The hep I peptide of thrombospondin-1 is known to induce the disassembly of focal adhesions, a critical step in regulating cellular adhesive changes needed for cell motility. Fibroblasts that are heterogeneous with respect to the surface expression of Thy-1 differ markedly in morphology, cytoskeletal organization, and migration, suggesting differential regulation of focal adhesion dynamics. Here we demonstrate that disassembly of focal adhesions mediated by both full-length thrombospondin-1 and the hep I peptide in fibroblasts requires the expression of Thy-1, although it does not appear to function as a stable member of the hep I receptor complex. Consistent with a known function of Thy-1 in regulating lipid raft-associated signaling, intact lipid rafts are necessary for hep I-mediated focal adhesion disassembly. Furthermore, we establish Src family kinase (SFK) activation as a novel component required for hep I-induced signaling leading to focal adhesion disassembly. Hep I induces transient phosphorylation of SFKs in Thy-1-expressing fibroblasts only. Therefore, we conclude that Thy-1 surface expression is required for thrombospondin-1-induced focal adhesion disassembly in fibroblasts through an SFK-dependent mechanism. This represents a novel role for Thy-1 in the regulation of fibroblast-matrix interactions critical to tissue homeostasis and remodeling.

Thrombospondin-1 (TSP-1) is a matricellular protein expressed in response to injury and has been shown to regulate cellular functions essential to the early phases of wound healing (for a full review see Ref. 1). One critical function of this protein is the regulation of the turnover of focal adhesions. TSP-1 binding to the co-receptor complex of calreticulin and low density lipoprotein receptor-related protein (LRP) activates phosphatidylinositol 3-kinase (5) and extracellular signal-regulated kinase (6) in a Gi protein-dependent manner, inducing the disassembly of focal adhesions and the transition of cells toward an intermediate adhesive state. Intermediate adhesion is required for efficient fibroblast migration (7), which is a critical step in early wound healing and must be tightly regulated to ensure proper and timely resolution of wounds (8, 9).

Fibroblasts are known to exhibit significant phenotypic heterogeneity (10), which may further direct the outcome of wound healing. One of the best defined models of fibroblast heterogeneity is based on surface expression of the 27-kDa glycosylphosphatidylinositol-linked glycoprotein, Thy-1 (11–14). Primary fibroblasts (sorted based on the surface expression of Thy-1) differ in proliferative responses and signal transduction events as well as cell morphology (15–17). Thy-1 expression has been linked in other cell types to adhesive events, yet the mechanisms of action have remained elusive. Thy-1 has been shown to inhibit neurite outgrowth on astrocytes (18), facilitate thymocyte adhesion to thymic epithelium (19), and facilitate the binding of leukocytes and monocytes to endothelial cells and fibroblasts (20). Previous studies (21–23) have demonstrated significant interactions between Thy-1 and members of the Src family of protein tyrosine kinases. Thy-1 is enriched in lipid raft microdomains, within which activation of multiple signaling cascades occurs (24). The location of Thy-1 in lipid rafts affects its signaling functions (25). Based on these known properties and our own observation that Thy-1 affects Src-mediated Rho GTPase activation (and subsequent fibroblast focal adhesion, cytoskeletal redistribution, and migration) (26), we hypothesized that Thy-1 expression regulates focal adhesion dynamics in fibroblasts, including disassembly of focal adhesions by thrombospondin-1. In this report, we describe clearly discordant responses of Thy-1 fibroblast subpopulations to TSP-1-mediated focal adhesion disassembly, identify that both lipid raft integrity and Src family kinase (SFK) activation are critical to the TSP-1-mediated focal adhesion disassembly signaling cascade, and demonstrate that Thy-1 surface expression is required for SFK activation by TSP-1 and subsequent focal adhesion disassembly in fibroblasts.

EXPERIMENTAL PROCEDURES

Materials—A hep I peptide (ELTGAARKGSGRRLVKGPD) and modified hep I peptide (ELTGAARKGSGRRLVAPDC) were synthesized, purified, and analyzed by Anaspec, Inc. (San Jose, CA) (24). Throm-
bospodin-1 was purified from human platelets as described by Murphy-Ullrich et al. (27). Wortmannin, LY294002, grade 1 glutaraldehyde, methyl-β-cyclodextrin (MBCD), and cholesterol were purchased from Sigma. Antibodies against low density LRP were kindly provided by Dr. Dudley Strickland (Department of Vascular Biology, Holland Laboratory, American Red Cross, Rockville, MD). Antibodies against phosphorylated Src (Y416) were purchased from Cell Signaling Technology (Beverly, MA). FITC-labeled antibodies against murine Thy-1.1 and Thy-1.2 and horseradish peroxidase-labeled goat anti-rabbit IgG were purchased from BD Biosciences, and the Src2 antibody was provided by Dr. Dudley Strickland (Department of Vascular Biology, Holland Laboratory, American Red Cross, Rockville, MD). Antibodies against low density LRP were kindly provided by Dr. Dudley Strickland (Department of Vascular Biology, Holland Laboratory, American Red Cross, Rockville, MD). Antibodies against phosphorylated Src (Y416) were purchased from Cell Signaling Technology (Beverly, MA). FITC-labeled antibodies against murine Thy-1.1 and Thy-1.2 and horseradish peroxidase-labeled goat anti-rabbit IgG were purchased from BD Biosciences, and the Src2 antibody was purchased from Proteins associated with biotin-tagged hep I were co-precipitated by incubation overnight at 4 °C with a 100-μl slurry of neutravidin beads (Pierce). The samples were washed seven times in DTO buffer (Dulbecco’s modified Eagle’s medium, 0.5% Tween 20, and 0.1% ovalbumin). Bound proteins were solubilized in Laemmli buffer and separated by SDS-PAGE on 6% gels. After transfer to nitrocellulose, Thy-1 co-prediction with the biotin peptide complexes was detected by immunoblotting with anti-Thy-1 antibody. The blots were stripped and checked for CRT presence using anti-CRT antisemur.

Immunoblotting—For cell signaling immunoblots, cells were plated and grown to 70% confluence in full growth medium, at which point the cells were then harvested by scraping. Membrane proteins were solubilized with 50 mM Bri98. Equal amounts of protein (1 mg) from each cell type were analyzed. Proteins associated with biotin-tagged hep I were co-predecipated by incubation overnight at 4 °C with a 100-μl slurry of neutravidin beads (Pierce). The samples were washed seven times in DTO buffer (Dulbecco’s modified Eagle’s medium, 0.5% Tween 20, and 0.1% ovalbumin). Bound proteins were solubilized in Laemmli buffer and separated by SDS-PAGE on 6% gels. After transfer to nitrocellulose, Thy-1 co-prediction with the biotin peptide complexes was detected by immunoblotting with anti-Thy-1 antibody. The blots were stripped and checked for CRT presence using anti-CRT antisemur.

Immunoblotting—For cell signaling immunoblots, cells were plated and grown to 70% confluence in full growth medium, at which point the cells were rendered quiescent in medium containing 0.1% FBS for a minimum of 24 h. Following the quiescence period, the cells were stimulated with hep I peptide (5 μM) for 0, 2, 5, 10, and 15 min at 37 °C, 5% CO2. The cells were then washed briefly with ice-cold PBS, pH 7.2, and lysed using standard 2× SDS sample buffer or modified radioimmunoprecipitation assay buffer (150 mM NaCl, 50 mM Tris/HCl (pH 7.5), 1% Nonidet P-40, 0.25% sodium deoxycholate, 2 mM EGTA, 0.1 mM Na3VO4, protease inhibitor mixture (Sigma)). Immunoblots for LRP expression were generated from unstimulated fibroblasts in logarithmic growth. All lysates were sonicated for 10 s and immediately boiled for 5 min and then stored at −20 °C until used. The lysates were electrophoresed in 10% (for SPK signaling) or 5% (for LRP expression) SDS-polyacrylamide gels as indicated, transferred to polyvinylidene difluoro-
ride membranes (PVDF-Plus, Osmonics, Washington, MA), and probed as described in Ref. 26. To account for gel-loading errors in signaling experiments, as well as variations in cell density, the membranes were stripped and re-blotted for total Src family kinases using the broad Src family antibody Src2 (Santa Cruz).

Immunofluorescence—Subconfluent monolayers of Thy-1(+) fibroblasts were cultured on glass coverslips and then rendered quiescent. Fibroblasts were stimulated with hep I peptide (5 μM) for 5 min at 37 °C, 5% CO2. Coverslips were then washed briefly with ice-cold PBS, blocked with 10% normal goat serum/PBS, incubated with anti-Thy-1.1-FITC for 1 h at 4 °C in the presence of 0.1 mM NaN3, and then fixed in methanol at −20 °C and incubated with polyclonal rabbit anti-human phospho-Src (Y416) for 16 h at 4 °C followed by Texas Red X-conjugated goat anti-rabbit IgG for 40 min at room temperature. The controls for antibody specificity were mouse IgG1-FITC (BD Biosciences) and rabbit IgG. Coverslips were mounted on glass microscope slides and examined with a Leitz Axioplan epifluorescence microscope. Images were acquired with a SenSys-cooled charge-coupled device, high resolution, monochromatic digital camera (Photometrics, Tucson, AZ) and analyzed with IPLab Spectrum software (Signal Analytics Corp., Fairfax, VA).

Statistical Analysis—Comparisons involving three or more groups were analyzed using one-way ANOVA (Student-Newman-Keuls method for multiple comparisons). Student’s t test was employed when determining the significance of single treatments compared with untreated samples. In both cases a p value < 0.05 was used to determine statistical significance.

RESULTS

hep I Mediates Focal Adhesion Disassembly Only in Thy-1(+) Primary Fibroblasts—Focal adhesion disassembly in response to TSP-1 or the hep I peptide was analyzed in Thy-1(−) and Thy-1(+) sorted primary Lewis rat lung fibroblasts using IRM. Thy-1(+) fibroblasts actively underwent focal adhesion disassembly in response to a 30-min stimulation with hep I (1 μM; Fig. 1) or TSP-1 (250 nM), whereas Thy-1(−) fibroblasts failed to respond. The specificity of the hep I/Thy-1 (+) response was verified by determining the focal adhesion disassembly following treatment with a control-modified hep I peptide (1 μM) and PDGF-BB (545 nM), a potent stimulator of focal adhesion disassembly in fibroblasts (29). Consistent with qualitative findings, the quantitative analysis indicates that TSP-1 and its active peptide, hep I, mediate focal adhesion disassembly in Thy-1(+) (but not Thy-1(−)) fibroblasts (Fig. 2A). Statistical comparison by one-way ANOVA (power = 1.00, α = 0.05) demonstrates that modified hep I does not elicit focal adhesion disassembly responses in either fibroblast subpopulation, whereas PDGF-BB induced focal adhesion disassembly in both Thy-1(+) and Thy-1(−) fibroblasts, indicating that the Thy-1-dependent response is specific to the TSP-1/hep I-mediated signaling pathway (Figs. 1 and 2A). As reported previously (5) for bovine aortic endothelial cells, the hep I-mediated response in Thy-1(+) fibroblasts was sensitive to phosphatidylinositol 3-kinase signaling, indicating a critical role for this signaling pathway in the observed response (Fig. 2B). In endothelial cells, the receptor complex mediating focal adhesion disassembly in response to hep I is known to include cell-surface CRT and LRP. Both Thy-1(−) and Thy-1(+) fibroblast subpopulations were shown to express both CRT and LRP (Fig. 3).

Src Family Kinases Are Activated by hep I and Are Required for Focal Adhesion Disassembly Mediated by Both hep I and PDGF-BB—Src family kinases have been shown to be activated following Thy-1 cross-linking in lymphocytes and are essential in Thy-1-dependent T-cell activation following Fe receptor engagement (22, 30). Furthermore, Src family kinases have both signaling and structural roles in the maintenance and turnover of focal adhesions (31, 32). We identified a requirement for Src family kinase downstream signaling in Thy-1-dependent hep I-mediated fibroblast focal adhesion disassembly by the addition of the Src-specific inhibitor PP2 (10 nM, Fig 4A). The addition of PP2, which binds the kinase domain of all Src family members prior to hep I stimulation, was sufficient to inhibit hep I-mediated focal adhesion disassembly in Thy-1(+) fibroblasts. Furthermore, PP2 treatment was also sufficient to block PDGF-BB (which is a potent stimulator of Src in both subpopulations) stimulated (26) focal adhesion disassembly in both Thy-1(+) and Thy-1(−) fibroblasts, indicating that Src may play a more general role in focal adhesion disassembly (Fig. 4B). Western blotting for active Src family kinases following hep I stimulation demonstrates that Src family kinases are transiently phosphorylated on tyrosine 416 following stimulation. Only Thy-1(+) fibroblasts demonstrate Src activation following hep I stimulation (Fig. 5B). Immunofluorescence confirms Src activation following hep I stimulation (Fig. 5A).

Lipid Raft Integrity Is Essential for hep I-mediated Focal Adhesion Disassembly in Thy-1(+) Fibroblasts—Thy-1 is known to localize to lipid raft microdomains because of its GPI linkage, which is necessary for many of its effects on signaling. We tested whether lipid raft integrity is required for hep I-mediated focal adhesion disassembly in Thy-1(+) fibroblasts by lipid raft disruption with MBCD and lipid raft reconstitution by the addition of exogenous cholesterol. In a dose-dependent manner, MBCD was shown to inhibit hep I-mediated focal

![Fig. 2. The differential focal adhesion disassembly response of Thy-1 fibroblast subpopulations is specific to TSP-1/hep I and dependent on phosphatidylinositol 3-kinase signaling. A, sorted Thy-1(+) fibroblasts were grown on coverslips as described above. controls were treated with PDGF-BB (545 nM) or MEM for 30 min. B, Thy-1(+) fibroblasts were grown on coverslips as described above and preincubated with MEM, LY294002 (10 μM), or Wortmannin (2.5 nM) for 15 min followed by stimulation with hep I (1 μM) or MEM for 30 min. Coverslips were examined by IRM and assayed for the percentage of cells positive for focal adhesions ± S.D., n ≥ 3. A minimum of 300 cellscollection was evaluated. One-way ANOVA demonstrates that only Thy-1(+) fibroblasts treated with hep I, TSP-1, and PDGF-BB and Thy-1(−) fibroblasts treated with PDGF-BB were significantly different from the base-line controls. Control’s t test demonstrates that both phosphatidylinositol 3-kinase inhibitors are sufficient to block hep I-mediated focal adhesion disassembly. In both statistical analyses, *** denotes p < 0.001 versus control.](http://www.jbc.org/content/1)
adhesion disassembly (Fig. 6A). Furthermore, the addition of excessive cholesterol with MBCD rescued the hep I response in these cells (Fig. 6B).

**Thy-1 Is Not a Stable Component of the Known Receptor Complex**—To further address the role of Thy-1 in regulating hep I-mediated cell responses, we looked to see if Thy-1 associated with the hep I receptor complex following hep I stimulation. Using a modification of a previously published technique utilizing biotin-labeled hep I peptide, we stimulated Thy-1(+) and Thy-1(−) fibroblasts and pulled down the components of the hep I receptor complex (e.g. CRT and LRP) as published previously (2–4). Immunoblots of hep I complexes failed to demonstrate the presence of Thy-1 (data not shown).

**De Novo Thy-1 Expression Imparts Sensitivity of Focal Adhesions to hep I-mediated Disassembly**—Although only Thy-1(+) fibroblasts disassembled focal adhesions in response to hep I despite the presence of the known receptor components in both Thy-1(+) and Thy-1(−) cells, the role of Thy-1 remained unclear, because the primary fibroblast subpopulations could presumably differ in the expression of an unidentified receptor component. To address this issue, we used an ectopic Thy-1 expression system. Heterologous Thy-1 surface expression was achieved in the Thy-1(−) embryonic rat lung fibroblast cell line (RFL6) to examine more specifically the role of Thy-1 in hep I-mediated focal adhesion disassembly in fibroblasts. Following transfection with full-length Thy-1 cDNA and the selection of stable clones, focal adhesions in the cells were analyzed with IRM, with and without hep I treatment (1 μM). These stable transfecants demonstrate that Thy-1 expression imparts sensitivity to hep I-mediated focal adhesion disassembly in these fibroblasts (Fig. 7). The focal adhesions present following de novo Thy-1 expression are sensitive to hep I treatment (p ≤ 0.05; RFL6.Thy-1 control versus hep I treatment), whereas the focal adhesions in stable empty vector transfec-

**DISCUSSION**

Our data indicate that the TSP-1-mediated disassembly of focal adhesions in fibroblasts is dependent on the expression

**FIG. 3.** Thy-1(+) and Thy-1(−) fibroblasts express both known hep I receptor components. A, Thy-1(+) and Thy-1(−) fibroblasts were plated on standard tissue culture-treated plastic in MEM with 10% FBS and grown to ~80% confluence. The cells were lysed in 2× reducing Laemmli sample buffer, immediately boiled and separated by gel electrophoresis (5% acrylamide), and transferred for Western blotting. Rabbit anti-LRP was used to detect LRP expression in both subpopulations. B, Thy-1(+) and Thy-1(−) fibroblasts were plated on tissue culture-treated plastic to 80% confluence and removed by EDTA. Cell suspensions were stained for cell-surface CRT by indirect immunofluorescence and analyzed by flow cytometry (lower histograms), compared with secondary antibody-only controls (upper left). To control for the staining of intracellular CRT due to membrane permeability, the samples were analyzed for the intracellular Src family kinase member fyn (upper right). Flow histograms show no significant difference in the expression of cell-surface CRT in either subpopulation.

**FIG. 4.** hep I-mediated focal adhesion disassembly in Thy-1(+) fibroblasts requires Src family kinase activity. Thy-1(+) fibroblasts (>90% purity) were plated on coverslips in the presence of full growth medium, allowed to spread overnight, and then washed with warm MEM and incubated at 37 °C to recover from shear stress for 30 min. The broad Src family kinase inhibitor, PP2 (10 nM), was added at the time of the 30-min incubation to recover from shear stress. Following the recovery time, the cells were treated with hep I (1 μM), PDGF-BB (545 nM), or MEM alone for 30 min at 37 °C. The cells were examined by IRM and assayed for the percentage of cells positive for focal adhesions ± S.D., n ≥ 3. A minimum of 300 cells/condition was evaluated. Statistical significance was determined using Student’s t test; ***, p ≤ 0.001 versus control.

**FIG. 5.** Thymocytes are sensitive to hep I treatment. A, Thymocytes were washed in MEM and treated with hep I (1 μM) for 30 min at 37 °C. These data indicate that Thy-1 is necessary for TSP-1 signaling of focal adhesion disassembly in fibroblasts.
of the GPI-linked glycoprotein Thy-1, demonstrating a novel level of control of the regulation of fibroblast adhesion/des- adhesion. Furthermore, we demonstrate that Thy-1-dependent TSP-1-mediated focal adhesion disassembly requires Src family kinase signaling. The initial analysis of Thy-1 fibroblast subpopulations in response to TSP-1 demonstrated that Thy-1(+) (but not Thy-1(-)) fibroblasts respond to the focal adhesion disassembly-specific domain of TSP-1, hep I. The differential response was found to be specific for TSP-1, as focal adhesion disassembly responses to modified hep I (neither subset) and PDGF-BB (both subsets) were identical in both fibroblast subpopulations.

To directly address the role of Thy-1, we employed a heterologous expression system by stably transfecting murine Thy-1 into RFL6 cells. These results indicate that expression of Thy-1 in Thy-1(-) cells is sufficient to impart the focal adhesion disassembly response to TSP-1 in fibroblasts. These data support a mechanistic role for Thy-1 in modulating signaling of focal adhesion disassembly and suggest that the failure of Thy-1-negative fibroblasts to respond is directly attributable to the lack of Thy-1 rather than to some other difference between the fibroblast subsets. The regulation of fibroblast TSP-1 synthesis by Thy-1, such that Thy-1-negative fibroblasts have disassembled their focal adhesions in response to endogenous TSP-1 at base line and are thus unresponsive to additional exogenous TSP-1, is an unlikely mechanism, as we have determined equivalent TSP-1 protein expression in the two subpopulations by immunoblotting (data not shown). Additionally, in previous studies (27, 28), endogenous TSP-1 synthesis by cells did not affect responsiveness to hep I- or TSP-1-stimulated focal adhesion disassembly. Although several reports published previously describe the effects of Thy-1 on cell-cell adhesion, such as thymocyte adhesion to thymic epithelium (19), leukocyte and monocyte adhesion to fibroblasts and endothelial cells (20), and Thy-1 binding to integrin β1 on astrocytes (33), this is the first report of Thy-1 affecting the regulation of cell adhesion to the extracellular matrix through a receptor-mediated signaling event.

The signaling complex for TSP-1-mediated focal adhesion disassembly has been shown to consist of CRT and LRP (2–4). CRT, a nontransmembrane protein expressed on the cell surface and the endoplasmic reticulum, was identified as being a critical component of TSP-1-mediated focal adhesion disassembly through interactions with a 19-amino acid NH2-terminal sequence from the heparin binding domain of TSP-1. LRP had been demonstrated previously to internalize and degrade TSP-1 by binding the NH2-terminal domain of TSP. More recently, CRT and LRP have been shown to interact, and their interaction is critical to TSP-1-mediated intracellular signaling and the downstream disassembly of focal adhesions (4). Whether these two components comprise the extent of the TSP-1 receptor complex mediating focal adhesion disassembly remains unknown. Significant differences in the expression of these two proteins were not seen in the Thy-1 fibroblast subpopulations. The interaction of Thy-1 with the receptor complex was examined by a pull-down of the receptor complex with biotinylated hep I as in previous studies (4). Failure to identify Thy-1 in these assays indicates that Thy-1 is not a likely part of the stable receptor complex, although the data do not exclude the possibility of Thy-1 interacting indirectly with the receptor complex to induce downstream signaling. These results indicate that the active mechanism of Thy-1 takes place through the modulation of a signaling event parallel to or downstream of the main receptor complex. The mechanism by which Thy-1 (which lacks a cytoplasmic domain) regulates intracellular signaling has not been fully elucidated. Thy-1 has been co-immunoprecipitated with the SFK Fyn from noncaveolar rafts in PC 12 cells and with active Fyn and Lyn SFKs in mesangial cells (34, 35). Two models have been described for Thy-1-SFK interaction. The first is the association of the lipid anchor of GPI-linked proteins in lipid rafts with palmitoylated cysteines on the NH2-terminal region of Thy-1 fibroblasts grown on coverslips to 60% confluence and rendered quiescent by serum starvation for 48 h at 37 C. The cells were then treated with hep I (1 μM) for 5 min and immediately fixed with 3% formaldehyde. The fixed cells were stained for Thy-1 and phospho-Src (pY416) and imaged on a Leitz inverted microscope with a 100× objective. B, Thy-1 fibroblast subpopulations were grown on tissue culture plastic in full growth medium. At 80% confluence, the cells were rendered quiescent for 48 h by serum starvation. The monolayers were immediately stimulated by the addition of hep I (1 μM) for 0, 2, 5, 10, and 15 min. Following stimulation, the cells were immediately lysed in buffer containing phosphatase inhibitor mixture (Sigma) and analyzed by Western blotting for total Src family kinases and phosphorylated Src family kinases. A single representative blot is shown. Statistics represent the average increase in Src phosphorylation over base line (0 min) of quadruplicate experiments; * denotes p < 0.05.

Fig. 5. hep I treatment induces transient phosphorylation of Src family kinases in Thy-1(+) (but not Thy-1(-)) fibroblasts. A, Thy-1(+) fibroblasts were grown on coverslips to 60% confluence and rendered quiescent by serum starvation for 48 h at 37 C. The cells were then treated with hep I (1 μM) for 5 min and immediately fixed with 3% formaldehyde. The fixed cells were stained for Thy-1 and phospho-Src (pY416) and imaged on a Leitz inverted microscope with a 100× objective. B, Thy-1 fibroblast subpopulations were grown on tissue culture plastic in full growth medium. At 80% confluence, the cells were rendered quiescent for 48 h by serum starvation. The monolayers were immediately stimulated by the addition of hep I (1 μM) for 0, 2, 5, 10, and 15 min. Following stimulation, the cells were immediately lysed in buffer containing phosphatase inhibitor mixture (Sigma) and analyzed by Western blotting for total Src family kinases and phosphorylated Src family kinases. A single representative blot is shown. Statistics represent the average increase in Src phosphorylation over base line (0 min) of quadruplicate experiments; * denotes p < 0.05.
domain containing proteins that include SFKs (39). We determined whether TSP-1 affects Src signaling in fibroblasts and whether the response is dependent on Thy-1 expression by measuring the phosphorylation of Src Y416 following hep I stimulation in fibroblast Thy-1 subpopulations. The results indicate that only fibroblasts expressing Thy-1 demonstrate
transient SFK phosphorylation in response to heparin stimulation. Furthermore, blocking Src family kinase signaling with the Src-specific inhibitor, PP2, completely inhibits heparin-1-mediated focal adhesion disassembly in Thy-1(+) fibroblasts and PDGF-BB-mediated focal adhesion disassembly in both fibroblast subsets, indicating a critical role for Src in this focal adhesion disassembly signaling event. Although Src kinases are known to modulate focal adhesion turnover in cell motility, this is the first report of a role for this family of protein tyrosine kinases in the TSP-1-mediated focal adhesion signaling pathway. A possible mechanism involves recently described signaling interactions between Src family kinases and LRP, a critical part of the TSP-1 focal adhesion disassembly signaling complex. PDGF stimulates SFK-dependent phosphorylation of LRP in WT-38 fibroblasts, resulting in increased association of LRP with the adaptor protein Shc (40). More recently, LRP was shown to be tyrosine-phosphorylated in v-Src-transformed fibroblasts (41).

The regulation of cell adhesion directly affects fibroblast migration and matrix reorganization, which in turn are critical in development, wound healing, fibrogenesis, and tumor stroma formation. These experiments describe a novel role for Thy-1 in the regulation of the adhesive state of fibroblasts. In addition to affecting base-line cell adhesion and migration in fibroblasts (26), we demonstrate here that Thy-1 exerts an additional level of control in regulating cell-matrix interaction by modulating the disassembly of focal adhesions mediated by the matricellular protein, TSP-1, in an SFK-dependent mechanism.

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Thrombospondin-1-induced Focal Adhesion Disassembly in Fibroblasts Requires Thy-1 Surface Expression, Lipid Raft Integrity, and Src Activation

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