The cell surface retention sequence (CRS) binding protein-1 (CRSBP-1) is a newly identified membrane glycoprotein which is hypothesized to be responsible for cell surface retention of the oncogene v-sis and c-sis gene products and other secretory proteins containing CRSs. In simian sarcoma virus-transformed NIH 3T3 cells (SSV-NIH 3T3 cells), a fraction of CRSBP-1 was demonstrated at the cell surface and underwent internalization/recycling as revealed by cell surface $^{125}$I labeling and its resistance/sensitivity to trypsin digestion. However, the majority of CRSBP-1 was localized in intracellular compartments as evidenced by the resistance of most of the $^{35}$S-metabolically labeled CRSBP-1 to trypsin digestion, and by indirect immunofluorescent staining. CRSBP-1 appeared to form complexes with proteolytically processed forms (generated at and/or after the trans-Golgi network) of the v-sis gene product and with a $\sim$140-kDa proteolytically cleaved form of the platelet-derived growth factor (PDGF) $\beta$-type receptor, as demonstrated by metabolic labeling and co-immunoprecipitation. CRSBP-1, like the v-sis gene product and PDGF $\beta$-type receptor, underwent rapid turnover which was blocked in the presence of 100 $\mu$m suramin. In normal and other transformed NIH 3T3 cells, CRSBP-1 was relatively stable and did not undergo rapid turnover and internalization/recycling at the cell surface. These results suggest that in SSV-NIH 3T3 cells, CRSBP-1 interacts with and forms ternary and binary complexes with the newly synthesized v-sis gene product and PDGF $\beta$-type receptor at the trans-Golgi network and that the stable binary (CRSBP-1-v-sis gene product) complex is transported to the cell surface where it presents the v-sis gene product to unoccupied PDGF $\beta$-type receptors during internalization/recycling.

Many secretory proteins, including growth factors and cytokines, have been demonstrated to undergo cell surface retention during biosynthesis and secretion in cultured cells (1–8). These proteins are secreted but remain tightly associated with the cell surface without being released into the medium or extracellular compartment. The cell surface-retained proteins may be released upon treatment with suramin, heparin, or high salt or by protease digestion (1, 5, 6, 8). Upon longer incubation (days), the cell surface-retained proteins may be released (mainly as degraded forms) or diffuse slowly and become associated with extracellular matrix (1, 8, 9). The proteins undergoing cell surface retention possess cell surface retention sequence (CRS)$^3$ motifs which include a stretch of basic amino acids; these motifs are responsible for their cell surface retention (1–8). The cell surface retention of secretory proteins is hypothesized to be mediated by high-affinity cell surface-binding sites which have been recently identified by binding and cross-linking of $^{125}$I-labeled peptides containing the CRS motifs of platelet-derived growth factor-B (c-sis gene product) and vascular endothelial cell growth factor (8). A major CRS-binding protein, designated CRSBP-1, has been purified to near homogeneity from plasma membranes of cultured cells and liver tissue (8). CRSBP-1 is a major binding protein for CRS peptides which is expressed in all cell types thus far examined including fibroblasts, endothelial cells, epithelial cells, and smooth muscle cells (8).$^2$

The viral oncogene v-sis product of simian sarcoma virus (SSV) was the first protein reported to undergo cell surface retention during secretion (1). The cell surface retention of the v-sis gene product appeared to facilitate its binding to the PDGF receptor at the cell surface of SSV-transformed cells (1). The c-sis gene product (PDGF-B), the cellular homologue of the v-sis gene product, was subsequently shown to undergo this novel secretion process and its major structural determinant (CRS) was localized near its C terminus (2, 6). Deletion of this major CRS in the PDGF-B molecule diminished its cell surface retention and enhanced its secretion but did not affect the transforming activity of the c-sis gene product as measured by an in vitro foci assay (6). This strongly argued that the major CRS was not absolutely required for the transforming activity of the c-sis gene product. However, the in vitro foci assay used to determine the transforming activity of the c-sis gene product did not yield information concerning its tumorigenicity. Furthermore, the c-sis gene product may contain other minor or cryptic CRSs which contribute to partial cell surface retention even after deletion of the major CRS near its C terminus (2, 6, 8). The CRSs of polypeptide growth factors are well conserved in evolution (2, 6–10), suggesting biological importance of cell surface retention of these proteins.

v-sis- and c-sis-transformed fibroblasts have been the best studied systems of autocrine transformation (1, 10, 11, 21–30).

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The abbreviations used are: CRS, cell surface retention sequence; CRSBP, CRS-binding protein; SSV, simian sarcoma virus; ER, endoplasmic reticulum; TGN, trans-Golgi network; STI, soybean trypsin inhibitor; DMEM, Dulbecco’s modified Eagle’s medium; PAGE, polyacrylamide gel electrophoresis; PBS, phosphate-buffered saline; NBR, normal rat kidney.

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$^1$ The abbreviations used are: CRS, cell surface retention sequence; CRSBP, CRS-binding protein; SSV, simian sarcoma virus; ER, endoplasmic reticulum; TGN, trans-Golgi network; STI, soybean trypsin inhibitor; DMEM, Dulbecco’s modified Eagle’s medium; PAGE, polyacrylamide gel electrophoresis; PBS, phosphate-buffered saline; NBR, normal rat kidney.

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$^2$ C. Boensch, S. S. Huang, D. T. Connolly, and J. S. Huang, unpublished results.

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In v-sis- and v-sis-transformed cells, the newly synthesized PDGF β-type receptor is activated by the newly synthesized v-sis or v-sis gene product during cellular routing of both proteins from the endoplasmic reticulum (ER) to plasma membranes or extracellular compartment (1, 8, 22, 23, 28–30). The ER and Golgi complex interactions of the PDGF β-type receptor and v-sis or v-sis gene product result in the rapid turnover of both proteins (1, 22, 23). A fraction of the newly synthesized v-sis or v-sis gene product, which does not interact with the PDGF β-type receptor in these intracellular compartments, is secreted and retained at the cell surface (1). The cell surface-released v-sis or v-sis gene product is then efficiently transferred to unoccupied cell surface PDGF β-type receptors (1). To test the hypothesis that CRSBP-1 is responsible for cell surface retention of the v-sis gene product, we investigated the interactions of CRSBP-1 with the v-sis gene product and PDGF β-type receptor in SSV-transformed cells. If CRSBP-1 is able to present the v-sis gene product to the PDGF β-type receptor at the cell surface and/or in intracellular compartments (22), CRSBP-1 should have the potential to form complexes with the v-sis gene product and PDGF β-type receptor in SSV-transformed cells. In this article, we demonstrate that the majority of CRSBP-1 is present in intracellular compartments (endosomes/presynaptic compartments and TGN) of SSV-NH 3T3 cells and normal NIH 3T3 cells. CRSBP-1 forms complexes with the v-sis gene product in SSV-NH 3T3 cells as demonstrated by metabolic labeling and co-immunoprecipitation. CRSBP-1 also forms complexes specifically with a ~140-kDa PDGF β-type receptor which represents an activated (phosphorylated) and proteolytically cleaved form of the PDGF β-type receptor as demonstrated by co-immunoprecipitation/immunocomplex phosphorylation analysis. Like the v-sis gene product and PDGF β-type receptor, CRSBP-1 undergoes rapid turnover which can be blocked by suramin. The cell surface CRSBP-1 exhibits ligand-dependent internalization and recycling. These results raise the possibility that CRSBP-1 may play an important role in the autocrine growth of SSV-transformed cells. The finding of a proteolytically cleaved form (identified as a ~140-kDa phosphorylated protein) of the PDGF β-type receptor specifically complexed with CRSBP-1 suggests a novel mechanism of signal transduction in autocrine growth.

**EXPERIMENTAL PROCEDURES**

**Materials—**Na125I (17 Ci/mg), Tran 35S-label (>1,000 Ci/mmol), I[35S]cytosteine (>800 Ci/mmol), and I[35S]ATP (4,500 Ci/mmol) were purchased from ICN Biochemicals (Irvine, CA). Suramin was obtained from FBA Pharmaceuticals (West Haven, CT). Iodoacetamide, N-ethylmaleimide, glucose oxidase, lactoperoxidase, trypsin, soybean trypsin inhibitor (STI), Triton X-100, phenylmethylsulfonyl fluoride, bovine serum albumin, chloramine T, high molecular mass protein standards (myosin, 205 kDa; β-galactosidase, 116 kDa; phosphorylase b, 97 kDa; bovine serum albumin, 66 kDa; ovalbumin, 45 kDa; carbonic anhydrase, 29 kDa), and low molecular weight standards (bovine serum albumin, 66 kDa; ovalbumin, 45 kDa; pepsin, 34.7 kDa; trypsinogen, 24 kDa; β-lactoglobulin, 18.4 kDa; lysozyme, 14.3 kDa) were obtained from Sigma. IgGsorb was obtained from the Enzyme Center (Malden, MA), and protein A-Sepharose beads were purchased from Life Technologies, Inc. (Grand Island, NY). Antiserum to PDGF-Bβ-sis gene product was raised in rabbits as described previously (1, 31). Antiserum to the PDGF β-type receptor was raised in rabbits according to our published procedure (22) and was used for most experiments, or purchased from Oncogene Research Products (Cambridge, MA). For preparation of anti-serum to CRSBP-1, a C-terminal cysteinated peptide whose amino acid sequence was derived from the N-terminal amino acid sequence of CRSBP-1 was synthesized and conjugated to bovine thyroglobulin (8). CRSBP-1 was synthesized and conjugated to bovine thyroglobulin (8). The cell surface labeled with Tran 35S-label and chased for 2 h or cell surface labeled with Na125I as described above. Labeled cells were cooled on ice and washed twice with ice-cold bicarbonate-free DMEM. Following the washes, the cells were treated with trypsin (2 mg/ml) on ice for 2 h. STI was then added to a final concentration of 6 mg/ml to stop the digestion reaction. In the control in which the cells were treated without trypsin, STI was added prior to the addition of trypsin. Cells treated with and without trypsin were collected, washed twice with bicarbonate-free DMEM containing 6 mg/ml STI, treated with RIPA buffer, and immunoprecipitated using antiserum to CRSBP-1.

**Indirect Immunofluorescent Staining—**Cells were grown on glass coverslips and fixed with methanol at −20°C for 15 min. Fixed cells were washed and rehydrated in PBS and blocked with 6% bovine serum albumin and 3% normal goat serum for 45 min at room temperature. For the first antibody reaction, cells were incubated with either normal rabbit serum or antiserum to CRSBP-1 (1:200 dilution) in the presence and absence of 2 μg peptide antigen (8) for 2 h at room temperature. Cells were washed and incubated with fluorescein isothiocyanate-conjugated goat anti-rabbit IgG (Cappel, NC) at 1:50 h at room temperature. Coverslips were washed and mounted in VECTASHIELD (Vector Laboratories, CA) and viewed under an Olympus AHRB3 microscope using interference blue filters.

**Internalization and Recycling of Cell Surface CRSBP-1—**Following cell surface iodination, cells were washed once with bicarbonate-free DMEM (pHwarmed to 37°C). The cells were then incubated at 37°C for various time periods as indicated. After incubation, cells were cooled...
to 0 °C on ice and treated with 2 mg/ml trypsin on ice for 2 h as described above. Control cells were kept on ice after cell surface iodination and treated with trypsin in the presence of STI (6 mg/ml). Finally, cells were solubilized with RIPA buffer and the cell extracts were immunoprecipitated with antiserum to CRSBP-1.

**Autophosphorylation of the PDGF β-Type Receptor in the Immunoprecipitates**—SSV-NIH 3T3 cells were lysed in 1% Triton X-100 buffer, diluted 10-fold with 20 mM Hepes-NaOH, pH 7.4, 50 mM NaCl and immunoprecipitated with antiserum to the PDGF β-type receptor or antiserum to CRSBP-1 in the presence of protein A-Sepharose. The immunoprecipitates were washed with 0.1% Triton X-100 buffer. The autophosphorylation reaction of the PDGF β-type receptor was started by addition of 5 μCi/μl [γ-32P]ATP and 3.2 mM MnCl₂. After 10 min at 0 °C, the immunoprecipitates were washed three times with 0.1% Triton X-100 buffer. The antibody-antigen complexes in the immunoprecipitates were then dissociated in the presence of 1% SDS and re-immunoprecipitated with antiserum to the PDGF β-type receptor as described above.

**RESULTS**

**CRSBP-1 Is Expressed at the Cell Surface of SSV-NIH 3T3 Cells and Normal NIH 3T3 Cells**—The newly synthesized v-sis gene product is secreted but retained at the cell surface of SSV-NIH 3T3 cells (1). If CRSBP-1 is the protein responsible for cell surface retention of the v-sis gene product, SSV-NIH 3T3 cells should express CRSBP-1 at its cell surface. To determine the cell surface expression of CRSBP-1, proteins on the cell surface of SSV-NIH 3T3 cells were labeled with [35S]methionine at 0 °C using the lactoperoxidase method (32). The [35S]-labeled cell lysates were then immunoprecipitated with antiserum to CRSBP-1 which reacts with an N-terminal region in the cytoplasmic domain of CRSBP-1, a type II membrane glycoprotein (8, 33). These were analyzed by SDS-PAGE and autoradiography. As shown in Fig. 1, both monomeric and dimeric forms (Fig. 1A, lane 1), and the monomeric form (Fig. 1B, lane 1) of CRSBP-1 were identified in the cell lysates by analysis on SDS-PAGE under nonreducing and reducing conditions, respectively. This result suggests that CRSBP-1 is present at the cell surface and can form disulfide-linked dimers in SSV-NIH 3T3 cells. Only the monomeric form of CRSBP-1 was detected in normal NIH 3T3 cells by analysis on SDS-PAGE under either reducing or nonreducing conditions (data not shown).

A Majority of CRSBP-1 Is Present in Intracellular Compartment—We hypothesized that CRSBP-1 forms complexes with the newly synthesized v-sis gene product in the TGN before the complex is transported to the cell surface (1, 8). To see if CRSBP-1 is present in intracellular compartments, CRSBP-1 was metabolically labeled with Tras35S-label in SSV-NIH 3T3 cells. After pulse and chase for 5 h to allow steady-state cellular distribution of CRSBP-1, cells were treated with trypsin. 35S-Labeled cell lysates were then immunoprecipitated using antiserum to CRSBP-1. The immunoprecipitates were analyzed by SDS-PAGE and fluorography. As shown in Fig. 2, the trypsin treatment of cells did not greatly affect the recovery of 35S-labeled CRSBP-1 in immunoprecipitates. Nearly all of 35S-labeled CRSBP-1 appeared to be recovered in the immunoprecipitates of cells treated with trypsin when compared with the amount of 35S-labeled CRSBP-1 in the immunoprecipitates of cells treated without trypsin (Fig. 2A, lane 2 versus lane 1). In a control experiment, the trypsin treatment completely abrogated the appearance of 35S-labeled cell surface CRSBP-1 in the immunoprecipitates (Fig. 2B, lane 2 versus lane 1). These results suggest that a majority of metabolically labeled cellular CRSBP-1 (>95%) is present in intracellular compartments, explaining its resistance to trypsin digestion.

To define the subcellular localization of CRSBP-1, we performed indirect immunofluorescent staining of SSV-NIH 3T3 cells using antiserum to CRSBP-1 and fluorescein isothiocyanate-conjugated goat anti-rabbit IgG. As shown in Fig. 3A, CRSBP-1 was localized in the vesicles near extranuclear membranes which may represent TGN and endosomes/prelysoso-
plus 2 mM peptide antigen (cubated with antiserum to CRSBP-1 (by indirect immunofluorescent staining. Cells were fixed and in-
labelled with [35S]cysteine. The 35S-labeled cells were then ex-
tracted with Triton X-100 or RIPA buffer (1, 22). The Triton
X-100 or RIPA buffer extracts were immunoprecipitated with
antisera to CRSBP-1 and/or after exit from TGN. p27 is a proteolytic product of the cell surface-bound p44 and does not contain the major CRS (1). However, under
mild conditions (cell lysis in 0.1% Triton X-100), the various
size forms of the v-sis gene product (p58, p50, p44, p36, and
p27) were detected in SSV-NIH 3T3 cells in this study (Fig. 4,
lanes 1 and 4). p50 and p36 were not detected in the previous more
restrictive conditions of cell lysis (0.1% SDS) (1) and may rep-
resent degradation products of p58 and p44, respectively. p27
was not detected on the autoradiogram of the co-immunopre-
icipitates (even after longer time exposure of the autoradi-
ogram). These results suggest that CRSBP-1 interacts mainly
with the v-sis gene products p44 and p36 which are produced at
and/or after TGN (1).

CRSBP-1 Undergoes Rapid Turnover in SSV-NIH 3T3 Cells
but Not in Normal NIH 3T3 Cells—The v-sis gene product
undergoes rapid turnover in SSV-transformed cells (1). If
CRSBP-1 forms complexes with the v-sis gene product in SSV-
NIH 3T3 cells, CRSBP-1 should undergo rapid turnover as the
v-sis product does (1). We, therefore, determined the turnover
rate of CRSBP-1 in SSV-NIH 3T3 cells and normal NIH 3T3
cells using pulse and chase experiments. As shown in Fig. 5,
CRSBP-1 underwent rapid turnover with a $t_{1/2}$ of $\sim$2.5 h in
SSV-NIH 3T3 cells (Fig. 5, A and C). In contrast, CRSBP-1 was
relatively stable even after a 4-h chase in normal NIH 3T3 cells
(Fig. 5, B and C). The rapid turnover of CRSBP-1 in SSV-NIH 3T3
cells was completely blocked by suramin, which is also
known to block the intracellular turnover of the v-sis gene
product and PDGF $\beta$-type receptor in SSV-transformed cells (1,
Interestingly, suramin not only blocked the turnover of CRSBP-1 but also increased the accumulation of CRSBP-1 in normal NIH 3T3 cells, which do not express the v-sis gene product (Fig. 5, A and C). Suramin did not affect the stability of CRSBP-1 in normal NIH 3T3 cells which do not express the v-sis gene product (Fig. 5, A and C). The suramin blocking of CRSBP-1 turnover appeared to be specific since it did not alter the stability of overall cellular proteins as determined by measuring total trichloroacetic acid precipitable 35S-labeled proteins (data not shown). Since suramin is also an inhibitor for the ligand binding activity of CRSBP-1 (8), these results suggest that suramin may abolish the turnover of CRSBP-1 by blocking the complex formation of CRSBP-1 and the v-sis gene product in SSV-NIH 3T3 cells. The slower turnover rate of CRSBP-1 compared with those of the v-sis gene product and PDGF β-type receptor (1, 22) may be due to the large size of its pool.

Cell Surface CRSBP-1 Undergoes Internalization and Recycling in SSV-NIH 3T3 Cells but Not in Normal NIH 3T3 Cells—Since the majority of CRSBP-1 is present intracellularly, the turnover of CRSBP-1 should occur intracellularly as well (Fig. 5, A and C). It was therefore of interest to see if cell surface CRSBP-1 undergoes ligand-dependent turnover. To examine the stability of cell surface CRSBP-1, CRSBP-1 on the cell surfaces of SSV-NIH 3T3 cells and normal NIH 3T3 cells was labeled with [125I] at 0 °C using the lactoperoxidase procedure (32). The [125I] labeled cells were warmed to 37 °C for various time periods to allow internalization of [125I]-labeled cell surface CRSBP-1. Following these warming time periods, cells were cooled to 0 °C and subjected to trypsin digestion. The cell lysates were then analyzed by SDS-PAGE and autoradiography under reducing conditions. As shown in Fig. 6A, [125I]-labeled cell surface CRSBP-1 in SSV-NIH 3T3 cells was sensitive to trypsin digestion prior to warming at 37 °C and became almost completely resistant to trypsin digestion after a 6-min incubation at 37 °C (Fig. 6A, lane 4), indicating that internalization of cell surface CRSBP-1 had occurred. After further incubation at 37 °C, the internalized [125I]-CRSBP-1 gradually recycled back to the cell surface and became sensitive to trypsin digestion again. After an 18-min incubation at 37 °C, [125I]-labeled cell surface CRSBP-1 achieved a second full internalization (Fig. 6A, lane 8). This result indicates that the cell surface CRSBP-1 undergoes rapid internalization and recycling with a cycling time of ~12 min. A third peak of cell surface CRSBP-1 internalization occurred after a 30-min incubation at 37 °C (Fig. 6A, lane 10). Quantitative analysis by densitometric scanning revealed that approximately ~30% of the internalized CRSBP-1 was recycled and that the remaining ~70% underwent turnover. In normal NIH 3T3 cells, the [125I]-labeled cell surface CRSBP-1 did not undergo detectable internalization under similar conditions and was sensitive to trypsin digestion even after incubation up to 30 min at 37 °C (Fig. 6B, lanes 2–10). In SSV-NIH 3T3 cells, the internalization and recycling of [125I]-labeled cell surface CRSBP-1 appeared to be blocked by 100 μM suramin. In the presence of suramin, [125I]-labeled cell surface CRSBP-1 remained at the cell surface and was sensitive to tryptic digestion (Fig. 6A, lanes 12–20). Suramin did not affect the status of the cell surface CRSBP-1 in normal NIH 3T3 cells (Fig. 6B, lanes 12–20). The cell surface CRSBP-1 was sensitive to trypsin digestion during incubation at 37 °C either in the presence or absence of suramin (Fig. 6B). These results suggest that cell surface CRSBP-1 undergoes ligand (v-sis gene product)-dependent internalization and recycling. We have also examined the internalization and recycling of cell surface CRSBP-1 in other cell types. Only cells expressing the v- or c-sis gene product (SSV-NIH 3T3, SSV-NRK, and v-sis-NIH 3T3 cells) exhibited cell surface CRSBP-1 internalization and recycling while, in other cell types which did not express the v- or c-sis gene product (neu/erbB2-transformed NIH 3T3 cells, ras-transformed NIH 3T3 cells, normal NRK cells, myc-transformed NIH 3T3 cells), CRSBP-1 did not undergo detectable internalization and recycling (data not shown). These results indicate that the internalization and recycling of CRSBP-1 is not a general feature associated with transformation.

Fig. 5. Effect of suramin on the turnover of CRSBP-1 in SSV-NIH 3T3 and normal NIH 3T3 cells. SSV-NIH 3T3 (A) and normal NIH 3T3 (B) cells were pulse-labeled with Tran35S-label for 3 h and chased in 10 mM unlabeled methionine for 0, 2, and 4 h. The cells were pulse-labeled and chased in the presence and absence of 0.1 mM suramin. After pulse and chase, cells were lysed and the cell lysates were immunoprecipitated with antiserum to CRSBP-1 and analyzed by 8% SDS-PAGE and fluorography. The exposure times were 10 days (SSV-NIH 3T3 cells without suramin), 7 days (SSV-NIH 3T3 cells with suramin), 14 days (NIH 3T3 cells without suramin), and 16 days (NIH 3T3 cells with suramin), respectively. The relative level of [35S]-labeled CRSBP-1 was quantitated by densitometric scanning. The quantitative analysis is shown in C, with the relative level of [35S]-labeled CRSBP-1 plotted against the length of chase. The relative level of [35S]-labeled CRSBP-1 at 0 h chase time was taken as 100%.

CRSBP-1 Interacts with the PDGF β-Type Receptor in SSV-NIH 3T3 Cells—In SSV-transformed cells, the v-sis gene product interacts with the PDGF β-type receptor in the lumen of the ER and Golgi complex and at the cell surface (1, 22, 23, 29). Since CRSBP-1 was found to interact with the v-sis gene product, we believed it was possible that CRSBP-1 may form a transient ternary complex with the v-sis gene product and PDGF β-type receptor. To test this possibility, we investigated the interaction of CRSBP-1 and the PDGF β-type receptor by performing sequential immunoprecipitation using antisera to CRSBP-1 and the PDGF β-type receptor. SSV-NIH 3T3 cells were lysed with 1% Triton X-100 buffer. The Triton X-100 extracts were then immunoprecipitated with antisera to CRSBP-1, antiserum to PDGF β-type receptor or non-immune serum. The immunoprecipitates were incubated with [γ-32P]-ATP to label the PDGF β-type receptor (through autophospho-
were labeled with 125I and lactoperoxidase on ice and then warmed to 37 °C for 0, 3, 6, 9, 12, 15, 18, 21, or 30 min. Cells were then treated with trypsin for 2 h on ice. For suramin treatment, cells were incubated in the presence and absence of 0.1 mM suramin for 30 min at 37 °C prior to cell surface labeling with 125I. After trypsin digestion, cells were lysed and the cell lysates were immunoprecipitated with antiserum to CRSBP-1 and analyzed by 8% SDS-PAGE and autoradiography. The exposure time of the autoradiograms was 3 days.

**FIG. 6.** Effect of suramin on the internalization and recycling of 125I-labeled cell surface CRSBP-1 in SSV-NIH 3T3 and normal NIH 3T3 cells. Cell surface proteins of SSV-NIH 3T3 cells (A) and normal NIH 3T3 cells (B) were labeled with 125I and lactoperoxidase on ice and then warmed to 37 °C for 0, 3, 6, 9, 12, 15, 18, 21, or 30 min. Cells were then treated with trypsin for 2 h on ice. For suramin treatment, cells were incubated in the presence and absence of 0.1 mM suramin for 30 min at 37 °C prior to cell surface labeling with 125I. After trypsin digestion, cells were lysed and the cell lysates were immunoprecipitated with antiserum to CRSBP-1 and analyzed by 8% SDS-PAGE and autoradiography. The exposure time of the autoradiograms was 3 days.

**DISCUSSION**

CRSBP-1 is expressed in all cell types thus far examined and appears to be a major cell surface binding protein for 125I-labeled CRS peptides (8). The intracellular and cell surface localization and broad pH ligand binding activity of CRSBP-1 allow CRSBP-1 to function as a ligand-specific transport protein trafficking between intracellular organelles (TGN) and the plasma membrane. Interestingly, in contrast to other transport proteins (low density lipoprotein receptor, transferrin receptor, and surface 6-phosphatidylinositol-like growth factor II receptor) (34–36), CRSBP-1 undergoes ligand-dependent internalization and recycling at the cell surface. This ligand-dependent internalization and recycling is supported by the following evidence: 1) the internalization and recycling of CRSBP-1 occurs in cells expressing the ligand v-sis gene product (SSV-NIH 3T3 cells and SSV-NRK cells) but not in other cell types, including transformed cells, and 2) suramin, a potent inhibitor of the ligand binding activity of CRSBP-1 (8), abolishes the internalization and recycling of CRSBP-1 in v-sis-transformed cells. The mechanism of this ligand-dependent internalization and recycling is not known. We hypothesize that the ligand v-sis gene product stimulates internalization and recycling of
CRSBP-1 by inducing dimerization or oligomerization of CRSBP-1 through its homodimer structure. This is supported by the observation that a disulfide-linked dimer of CRSBP-1 was detected only in cells expressing the v-sis gene product but not in normal NIH 3T3 cells.

Several lines of evidence suggest that the v-sis gene product interacts with CRSBP-1 in SSV-NIH 3T3 cells. These include: 1) the v-sis gene products p44 and p36, which are generated by proteolysis at and/or after TGN were co-immunoprecipitated with antiserum to CRSBP-1. This is consistent with the observation that the majority of CRSBP-1 is localized in TGN and endosomes/prelysosomal compartments (37, 38), and 3) all three proteins (CRSBP-1, the v-sis gene product and the PDGF β-type receptor) exhibited rapid turnover in cells expressing these proteins. No turnover of the PDGF β-type receptor and CRSBP-1 was observed in cells lacking expression of the v-sis gene product (e.g. normal and other transformed NIH 3T3 cells). CRSBP-1 exhibits broad pH activity for ligand binding (8). pH 6.0–6.5 (the luminal pH of TGN and exocytic vesicles) is not optimal for binding of the v-sis gene product to the PDGF β-type receptor. However, it is possible that these slightly acidic pH environments would not have significant effects on the transfer of the v-sis gene product from the occupied CRSBP-1 to the PDGF β-type receptor. CRSBP-1 may play an important role in the interaction of the v-sis gene product and the PDGF β-type receptor in the lumen of TGN and exocytic vesicles especially under non-overexpression conditions.

The ~140-kDa PDGF β-type receptor which was co-immunoprecipitated with CRSBP-1 was previously identified by 32P-metabolic labeling followed by immunoprecipitation using antiserum to the PDGF β-type receptor in SSV-NIH 3T3 cells.
(22). The ~140-kDa PDGF β-type receptor is a proteolytically cleaved product of the 180-kDa form (mature form) of the PDGF β-type receptor which was resistant to endoglycosidase H digestion but sensitive to endoglycosidase F digestion (22). The endoglycosidase F digestion of the ~140-kDa PDGF β-type receptor yielded a product of molecular weight (~120,000) which is smaller than that (molecular mass ~140,000) of the endoglycosidase F-digested 180-kDa PDGF β-type receptor (22). Compared with the 180- and 160-kDa PDGF β-type receptors, this ~140-kDa PDGF β-type receptor appeared to have a high phosphorylation activity (>10 fold) based on the estimation of the ratio of [32P]orthophosphate/[35S]methionine metabolic labeling (22). Since the ~140-kDa PDGF β-type receptor was only found in v-sis-transformed cells (22, 36), it may play an important role in mediating the signal transduction involved in the autocrine transformation of SSV-NIH 3T3 cells.

The mechanism for the production of the ~140-kDa PDGF β-type receptor is unknown. We hypothesize that some of CRSBP-1 molecules at TGN form a transient ternary complex with the newly synthesized v-sis gene product and PDGF β-type receptor and that some of CRSBP-1 molecules form a binary complex with the v-sis gene product (Fig. 8). The PDGF β-type receptor in the ternary complex undergoes proteolysis at its ligand-binding domain to produce the v-sis gene product to unoccupied PDGF β-type receptors during its cell surface internalization and recycling (1) (Fig. 8). During transport through exocytotic vesicles which have acidic luminal pH, the CRSBP-1:v-sis gene product binary complex is stable (8).

Recently, Valgeirsdóttir et al. (30) characterized the PDGF receptor-mediated signal transduction in SSV-NIH 3T3 cells. Their analysis of a panel of known PDGF β-receptor signaling molecules revealed the presence of suramin-sensitive and -insensitive pathways of PDGF receptor-mediated signaling (30). Since suramin enters and accumulates in intracellular acidic compartments (37, 38), suramin-sensitive signaling should be mediated by the PDGF β-type receptor activated by the v-sis gene product at TGN as well as the cell surface. The suramin-insensitive signaling should be mediated by the PDGF β-type receptor activated by the v-sis gene product in cis and medial Golgi complex and the ER. No evidence has been found to suggest that suramin is able to enter these compartments. We propose that the CRSBP-1:v-sis gene product and proteolytically cleaved PDGF β-type receptor (identified as a 140-kDa protein) ternary complex reported here is involved in mediating suramin-sensitive signaling in the TGN and endosomes/prelysosomal compartments (Fig. 8).

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