**Interaction of the Adaptor Protein Shc and the Adhesion Molecule Cadherin**

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In mitogenic signaling pathways, Shc participates in the growth factor activation of Ras by interacting with activated receptors and/or the Grb-2-Sos complex. Using several experimental approaches we demonstrate that Shc, through its SH2 domain, forms a complex with the cytoplasmic domain of cadherin, a transmembrane protein involved in the Ca\(^{2+}\)-dependent regulation of cell-cell adhesion. This interaction is demonstrated in a yeast two-hybrid assay, by co-precipitation from mammalian cells, and by direct biochemical analysis in vitro. The Shc-cadherin association is phosphotyrosine-dependent and is abrogated by addition of epidermal growth factor to A-431 cells maintained in Ca\(^{2+}\)-free medium, a condition that promotes changes in cell shape. Shc may therefore participate in the control of cell-cell adhesion as well as mitogenic signaling through Ras.

Shc (1, 2) is an adaptor protein and tyrosine kinase substrate that contains an N-terminal phosphotyrosine-binding (PTB) domain (3), a central collagen-like region that contains three tyrosine phosphorylation sites (4–6), and a C-terminal src homology 2 (SH2) domain (see Fig. 1A). The SH2 domain recognizes phosphotyrosine but in a manner mechanistically and structurally distinct from the PTB domain. Although Shc is known to participate in Ras activation by growth factors, the properties of *Drosophila* Shc have suggested participation in other, unknown pathways (7). This is likely to occur through protein-protein associations because Shc has no catalytic function. In growth factor-dependent signal transduction, Shc phosphorylates residues mediate association with the Grb-2-Sos complex involved in Ras activation (8), whereas the PTB domain recognizes NPXY sequences in several autophosphorylated growth factor receptors and other tyrosine phosphorylated molecules (3). Nonphosphorylated residues within the collagen-like region of Shc mediate an interaction with α-adaptin, a coated-pit component (9) implicated in the endocytosis of growth factor receptors. The functional significance of this interaction is as yet unknown. Whereas the identity of association partners with the SH2 domain of Shc is unclear, over-expression of the Shc SH2 domain attenuates growth factor-induced mitogenesis in a dominant-negative manner (10–12). We present evidence that this SH2 motif mediates an interaction between Shc and cadherins, transmembrane cell-cell adhesion receptors, suggesting a function of Shc in the maintenance of cell-cell adhesion and cell shape.

**EXPERIMENTAL PROCEDURES**

**Materials**—The antibodies used were rabbit IgG fractions to phosphotyrosine (Transduction Laboratories, horseradish peroxidase-coupled), to cadherin (pan-cadherin, ICOS Corp.), and to Shc for Western blotting (Transduction Laboratories). For the immunoprecipitation of Shc, antisera to recombinant p52 Shc was produced. The coding sequence for the 52-kDa form of Shc was cloned into the pXH-LT-B baculovirus transfer vector (Pharmingen, Corp.) and transferred into sf9 insect cells. The His-tagged Shc protein was overexpressed in High 5 insect cells and purified via Ni\(^{2+}\) affinity chromatography (Qiagen). 150 μg of p52Shc from the 150 mM imidazole elution fraction was used as immunogen to subcutaneously inject a rabbit. Following three booster injections, immune serum was harvested. Sodium orthovanadate was purchased from Fisher, and hydrogen peroxide was from Sigma.

**Yeast Two-hybrid Screen**—The SH2 domain (residues 373–469) of Shc (1) was fused to the LexA DNA-binding domain and used as bait to screen a mouse 10-day embryo library fused to VP16 transcription activation domain. The yeast two-hybrid assay with this library was carried out essentially as described elsewhere (13, 14) except that to tyrosine phosphorylate library proteins a constitutive active form of c-Src under the control of ADH1 promoter (15) was cloned into the *NaeI* site of the same bait plasmid containing the LexA-Shc-SH2 construct (pBTM116 Shc-SH2+c-Src).

**Immunoprecipitation**—A-431 and NIH 3T3 cells were grown in Dulbecco’s modified Eagle’s medium with 10% calf serum at 37 °C under 5% CO\(_2\). Upon reaching confluence and growth factor treatment (where indicated), cells were lysed in TGB buffer (1% Triton X-100, 10% glycerol, 50 mM Hepes, pH 7.2, and 100 mM NaCl) supplemented with 10 ng/ml leupeptin, 10 ng/ml aprotinin, 544 μM iodoacetamide, 1 mM phenylmethylsulfonyl fluoride, and 1 mM sodium vanadate. 5 μl preimmune serum or serum containing p52Shc antibody were added to 500 μg of cell lysate, and after incubation at 4 °C for 2 h, immunocomplexes were washed with TBST buffer (50 mM Tris-HCl, pH 7.4, 150 mM NaCl, 1% Tween 20, and 3% bovine serum albumin) and incubated with primary antibody in TBST buffer for 1 h at room temperature. The blots were washed with TBST buffer without 3% bovine serum albumin, followed by incubation with secondary antibody conjugated to horseradish peroxidase (Zymed) for 1 h. The filters were then washed with TBST buffer, incubated with ECL working solution (Amersham Corp.) for 1 min, and exposed to x-ray film.

**In Vitro Tyrosine Phosphorylation of GST-N-Cadherin C Terminus**—Clone S24 corresponding to the N-cadherin intracellular domain (residue 792–906) was fused in-frame to GST, expressed in *Escherichia coli*, and purified on glutathione-Sepharose 4B beads according to the manufacturer’s manual (Pharmacia Biotech Inc., pGEX-5X-1 vector). Purified c-Src (Upstate Biotechnology Inc.) was used in kinase assays according to the manufacturer’s instructions.

**Gel Overlay Assay**—The nitrocellulose filter was treated with 6 M guanidine hydrochloride to denature proteins at 4 °C for 10 min in Hyb buffer (20 mM Hepes, pH 7.6, 75 mM KCl, 0.1 mM EDTA, 2.5 mM MgCl\(_2\), 1 mM dithiothreitol, and 0.05% Nonidet P-40), and proteins were renatured at 4 °C by five successive dilutions (40 min each) of the guanidine
These results indicate a putative recognition of cadherin by the SH2 domain of Shc in the presence and the absence of c-Src. Also, the central region of PLC-π interacts with clone S24 in the two-hybrid assay in the presence and the absence of c-Src. No evidence for Shc-cadherin association in vivo was observed under the same conditions as in the two-hybrid assay. Two-hybrid Assay—To identify tyrosine phosphorylated molecules that recognize the SH2 domain of Shc, a modified yeast two-hybrid screen was performed in a system that included the SH2 domain fused to the LexA DNA-binding domain, a mouse embryo library (13, 14) fused to the VP16 transactivating domain, and a constitutively active form of the tyrosine kinase c-Src regulated by the ADH1 promoter (15), because SH2 interacting molecules are expected to contain phosphotyrosine. One positive clone, clone S24 (Fig. 1B), contained sequences that when translated correspond to residues 792–906 within the cytoplasmic domain of mouse N-cadherin (Fig. 1B). Cadherins are transmembrane proteins that regulate cell-cell adhesion in a Ca\(^{2+}\)-dependent manner (16). The cytoplasmic domains of the three major cadherins are relatively conserved in sequence, particularly within the region corresponding to clone S24 (Fig. 1C). Clone S24 was subsequently retested in the two-hybrid assay in the presence and the absence of c-Src. No interaction of S24 with the Shc SH2 motif was detected in the absence of c-Src. Also, the central region of PLC-α1, which contains two SH2 domains, did not, when substituted for the Shc SH2 domain, interact with clone S24 in the presence of c-Src. These results indicate a putative recognition of cadherin by the SH2 domain of Shc.

**Shc-Cadherin Association in Vivo**—To determine whether the native Shc protein interacts with cadherin, co-immunoprecipitation assays were performed with A-431 and NIH 3T3 cells. The results shown in Fig. 2 demonstrate the specific co-precipitation of cadherin in Shc immunoprecipitates obtained from both cell types and in the absence of exogenous growth factor stimulation. Therefore, under typical cell culture conditions the association of Shc and cadherin is constitutive and likely dependent on the basal activity of tyrosine kinases.

The extracellular domain of cadherins binds Ca\(^{2+}\) and mediates Ca\(^{2+}\)-dependent cell-cell association. This recognition event involves the lateral dimerization of cadherin molecules (17–19) and the homophilic association of cadherin extracellular domains between adjacent cells (16). Cell-cell interaction then transmits biochemical signals through the cadherin cytoplasmic domain to effector molecules, such as the catenins, that bring about changes in actin cytoskeletal structure.

When placed in Ca\(^{2+}\)-free medium, adherent and spread-out A-431 cells undergo a rapid morphological change to a rounded morphology following the addition of epidermal growth factor (EGF) (20). Given the Ca\(^{2+}\) dependence of cadherin function in cell-cell association, we examined the state of Shc association with cadherin in the presence or the absence of extracellular Ca\(^{2+}\) and EGF. The results presented in Fig. 3A demonstrate that the addition of EGF to A-431 cells in Ca\(^{2+}\)-containing medium has no significant influence on cadherin co-precipitation with Shc (lanes 1 and 2). However, the cells were placed in a Ca\(^{2+}\)-free medium. Because the incubation period for this experiment was 30 min and cell rounding occurs within this time, the observed loss of cadherin association with Shc could be a consequence of the change in cell shape that occurs when EGF is added to cells in the Ca\(^{2+}\)-free medium. Therefore, under the same conditions A-431 cells were analyzed for Shc-cadherin association at much earlier times (1–30 min). As shown in Fig. 3B, cadherin association with Shc was significantly decreased 1 min (lane 2) after the addition of EGF to A-431 cells in this Ca\(^{2+}\)-free medium. Hence, cadherin interaction with Shc is disrupted prior to observable changes in cell morphology. However, the biochemical mechanism underlying dissociation of the Shc-cadherin complex under these experimental conditions is not known.

In this assay system, prolonged incubation in Ca\(^{2+}\)-free medium without EGF does decrease Shc-cadherin association to a moderate extent. However, the addition of EGF dramatically enhances the rapidity and extent of complex dissociation. The low level of cadherin that remains detectable in Shc immunoprecipitates obtained from cells treated with EGF in Ca\(^{2+}\)-free medium (Fig. 3B, lanes 4 and 6), is due, at least in part, to cadherin present nonspecifically in precipitates obtained from...
tyrosine on cadherin.

**She Association with Tyrosine Phosphorylated Cadherin in Vitro**—To determine whether Shc interacts directly with cadherin and to resolve the issue of whether cadherin must be tyrosine phosphorylated to affect this association, the *in vitro* experiments described in Fig. 5 were performed. The clone S24 was expressed as a GST fusion protein and purified by absorption on glutathione-Sepharose. As a control, GST was absorbed to the glutathione matrix. Aliquots of GST and GST-cadherin were then incubated with c-Src in the presence or the absence of ATP, eluted from the column with glutathione, separated by SDS-PAGE, and, following transfer to nitrocellulose, blotted with anti-phosphotyrosine (Fig. 5A) or anti-GST (Fig. 5B). The results show clearly that GST-cadherin is tyrosine phosphorylated in the presence of c-Src and ATP.

In a parallel experiment, following incubation with c-Src and/or ATP, GST and GST-cadherin were transferred to filters, denatured in 6 M guanidine HCl, and then gradually renatured. The filters were subsequently incubated with a baculovirus expressed, purified p52 form of Shc. After washing, the filters were incubated with anti-Shc. As shown in Fig. 5C, Shc association with GST-cadherin was detected only in those samples where GST-cadherin had been previously incubated with c-Src and ATP. This demonstrates a direct interaction between Shc and the tyrosine phosphorylated cytoplasmic domain of cadherin. As shown in Fig. 5D, this direct interaction was also observed when isolated p52 Shc was added to Sepharose beads coupled to GST or GST-cadherin, which had been preincubated with c-Src and/or ATP. Following washing of the beads, elution

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**Fig. 3.** Modulation of Shc-cadherin complexes by EGF and extracellular Ca\(^{2+}\). A, confluent A-431 cells incubated overnight in serum-free Dulbecco's modified Eagle's medium were washed with Ca\(^{2+}\)-free phosphate-buffered saline (PBS) and placed in Ca\(^{2+}\)-free PBS. After 10 min, EGF (200 ng/ml) was added as indicated. The cells were then incubated at 37 °C for 30 min (lanes 1–4). As described above, the cells were then lysed, and equal aliquots of lysate were incubated with anti-Shc. The immunoprecipitates (IP) were processed for Western blotting with either anti-cadherin (upper half of filter) or anti-Shc (lower half of filter). B, the same experiment described above analyzed at various times after the addition of EGF (200 ng/ml) to A-431 cells in Ca\(^{2+}\)-free medium.

**Fig. 4.** Influence of pervanadate on cadherin tyrosine phosphorylation and Shc association with cadherin. Pervanadate was prepared by mixing of sodium orthovanadate and hydrogen peroxide at a molar ratio of 1:1 and incubating at room temperature for 20 min. A, A-431 cells were incubated with or without pervanadate (0.5 mM) for 40 min at 37 °C. The cells were then lysed, and equal aliquots were subjected to precipitation with anti-cadherin and Western blotting (WB) with anti-phosphotyrosine (anti-pY) or anti-cadherin. Arrow indicates position of cadherin. B, lysates of cells incubated with or without pervanadate were precipitated with anti-Shc or anti-cadherin and then analyzed by Western blotting with the indicated antibody. Each filter was cut to allow simultaneous blotting with anti-cadherin (upper panels) and anti-Shc (lower panels). The arrow indicates p52 Shc. IP, immunoprecipitation.
Shc-Cadherin Association

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with glutathione, and SDS-PAGE, Western blotting showed that they associated only with tyrosine phosphorylated GST-cadherin.

Physiological requirements for cell proliferation, particularly within tissues, include the coordinated modulation of intracellular functions, such as nuclear transcription and cytoskeletal structure, with changes in the relationship of cells to their immediate extracellular environment, such as neighboring cells and the extracellular matrix. Cadherins represent a major molecular system by which cell-cell adhesion occurs. The results described in this manuscript demonstrate a Shc-cadherin association that is modulated by extracellular Ca^{2+} and EGF. This raises the possibility that Shc, a tyrosine kinase substrate, may participate in the control of cadherin function in addition to its known role in the mitogenic activation of Ras and thereby nuclear signaling. Interaction of cells with the extracellular matrix is mediated by receptors termed integrins. Recently, Shc association with the tyrosine phosphorylated β₁ integrin subunit has been reported (28). The β₁-Shc complex has been shown to be dissociated by EGF treatment of cells (29) and the loss of Shc association capacity by integrins results in aberrant cell cycle progression (30). Hence Shc may function to coordinate multiple alterations in cell physiology necessary for proliferation.

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