-*fps*, or in normal cells stimulated with epidermal growth factor, Shc proteins complex with a poorly phosphorylated 23-kDa polypeptide (p23). Activated tyrosine kinases therefore regulate the association of Shc proteins with p23 and may thereby control the stimulation of an Shc-mediated signal transduction pathway. The efficient phosphorylation of Shc proteins and the apparent induction of their p23-binding activity in v-*src*- and v-*fps*-transformed cells are consistent with the proposition that the SH2-containing Shc polypeptides are biologically relevant substrates of the oncogenic v-Src and v-Fps tyrosine kinases.**

A sizable class of retroviral and cellular oncogenes encode nonreceptor, cytoplasmic protein-tyrosine kinases, for which the prototypes are the products of the v-*src*, v-*fps*, and v-*abl* transforming genes (1, 2). The transforming activity of these oncoproteins requires the sustained activation of their tyrosine kinase domains (3, 4), suggesting that they induce the phenotypic changes characteristic of transformed cells by phosphorylating specific cellular targets. The activated Src tyrosine kinase contains other structural features that modify its transforming activity, including an N-terminal myristoylation site (5) and noncatalytic Src homology 2 and 3 (SH2 and SH3) domains (6–14).

SH2 domains are elements of ≈95 amino acids that apparently regulate protein–protein interactions in signal transduction (15), by virtue of their ability to bind with high affinity to specific tyrosine-phosphorylated sites (16–19). SH2 domains are conserved between nonreceptor tyrosine kinases of the Src, Fps/Fes, and Abl families (20) and are also found in a variety of intracellular signaling proteins, such as Ras GTPase activating protein (GAP) (21, 22), phospholipase C-γ (23), and the regulatory subunit of phosphatidylinositol 3-kinase (24–26). The SH2 domains of these polypeptides direct high-affinity interactions with autophosphorylated sites on activated growth factor receptors (18, 27–32) and with specific cytoplasmic phosphoproteins (6, 33, 34).

A number of the proteins that become tyrosine phosphorylated in v-*src*- and v-*fps*-transformed cells have been identified (2, 14, 35). Some of these substrates themselves possess SH2 or SH3 domains, including GAP (33, 36), tensin (37), the p85 subunit of phosphatidylinositol 3-kinase (ref. 38; X. Liu and T.P., unpublished observations), and a distinct 85-kDa SH3-containing protein (39). However, the nature of the targets responsible for specific aspects of the transformed phenotype induced by v-*src* and v-*fps* remains uncertain. We have recently identified a gene, *shc*, that encodes two overlapping proteins of 46 and 52 kDa ($p46^{shc}$ and $p56^{shc}$), both of which are translated from a widely expressed 3.4-kilobase mRNA (40). $p46^{shc}$ and $p52^{shc}$ possess a C-terminal SH2 domain most closely related to those of the Fer and Fps/Fes tyrosine kinases, and a more N-terminal glycine/proline-rich region, but do not possess any readily identified catalytic domain. $p46^{shc}$ and $p52^{shc}$ apparently utilize different translation initiation sites and therefore differ at their extreme N termini. A 66-kDa Shc protein ($p66^{shc}$) has also been detected with anti-Shc antibodies and is likely encoded by a distinct *shc* transcript (40). Overexpression of $p46^{shc}$ and $p52^{shc}$ in NIH 3T3 mouse fibroblasts induces malignant transformation (40) and prompts these cells to transit the $G_1$ phase of the cell cycle in the absence of growth factors (G.P., unpublished results), suggesting that the cytoplasmic *shc* gene products may normally activate a signaling pathway that controls proliferation in response to growth factor stimulation. Consistent with this possibility, all three Shc proteins associate with, and become phosphorylated by, the epidermal growth factor (EGF) receptor in EGF-stimulated cells (40).

The ability of *shc* gene products to induce cellular transformation when aberrantly overexpressed suggested that these proteins might be downstream targets of oncogenic tyrosine kinases. To test this possibility, we have investigated the phosphorylation of Shc proteins in v-*src*- and v-*fps*-transformed cells and have identified a mechanism by which Shc proteins might stimulate intracellular signaling.

## MATERIALS AND METHODS

**Cells.** Rat-2 cells transformed by wild-type v-*src* (derived from the Schmidt-Rupin A strain of Rous sarcoma virus) or the *gag–fps* oncogene (derived from Fujinami avian sarcoma virus) have been described (20, 34, 41). Rat-1 cells expressing the temperature-sensitive (ts)LA29 v-*src* mutant (42) and Rat-1 cells expressing the human EGF receptor (R1hER) (36) were a gift of M. Weber. We employed the tsAX9m v-*fps* mutant to analyze the effects of ts v-Fps kinase activity on Shc phosphorylation (20, 43). Rat-2 cells expressing this mutant are generally phenotypically normal regardless of

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Abbreviations: SH, Src homology; EGF, epidermal growth factor; ts, temperature-sensitive.

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temperature (43). However, one Rat-2 line, CNA7, expressing a very high level of the tsAX9m P130$^{gag-fps}$ protein, is tightly ts for both P130$^{gag-fps}$ tyrosine kinase activity and the transformed phenotype (44) and was used for temperature-shift experiments. Cells were cultured in Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal bovine serum at 37°C unless otherwise indicated. For experiments involving ts mutants, cells were maintained at the nonpermissive temperature (39–39.5°C) for at least 2 days before being shifted to 35°C.

**Metabolic Labeling.** Cells were labeled with $^{32}P_i$ (1 mCi/ml; 1 Ci = 37 GBq) for 4 hr in phosphate-free DMEM, or with [$^{35}$S]methionine (150 $\mu$Ci/ml) for 16 hr in methionine-free DMEM containing 0.5% dialyzed calf serum, unless otherwise noted.

**Antibodies and Immunoprecipitation.** Rabbit anti-Shc antiserum was raised to a glutathione *S*-transferase (GST) fusion protein containing residues 366–473 of the human p52$^{shc}$ protein, and anti-Shc antibodies were affinity-purified by using the immobilized GST–Shc fusion protein (40). Immunoprecipitations were carried out essentially as described (33). All cells were lysed in PLC lysis buffer [50 mM Hepes, pH 7.0/150 mM NaCl/10% (vol/vol) glycerol/1% (vol/vol) Triton X-100/1.5 mM $MgCl_2$/1 mM EGTA/100 mM NaF/10 mM $NaPP_i$/1 mM $Na_3VO_4$/1 mM phenylmethanesulfonyl fluoride with aprotinin and leupeptin at 10 $\mu$g/ml], except for metabolically labeled cells, for which 0.1% SDS was added to the PLC lysis buffer. Lysates were preincubated with protein A-Sepharose and then incubated with 1 $\mu$g of purified antibodies per 10-cm tissue culture plate. After 90 min at 4°C, immunoprecipitates were adsorbed to protein A-Sepharose, and washed three to five times with PLC lysis buffer, or with buffer containing 0.1% SDS for lysates of metabolically labeled cells.

Immunoprecipitates from metabolically labeled cells were analyzed by SDS/PAGE, followed by autoradiography for $^{32}$P-labeled samples. Gels containing [$^{35}$S]methionine-labeled proteins were treated with EN$^3$HANCE (Amersham), dried, and exposed to XAR-5 x-ray film (Kodak) at −80°C. For Western blotting, samples were isolated by SDS/PAGE, transferred to nitrocellulose with a semidry transfer apparatus, and immunoblotted (45). Anti-Shc antibodies were used at 2 $\mu$g/ml in Tris-buffered saline (TBS; 20 mM Tris·HCl, pH 7.5/150 mM NaCl) containing 5% Carnation skim milk powder; anti-phosphotyrosine antibodies were used at 1 $\mu$g/ml in TBS containing 5% bovine serum albumin and 1% ovalbumin.

For phospho amino acid analysis, a $^{32}$P-labeled sample was resolved by SDS/PAGE and transferred to Immobilon (Millipore). Shc bands were excised, acid-hydrolyzed, and subjected to phospho amino acid analysis by electrophoresis in two dimensions at pH 1.9 and pH 3.5 (36, 46).

## RESULTS

**Shc Proteins Are Phosphorylated on Tyrosine in v-*src*- and v-*fps*-Transformed Cells.** To investigate the phosphorylation of Shc proteins in v-*src*-transformed cells, we initially immunoprecipitated lysates of normal Rat-2 fibroblasts or v-*src*-transformed Rat-2 (Rat-2 v-*src*) cells with affinity-purified anti-Shc antibodies and immunoblotted the precipitated proteins with anti-Shc or anti-phosphotyrosine antibodies. Anti-Shc antibodies recognized proteins of 46 and 52 kDa and a more minor 66-kDa polypeptide in parental Rat-2 and Rat-2 v-*src* cells (Fig. 1*A*). Shc proteins isolated from normal Rat-2 cells contained little phosphotyrosine, as measured by anti-phosphotyrosine immunoblotting, whereas the tyrosine phosphorylation of the three Shc proteins immunoprecipitated from Rat-2 v-*src* cells was markedly increased (Fig. 1*A*). p52$^{shc}$ was the most prominent phosphotyrosine-containing protein in anti-Shc precipitates of v-*src*-transformed Rat-2 cells.

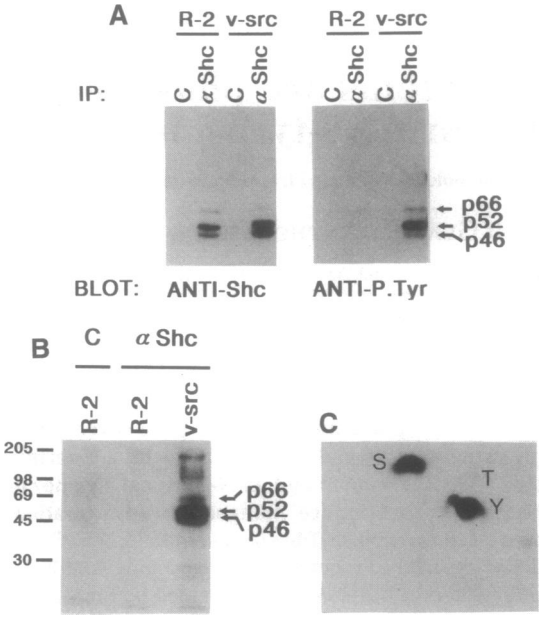


FIG. 1. Phosphorylation of Shc protein in v-*src*-transformed Rat-2 cells. (*A*) Rat-2 cells (R-2) or Rat-2 v-*src* cells (v-src) were lysed and immunoprecipitated with affinity-purified rabbit anti-Shc antibodies (αShc) or with purified rabbit anti-mouse immunoglobulin antibodies as a control (C). Immunoprecipitates (IP) were electrophoresed in an SDS/8.25% polyacrylamide gel and immunoblotted with anti-Shc or anti-phosphotyrosine (P.Tyr) antibodies. Blots were incubated with $^{125}$I-labeled protein A and then exposed to x-ray film for 4 hr. Mobilities of the 46-, 52-, and 66-kDa Shc proteins are indicated. (*B*) Rat-2 cells or Rat-2 v-*src* cells were metabolically labeled with $^{32}P_i$, lysed, and immunoprecipitated with control or anti-Shc antibodies. Mobilities and molecular masses (kDa) of size markers are indicated at left. (*C*) $^{32}$P-labeled p46$^{shc}$ and p52$^{shc}$ immunoprecipitated from radiolabeled Rat-2 v-*src* cells (*B*) were pooled, hydrolyzed, and subjected to phospho amino acid analysis by electrophoresis in two dimensions at pH 1.9 and pH 3.5. Migration of phosphotyrosine (Y), phosphothreonine (T), and phosphoserine (S) standards is indicated.

Phosphorylation of Shc proteins in Rat-2 v-*src* cells was examined directly by phospho amino acid analysis of $^{32}$P-labeled proteins. Rat-2 and Rat-2 v-*src* cells were metabolically labeled with $^{32}P_i$, lysed, and immunoprecipitated with anti-Shc antibodies (Fig. 1*B*). Consistent with the results obtained using anti-phosphotyrosine antibodies, the Shc proteins were poorly $^{32}$P-labeled in normal Rat-2 cells but displayed a striking increase in their total phosphorylation in v-*src*-transformed cells. In addition to phosphorylated Shc proteins, several more minor phosphoproteins were identified in the anti-Shc precipitates from Rat-2 v-*src* cells (Fig. 1*B*). These were probably Shc-associated proteins, since they were not recognized directly by anti-Shc antibodies in immunoblots. Similar polypeptides were detected upon prolonged exposure of anti-phosphotyrosine immunoblots of anti-Shc precipitates (data not shown). p46$^{shc}$ and p52$^{shc}$ from Rat-2 v-*src* cells contained similar levels of phosphotyrosine and phosphoserine (Fig. 1*C*). Hence, transformation by v-*src* induces elevated levels of Shc phosphorylation on both tyrosine and serine residues.

To investigate whether the tyrosine phosphorylation of Shc proteins might be of more general significance for the transforming activity of activated cytoplasmic tyrosine kinases, we used v-*fps*-transformed Rat-2 cells expressing the P130$^{gag-fps}$ oncoprotein encoded by Fujinami sarcoma virus (20, 47). All three Shc proteins were strongly phosphorylated in two dif-

ferent v-*fps*-transformed Rat-2 cell lines (Fig. 2), arguing that the *shc* gene products may be common substrates of the v-Src and v-Fps tyrosine kinase oncoproteins.

**Phosphorylation of Shc Proteins Correlates with Activation of the v-Src and v-Fps Tyrosine Kinases.** The increased tyrosine phosphorylation of Shc polypeptides in v-*src*- and v-*fps*-transformed cells suggests that they may be directly phosphorylated by the products of these oncogenes. To test this possibility, we employed a Rat-2 cell line (CNA7) expressing a high level of a ts v-*fps* mutant (tsAX9m) (20, 43). These cells are tightly ts both for $P130^{gag-fps}$ tyrosine kinase activity and for the transformed phenotype (44). CNA7 cells were held at the nonpermissive temperature (39.5°C) and then shifted to the permissive temperature (35°C). At various times after the temperature shift, cells were lysed, and immunoprecipitates were obtained with antibodies to Shc and analyzed by immunoblotting with anti-Shc or anti-phosphotyrosine antibodies. Residual tyrosine phosphorylation of $p46^{shc}$ and $p52^{shc}$ was observed in cells maintained exclusively at the nonpermissive temperature (Fig. 3*A*). However, tyrosine phosphorylation of $p46^{shc}$ and $p52^{shc}$ increased within 15 min of shifting to 35°C and continued to increase over 24 hr (Fig. 3). Phosphorylation of the Shc proteins occurred coordinately with the induction of v-Fps tyrosine kinase activity, as assessed by v-Fps autophosphorylation in the cells (data not shown). A similar study was undertaken using Rat-1 fibroblasts expressing a v-*src* mutant that is ts for both kinase and transforming activities (tsLA29) (42). Tyrosine phosphorylation of the *shc* gene products was rapidly induced upon shifting these ts v-*src* cells from the nonpermissive to the permissive temperature (Fig. 3*B*). The rapid kinetics with which Shc proteins become tyrosine phosphorylated upon shifting cells expressing ts v-*src* or ts v-*fps* mutants to the permissive temperature are consistent with the possibility that they are immediate v-Src and v-Fps substrates.

**Tyrosine Phosphorylation of Shc Proteins Correlates with Binding to a 23-kDa Protein.** $p46^{shc}$ and $p52^{shc}$ do not possess an obvious catalytic domain, suggesting that they may be adaptors that couple tyrosine kinases to a downstream Shc-binding protein. Such a protein might not have been detected in previous experiments, especially if it were not highly phosphorylated. To investigate this possibility, we metabolically labeled v-*src*- or v-*fps*-transformed cells with [$^{35}$S]methionine and examined the spectrum of polypeptides that precipitated with anti-Shc antibodies (Fig. 4). Immunoprecipitates of normal Rat-2 cells revealed only $p46^{shc}$, $p52^{shc}$, and $p66^{shc}$. However, in Rat-2 cells transformed by v-*src*, two principal differences were seen. First, a considerable fraction of all three Shc polypeptides shifted into species that migrated more slowly upon SDS/PAGE (Fig. 4*A*). This reduced

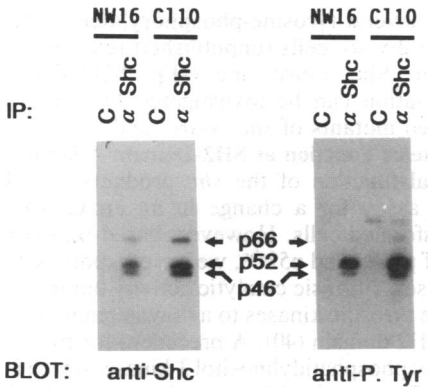


FIG. 2. Shc proteins are phosphorylated on tyrosine in v-*fps*-transformed cells. Two independently isolated *gag–fps*-transformed Rat-2 cell lines (clone 10 and NW16) were lysed and immunoprecipitated with affinity-purified anti-Shc antibodies (αShc) or with preimmune antiserum as a control (C). Immunoprecipitates (IP) were electrophoresed in an SDS/8.25% polyacrylamide gel and immunoblotted with anti-Shc or anti-phosphotyrosine (P.Tyr) antibodies. Blots were incubated with $^{125}$I-protein A and exposed to x-ray film for 24 hr.

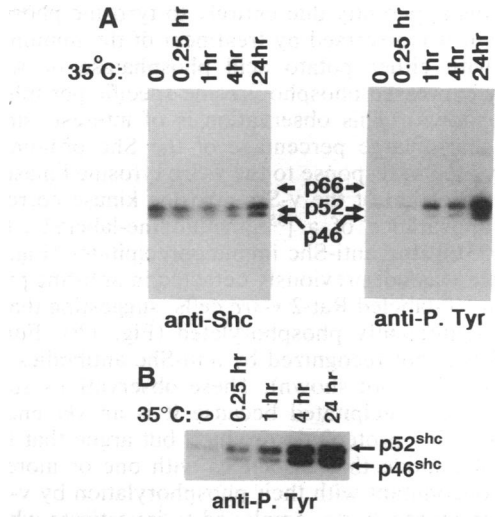


FIG. 3. Shc proteins are rapidly phosphorylated on tyrosine following activation of the v-Fps and v-Src kinases. (*A*) Rat-2 cells expressing the tsAX9m v-*fps* mutant (CNA7 cells) were held for 48 hr at the nonpermissive temperature (39–39.5°C). Cells were then shifted to the permissive temperature (35°C) for the indicated period (0 represents cells maintained at 39.5°C throughout). Cells were lysed, immunoprecipitated with anti-Shc antibodies, and immunoblotted with anti-Shc or anti-phosphotyrosine (P.Tyr) antibodies. (*B*) Rat-1 cells expressing the ts v-*src* mutant were held for 24 hr at the nonpermissive temperature for kinase and transforming activities (39.5°C). Cells were then shifted to the permissive temperature (35°C) for the indicated period (0 represents cells maintained at 39.5°C throughout). Cells were lysed, immunoprecipitated with anti-Shc antibodies, and immunoblotted with anti-phosphotyrosine antibodies. Only that portion of the blot containing the Shc proteins is shown.

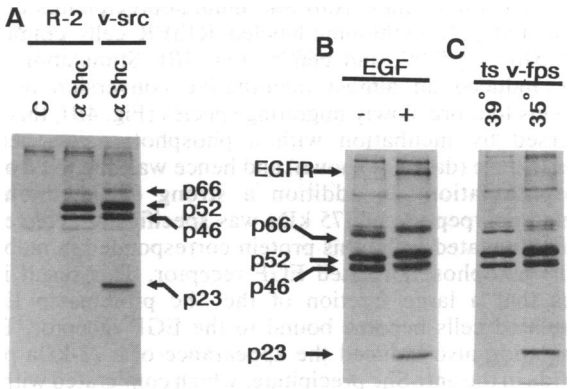


FIG. 4. (*A*) Rat-2 cells (R-2) or Rat-2 v-*src* cells (v-src) were metabolically labeled with [$^{35}$S]methionine for 4 hr, lysed, and immunoprecipitated with affinity-purified anti-Shc antibodies (αShc) or with rabbit anti-mouse immunoglobulin antibodies as a control (C). Immunoprecipitates were analyzed by SDS/10% PAGE followed by fluorography. (*B*) Rat-1 cells expressing the human EGF receptor (EGFR) were metabolically labeled with [$^{35}$S]methionine in 0.5% serum for 16 hr. Serum-starved, radiolabeled cells were either unstimulated (−), or stimulated for 15 min with EGF (+). Cells were lysed and immunoprecipitated with anti-Shc antibodies. (*C*) CNA7 Rat-2 cells expressing the tsAX9m v-*fps* mutant were maintained at 39.5°C (nonpermissive temperature) or shifted from 39.5°C to 35°C for 18 hr (permissive temperature). Cells were labeled with [$^{35}$S]methionine from the start of the temperature shift, lysed, and immunoprecipitated with anti-Shc antibodies.

mobility was apparently due entirely to tyrosine phosphorylation, as it was reversed by treatment of the immunoprecipitates with either potato acid phosphatase or with a bacterially expressed phosphotyrosine-specific phosphatase (data not shown). This observation is of interest, since it indicates that a large percentage of the Shc proteins are phosphorylated in response to the v-Src tyrosine kinases. In addition, activation of the v-Src tyrosine kinase correlated with the appearance of a [$^{35}$S]methionine-labeled 23-kDa protein (p23) in the anti-Shc immunoprecipitates (Fig. 4*A*). This protein was not previously detected in anti-Shc precipitates from $^{32}$P-labeled Rat-2 v-*src* cells, suggesting that it is not itself significantly phosphorylated (Fig. 1*B*). Furthermore, p23 was not recognized by anti-Shc antibodies in an immunoblot (data not shown). These observations suggest that p23 is not precipitated because it is an *shc*-encoded protein, or a Shc proteolytic product, but argue that it is a distinct polypeptide that associates with one or more Shc proteins concomitant with their phosphorylation by v-Src.

A similar approach was employed to investigate whether transformation by v-*fps* induces association of Shc proteins with p23. For this purpose Rat-2 cells expressing the tsAX9m v-*fps* mutant were metabolically labeled with [$^{35}$S]methionine and either maintained at the nonpermissive temperature (39.5°C), or shifted for 18 hr to the permissive temperature (35°C). Activation of the P130$^{gag-fps}$ tyrosine kinase, induced by shifting to the permissive temperature, correlated with a decreased mobility of the Shc proteins and with the appearance of p23 in the anti-Shc immunoprecipitate (Fig. 4*C*). These results suggest that the v-Fps tyrosine kinase, like v-Src, induces the extensive tyrosine phosphorylation of Shc proteins and their association with p23. The interaction of Shc proteins with p23 may therefore be a general feature of cells transformed by oncogenic tyrosine kinases.

Transformation by v-*src* and v-*fps* most likely results from the constitutive activation of signaling pathways that normally drive cell proliferation in response to growth factor stimulation. We therefore examined EGF-stimulated cells for a 23-kDa Shc-associated protein. Rat-1 cells engineered to express the human EGF receptor (R1hER) were radiolabeled with [$^{35}$S]methionine, serum-starved, and then stimulated with EGF for 15 min. Anti-Shc immunoprecipitates of unstimulated [$^{35}$S]methionine-labeled R1hER cells contained only p46$^{shc}$, p52$^{shc}$, and p66$^{shc}$ (Fig. 4*B*). Stimulation with EGF induced an almost quantitative conversion of Shc proteins to more slowly migrating species (Fig. 4*B*); this was reversed by incubation with a phosphotyrosine-specific phosphatase (data not shown) and hence was due to tyrosine phosphorylation. In addition a strong [$^{35}$S]methionine-labeled polypeptide of 175 kDa was specifically detected in EGF-stimulated cells; this protein corresponded in mobility to the autophosphorylated EGF receptor. This result indicates that a large fraction of the Shc proteins in EGF-stimulated cells become bound to the EGF receptor. EGF stimulation also induced the appearance of a 23-kDa polypeptide in the anti-Shc precipitate, which comigrated with the p23 Shc-associated protein from v-*src*- and v-*fps*-transformed cells. Shc proteins therefore complex with p23 in response to both the oncogenic v-Src and v-Fps tyrosine kinases and the activated EGF receptor.

## DISCUSSION

**Shc Proteins Are Substrates for the Transforming v-Src and v-Fps Tyrosine Kinases.** The transforming activity of the v-Src and v-Fps oncoproteins is dependent on their kinase activity and hence, in all probability, on their ability to phosphorylate specific cellular proteins on tyrosine. Biologically relevant substrates of oncogenic tyrosine kinases that contribute to their transforming activity are expected to fulfill several criteria. Such targets should become rapidly tyrosine-phosphorylated to a high stoichiometry upon activation of the v-Src tyrosine kinase, and this phosphorylation should correlate with a demonstrable functional change in the presumptive target. Furthermore, a tyrosine kinase target that plays a central role in controlling cell proliferation might be anticipated to serve as a substrate for a variety of oncogenic tyrosine kinases and to be involved in the transmission of mitogenic stimuli in normal cells. Finally, a v-Src target that actively participates in v-*src* transforming activity might itself have oncogenic potential when aberrantly expressed.

The Shc proteins meet each of these requirements to a greater or lesser extent. The p46$^{shc}$, p52$^{shc}$, and p66$^{shc}$ polypeptides are all rapidly phosphorylated on tyrosine when cells expressing ts v-*src* or v-*fps* mutants are shifted to the permissive temperature for kinase activity. This suggests that Shc proteins may be direct substrates for the v-Src and v-Fps tyrosine kinases, although it does not exclude the possibility of an intermediate tyrosine kinase. A bacterially expressed Shc polypeptide can be phosphorylated *in vitro* by v-Src (A.C. and J.M., unpublished results), consistent with the notion that Shc proteins are substrates for v-Src *in vivo*. Indeed, a considerable fraction of each Shc polypeptide became tyrosine-phosphorylated in v-*src*- or v-*fps*-transformed cells, as evidenced by the appearance of more slowly migrating species. In addition to stimulating Shc tyrosine phosphorylation, v-*src* transformation increased Shc phosphorylation on serine, indicating that Shc proteins are also substrates for a protein-serine/threonine kinase activated by v-Src.

These results suggest that Shc proteins may be *in vivo* substrates for the v-Src and v-Fps nonreceptor tyrosine kinases. Consistent with the possibility that tyrosine phosphorylation of Shc proteins is a common feature of cells transformed by oncogenic tyrosine kinases, Shc polypeptides are highly tyrosine phosphorylated in hematopoietic cells and fibroblasts expressing the P210$^{bcr-abl}$ oncoprotein (L. Puil and J.M., unpublished observation). The relatively high stoichiometry of Shc tyrosine phosphorylation in v-*src*- and v-*fps*-transformed cells suggests an unusually specific interaction between these nonreceptor tyrosine kinases and the *shc* gene products. It is of interest that v-Src, v-Fps, and the Shc proteins contain SH2 domains, which might participate in such high-affinity interactions. The binding of Shc proteins to the EGF receptor, for example (see Fig. 4*B*), is apparently mediated by the Shc SH2 domain (40). Indeed, a bacterial fusion protein containing the Shc SH2 domain complexed *in vitro* with several tyrosine-phosphorylated proteins in a lysate of Rat-2 v-*src* cells (unpublished results). The involvement of the Shc, v-Src, and v-Fps SH2 domains in Shc phosphorylation can be investigated by using appropriate site-directed mutants of *shc*, v-*src*, and v-*fps*.

**Shc Proteins Function as SH2-Domain Adaptors.** Since the biochemical function of the *shc* products is unknown, we could not assay for a change in an enzymatic activity in v-*src*-transformed cells. However, based on the primary sequences of p46$^{shc}$ and p52$^{shc}$, we have hypothesized that they do not possess intrinsic catalytic activity but may be adaptors that couple tyrosine kinases to a downstream target that itself lacks an SH2 domain (40). A precedent for this suggestion is provided by phosphatidylinositol 3-kinase, which is composed of an 85-kDa receptor-binding subunit containing SH2 and SH3 domains, associated with a 110-kDa catalytic subunit (24–26, 51). Indeed, a number of polypeptides have been identified that possess SH2 and SH3 domains but have no covalently linked enzymatic domain. Such proteins, including Crk (48), Nck (49), and Sem-5 (50), may couple tyrosine kinases to as yet unidentified catalytic subunits.

To test the possibility that Shc polypeptides might fit this model, we searched for Shc-binding proteins, other than

activated tyrosine kinases, by labeling with [$^{35}$S]methionine. The Shc proteins coprecipitated with a 23-kDa polypeptide in cells transformed by v-*src* or v-*fps* or in cells stimulated with EGF. The association of Shc proteins with p23 is therefore a normal event following EGF stimulation, which occurs constitutively in v-*src*- or v-*fps*-transformed cells. p23 is not recognized directly by available anti-Shc antibodies and therefore is presumed to coprecipitate in a complex with Shc proteins. The site of p23 binding on the Shc proteins is not known, nor can we be sure whether p23 associates with all three *shc* gene products. However, the observation that p23 is poorly phosphorylated argues against the Shc SH2 domain associating with a phosphorylated site on p23, raising the possibility that p23 interacts with the N-terminal Shc domain. These results provoke the speculation that p23 is an Shc target whose function is modified by its interactions with Shc.

From a variety of perspectives, Shc proteins are good candidates for biologically relevant substrates of the v-Src and v-Fps tyrosine kinases. In addition to becoming phosphorylated in fibroblasts transformed by v-*src* or v-*fps* or stimulated by EGF, *shc* gene products are strongly phosphorylated in primary mast cells stimulated with the ligand for the Kit receptor tyrosine kinase and in activated T and B cells (L. Puil, J.M., and G.P., unpublished results). The broad range of extracellular stimuli that elicit Shc tyrosine phosphorylation argues that these SH2-containing proteins are important regulators of signal transduction in a variety of normal cells and potential targets of oncogenic tyrosine kinases in transformed cells.

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