Low density lipoprotein receptor-related protein-1 (LRP-1) mediates the endocytosis of multiple plasma membrane proteins and thereby models the composition of the cell surface. LRP-1 also functions as a catabolic receptor for fibronectin, limiting fibronectin accumulation in association with cells. The goal of the present study was to determine whether LRP-1 regulates cell surface levels of the β1 integrin subunit. We hypothesized that LRP-1 may down-regulate cell surface β1 by promoting its internalization; however, unexpectedly, LRP-1 expression was associated with a substantial increase in cell surface β1 integrin in two separate cell lines, murine embryonic fibroblasts (MEFs) and CHO cells. The total amount of β1 integrin was unchanged because LRP-1-deficient cells retained increased amounts of β1 in the endoplasmic reticulum (ER). Expression of human LRP-1 in LRP-1-deficient MEFs reversed the shift in subcellular β1 integrin distribution. Metabolic labeling experiments demonstrated that the precursor form of newly synthesized β1 integrin (p105) is converted into mature β1 (p125) more slowly in LRP-1-deficient cells. Although low levels of cell surface β1 integrin, in LRP-1-deficient MEFs, were associated with decreased adhesion to fibronectin, the subcellular distribution of β1 integrin was most profoundly dependent on LRP-1 only after the cell cultures became confluent. A mutagen-treated CHO cell line, in which LRP-1 is expressed but retained in the secretory pathway, also demonstrated nearly complete ER retention of β1 integrin. These studies support a model in which LRP-1 either directly or indirectly promotes maturation of β1 integrin precursor and thereby increases the level of β1 integrin at the cell surface.

The β1 integrin subunit associates with multiple α-subunits to form transmembrane adhesion receptors for extracellular matrix proteins, including collagen, fibronectin, vitronectin, and laminin (1). Once present at the cell surface, mature integrins anchor the plasma membrane to the actin cytoskeleton and promote cell signaling (2, 3). Integrin and growth factor-initiated cell signaling responses are integrated by the cell to regulate gene expression, cell migration, cell growth, apoptosis, and development (2). Altered cell surface integrin expression may be particularly important in cancer, impacting on various aspects of cancer metastasis (4–6). Understanding mechanisms that regulate the concentration of integrins in the plasma membrane is an important problem.

The β1 integrin subunit is synthesized as an 87-kDa polypeptide that undergoes glycosylation in the endoplasmic reticulum (ER) and in the Golgi apparatus (6, 7). In the ER, the most prevalent, incompletely glycosylated form of β1 has a mass of 105 kDa and is thus referred to as p105. Mature β1 has a mass of ~125 kDa (p125). In some cells, p105 is the primary form of β1 integrin identified; however, p105 is not found at the cell surface and cannot function in cell adhesion or cell signaling (6, 8).

Many factors control maturation of β1 and its transfer to the cell surface, including the availability of α-subunits, growth factors such as transforming growth factor-β (TGF-β), and the state of activation of Ras (9–11). The membrane-bound protein chaperone, calnexin, associates with β1 in the ER, promoting integrin assembly and inhibiting integrin transfer to the cell surface (12, 13). Other protein chaperones, including calreticulin and receptor-associated protein (RAP), may associate with β1-containing integrins or integrin-based adhesion complexes; however, a role for these proteins in integrin maturation has not been defined (14, 15). Talin, which is best known for its role in focal adhesion assembly, and HEMCAM/gicerin, an immunoglobulin superfamily protein, also may regulate β1 maturation (16, 17).

LRP-1 is a receptor for over 40 soluble ligands, which undergoes rapid and constitutive endocytosis in clathrin-coated pits, delivering most bound ligands to lysosomes for degradation (18). In some cells, LRP-1 may also localize in caveolae/lipid rafts, where it functions in cell signaling (19, 20). In addition to soluble ligands, LRP-1 mediates the endocytosis of other plasma membrane proteins, including the urokinase receptor (uPAR), tissue factor, and amyloid precursor protein (APP), and thereby down-regulates the plasma membrane levels of these proteins (21–25). Regulation of the concentration and activity of membrane proteins represents an indirect mechanism by which LRP-1 may control cell signaling. For example, by down-regulating cell surface uPAR in murine embryonic...
fibroblasts (MEFs), LRP-1 suppresses activation of the small GTPase, Rac1, and inhibits cell migration (22, 26).

We previously demonstrated that LRP-1 binds fibronectin and mediates its endocytosis, limiting fibronectin accumulation in association with cell surfaces (27). LRP-1 also is an endocytic receptor for thrombospondin-1 (28). By modifying the composition of the extracellular matrix, LRP-1 may regulate processes such as cell adhesion and migration. In this study, we sought to determine whether LRP-1 regulates cell surface integrin expression. We hypothesized that LRP-1 may down-regulate basal levels of integrin subunits, at the cell surface, by facilitating integrin endocytosis. In support of this hypothesis, Czekay et al. (29) recently reported that plasminogen activator inhibitor-1 promotes α5 integrin endocytosis by forming an integrin-containing multiprotein complex that is recognized and internalized by LRP-1. In this report, we demonstrate the unanticipated finding that LRP-1 expression is associated with a substantial increase in cell surface β1 integrin. The effects of LRP-1 are observed principally in confluent cell cultures and reflect the ability of LRP-1 to directly or indirectly promote maturation of newly synthesized β1 integrin in the secretory pathway. Regulation of β1 integrin maturation represents a novel mechanism by which LRP-1 may influence interactions of the cell with its microenvironment.

EXPERIMENTAL PROCEDURES

Reagents and Proteins—Purified fibronectin, vitronectin and αβ1 integrin were purchased from Chemicon International (Temecula, CA). Type I collagen was obtained from BD Biosciences (Palo Alto, CA). Glutathione S-transferase (GST)-RAP was expressed in bacteria and purified as previously described (27) using a construct obtained from Dr. Joachim Herz (University of Texas Southwestern Medical Center, Dallas, TX). As a control, GST without fused RAP, was also expressed and purified from bacteria transformed with the empty vector, pCEX-H2T. Endoglycosidase H (Endo-H) and peptide-N-glycosidase F (PNGase F) were purchased from Roche Applied Science (Mannheim, Germany) and Sigma, respectively. Polyclonal antibody PAB1952, which recognizes the C-terminal cytoplasmic domain of β1 integrin, was obtained from Chemicon International (Temecula, CA). β1 integrin-specific polyclonal antibody 363 was kindly provided by Dr. Douglas DeSimone (University of Virginia). Polyclonal anti-epidermal growth factor receptor (EGFr) antibody was purchased from Upstate Biotechnology (Lake Placid, NY) and polyclonal anti-extracellular signal-regulated kinase (ERK/MAPK) antibody was from Zymed Laboratories Inc. (San Francisco, CA). TOP/21,2-9-BSA-neutralizing antibody 1D11 was from R&D Systems. The activity of this antibody was confirmed in endothelial cell growth assays, as previously described by our laboratory (30). Streptavidin-Sepharose, peroxidase-conjugated donkey anti-rabbit IgG and Protein A-Sepharose were from Amersham Biosciences. Trans-35S-label, for metabolic labeling, was from ICN Biochemicals (Irvine, CA). The membrane-impermeable biotinylation reagent, sulfo-NHS-LC-biotin, was from Pierce. Cell culture media was from Invitrogen Life Technologies, Inc.

Cell Culture—MEFs that are genetically deficient in LRP-1 (interchangeably called MEF-2 or PEA 13 cells), LRP-1−/−/− MEFs (PEA10), and wild-type MEFs (MEF-1 cells) were obtained from the ATCC. PEA10 and MEF-2 cells were cloned from the same culture of MEFs, heterozygous for LRP-1 gene disruption, after selection with Pseudo- monas exotoxin A (31). MEF-1 cells were isolated from the same mouse strain. All MEFs were cultured in Dulbecco’s modified Eagle’s medium with 10% FBS (HyClone Laboratories, Logan, UT). B41 cells are MEF-2 cells that were transfected for stable expression of full-length human LRP-1 (27). Wild-type Chinese hamster ovary (CHO K1) cells, LRP-1-deficient CHO 14-2-1 cells, and CHO-A-Sepharose were cultured in association with cell surfaces (27). Cells were extracted in 1% Triton X-100, 0.125% Tween-20, 0.5% deoxycholate, 50 mM HEPES, pH 7.5, 0.5 mM CaCl2, 10 mM aprotinin, 10 μg/ml E64, and 10 μg/ml leupeptin. To detect β1 integrin glycoforms, equal amounts of cellular protein were subjected to SDS-PAGE under non-reducing conditions in 7.5% gels, transferred to nitrocellulose membranes, and probed with primary antibody PAB1952 for 12 h, followed by 2,4-dinitrophenylated anti-rabbit IgG. Secondary antibody was visualized by enhanced chemiluminescence (Renaissance-ECL, PerkinElmer Life Sciences).

Biotinylation and Recovery of Cell Surface Integrin Subunits—Monolayer cultures of MEFs and CHO cells were washed three times with ice-cold 20 mM sodium phosphate, 150 mM NaCl, pH 7.4 (PBS) to remove contaminating FBS and other soluble proteins and then treated with the membrane-impermeable biotinylation reagent, sulfo-NHS-LC-biotin (0.1 mg/ml), for 15 min at 22 °C, as described previously (27). Biotinylation reactions were terminated by adding 50 mM Tris-HCl, 150 mM NaCl, 100 mM glycine, pH 7.5 for 15 min at 22 °C. After washing with PBS, the cells were counted and lysed in extraction buffer. Biotinylated cell surface proteins were precipitated with Streptavidin-Sepharose (Amersham Biosciences). The affinity precipitates were recovered by centrifugation, washed, boiled in SDS sample buffer, and subjected to SDS-PAGE and immunoblot analysis to detect β1 integrin.

Endoglycosidase Digestion—For PNGase F treatment, MEF extracts were diluted to a concentration of 1 mg/ml in 100 mM Tris-Cl, pH 7.4 with protease inhibitors, 50 mM β-mercaptoethanol and 0.1% SDS. The samples were then boiled for 2 min. After neutralization with Tris-X100 (0.5%), the samples were incubated with PNGase F (0.025–25 units/ml) at 37 °C for 12 h. A second dose of enzyme was added for an additional 12 h. For Endo-H treatment, MEF extracts were diluted to a concentration of 1 mg/ml in 100 mM sodium acetate, pH 5.5, containing 0.1% N-lauroylsarcosine and protease inhibitors. Samples were treated with Endo-H (30 milliunits/ml) at 37 °C for 12 h and then with a second dose of Endo-H for an additional 12 h. Enzymatic reactions were terminated by protein precipitation with ice-cold acetone. Pellets were air-dried. The samples were then neutralized and resuspended in SDS sample buffer for SDS-PAGE and immunoblot analysis.

Metabolic-labeling of β1 Integrin—MEFs were seeded in 60-mm dishes in serum-containing medium, and allowed to grow until 100% confluent. Depletion of intracellular methionine was achieved by culturing in 1-l-methionine/1-cysteine-free Dulbecco’s modified Eagle’s medium containing 5% FBS for 1 h. The FBS was predialyzed to remove unmodified l-cysteine and methionine. The cells were then labeled with 0.15 mCi/ml of TRAN35S-Label (which includes radioactive L-cysteine and L-methionine) at 37 °C for 12 h, which is the same method. After labeling, the cells were harvested immediately (time 0) or chased for the indicated times in DMEM supplemented with 10% FBS and excess of non-radioactive 1-l-methionine/-cysteine (2 μM). Cell extracts were prepared by boiling for 10 min in 1% SDS, 20 mM Tris-HCl, 150 mM NaCl (TBS) (5 μl EDTA). The extracts were diluted 1:10 in TBS with 1% (v/v) Triton X-100 and protease inhibitor mixture (Roche Applied Science), subjected to centrifugation, and precleared protein-A Sepharose (Amersham Biosciences). β1 integrin subunit was recovered by immunoprecipitation with polyclonal antibody 363. The precipitates were then subjected to SDS-PAGE on 6% gels. The gels were dried, fixed, and exposed to radiographic film. Radiolabeled cell surface integrin subunits were detected using a Storm 860 PhosphorImager and analyzed using ImageQuant software (Molecular Dynamics).

Cell Adhesion—Costar 96-well plates were coated with various concentrations of fibronectin in PBS overnight at 4 °C, rinsed, and then blocked with 2% (w/v) BSA (Sigma) in PBS for 2 h at room temperature. Cells (CHO 14-2-1, CHO-A-Sepharose, CHO 14-2-1 cells, and CHO-A-Sepharose) were washed 3 times in calcium/magnesium-free PBS containing 0.5 mM EDTA. The cells were pelleted at 1000 × g for 5 min and resuspended at a density of 105 cells/ml in Puck’s saline medium A, supplemented with 10 mM HEPES, pH 7.4, 0.5 mM CaCl2, 0.5 mM MgCl2, and, when indicated, 1 mM MnCl2. Cells were allowed to adhere for different periods of time at 37 °C in a humidified 5% CO2–95% air atmosphere. Nonadherent cells were washed away with PBS. Adherent cells were fixed for 20 min with 4% formaldehyde in PBS, rinsed with PBS, and stained with 0.2% crystal violet in 2% ethanol for 30 min. Excess stain was washed away. Cell associated stain was then released in 1% SDS (50 μl per well). The absorbance at 555 nm

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LRP-1 and β1 Integrin Maturation

**RESULTS**

**LRP-1-deficient MEFs Demonstrate Decreased p125/Cell**

**Surface β1 Integrin—β1 integrin was compared by immunoblot analysis**

in LRP-1-deficient MEF-2 cells and in two LRP-1-positive cell lines (MEF-1 and PEA10). PEA10 cells were cloned from cultures of LRP-1(-/-) embryonic fibroblasts that had been subjected to selection with Pseudomonas exotoxin A, as were MEF-2 cells (31). MEF-1 cells are LRP-1(+/−). As shown in Fig. 1A, the LRP-1-positive MEF-1 and PEA10 cells showed similar amounts of p125 and p105. In both cases, p125 accounted for more than 50% of the total β1 integrin. By contrast, LRP-1-deficient MEF-2 cells demonstrated decreased p125 and increased p105. In seven separate experiments, the amount of p125, as a fraction of the total amount of glycosylated β1 (p125/p105+p125), was 2.3 ± 0.3 fold higher in MEF-1 cells than in MEF-2 cells (p < 0.01).

B41 cells were MEF-2 cells that were transfected for stable expression of full-length human LRP-1. Expression of human LRP-1 in MEF-2 cells increased the fraction of p125 β1 integrin (Fig. 1B). In four separate experiments, p125/p105+p105 was increased by 2.5 ± 0.3-fold in the B41 cells compared with MEF-2 cells (p < 0.05). To confirm the identity of p105 as an intracellular β1 precursor in the ER, we treated MEF extracts with Endo-H. This enzyme dissociates N-linked mannose-rich glycans, which become Endo-H-resistant after modification by glycosyltransferases in the Golgi apparatus. As anticipated, Endo-H totally eliminated p105, replacing this band with a new band that migrated near unglycosylated β1 core protein. p125 was not modified by Endo-H, confirming that this species is the mature form of the integrin subunit.

Because LRP-1-deficient MEF-2 cells have decreased amounts of p125, we hypothesized that these cells have decreased amounts of cell surface β1 integrin. To test this hypothesis, we biotinylated cell surface proteins in MEFs, using the membrane-impermeable biotinylation reagent, sulfo-NHS-LC-biotin. Biotinylated proteins were affinity-purified with streptavidin-Sepharose and probed for β1 integrin. As shown in Fig. 1C (representative of four separate experiments), the LRP-1-positive cells (MEF-1 and B41) had increased amounts of biotinylated β1 compared with MEF-2 cells. As anticipated, biotinylated p105 was never recovered in the affinity precipitates, confirming that p105 is not found on cell surfaces in MEFs.

We considered a number of previously described mechanisms whereby LRP-1 may indirectly control maturation of p105. TGF-β establishes an autocrine pathway in some cell cultures (33) and has been shown to promote β1-chain maturation (10, 11). Furthermore, LRP-1 may function as a TGF-β receptor (34). To test whether alterations in endogenously produced TGF-β activity are responsible for differences in β1 integrin maturation in LRP-1-positive and -negative MEFs, we treated PEA10 and MEF-2 cells with TGF-β-neutralizing antibody (20 μg/ml) or with an equivalent concentration of preimmune mouse IgG for 12 h in serum-free medium. Fig. 1D shows that TGF-β-neutralizing antibody had no effect on β1 integrin distribution between p125 and p105 forms. In separate experiments, plating LRP-1-positive and -negative cells on different substrata, including vitronectin, fibronectin, type I collagen, and poly-L-lysine (negative control) had no effect on the distribution of β1 integrin into the p125 and p105 bands (results not shown). Furthermore, αβ1 function-blocking antibody (10 μg/ml for 48 h) failed to alter p105 and p125 in LRPs-1-positive and -negative MEFs. However, culture confluency did affect the distribution of β1 integrin into p125 and p105, and this result was only observed in LRP-1-positive cells.

We applied two strategies to test the effects of culture confluency on β1 integrin maturation. In one set of experiments, cells were plated at different densities and cultured for 24 h. By visual inspection, cells plated at 10^5/well remained sub-confluent. Cells plated at 10^6/well or 10^5/well were confluent; however, as anticipated, the cells plated at 2 × 10^5/well became confluent sooner. In a second set of experiments, cells were plated at the equivalent density (2 × 10^5/well) and analyzed after culturing for increasing periods of time. In both experiments, the fraction of β1 integrin migrating as mature p125 increased under conditions that favored development of cell...
culture confluency only in LRP-1-expressing cells (Fig. 2).

The results presented thus far suggest a model in which LRP-1 regulates the relative abundance of $\beta_1$ integrin glycoforms in MEFs. To confirm that the total amount of $\beta_1$ integrin is not altered, we treated extracts of MEF-1, MEF-2, and B41 cells with PNGase F, which hydrolyzes asparagyl-glycosamine bonds, releasing nearly all N-linked glycans. As shown in Fig. 3A, PNGase F modified both p125 and p105, converting both species into a common product that migrated near the mobility of core protein. The amount of product observed with each of the species into a common product that migrated near the mobility of core protein. The amount of product observed with each of the three cell lines was essentially identical, supporting the hypothesis that LRP-1 does not regulate the total amount of $\beta_1$ integrin. Densitometry was performed to quantitate p125 as a fraction of total $\beta_1$ integrin (p105+p125) in this representative study (bar graph to the right). B, 2 x 10^6 cells were plated in each well and cultured for the indicated times. The samples were then subjected to immunoblot analysis for $\beta_1$ integrin. Densitometry was performed to quantitate p125 as a fraction of total $\beta_1$ integrin (p105+p125) in this representative study (bar graph to the right).

**LRP-1 Expression Promotes $\beta_1$ Integrin Maturation**—To explain the results presented thus far, we hypothesized that LRP-1, either directly or indirectly, promotes maturation and transport of $\beta_1$ integrin in the secretory pathway. An alternative hypothesis, which was considered less likely, was that LRP-1 stabilizes cell surface $\beta_1$ integrin against catabolism and that in LRP-1-deficient cells, there is compensatory up-regulation of $\beta_1$ integrin expression.

To test whether LRP-1 expression alters $\beta_1$ integrin maturation in the secretory pathway, metabolic labeling experiments were performed in LRP-1-positive PEA10 cells and in LRP-1-negative MEF-2 cells. After pulse exposure to radioactive L-methionine/L-cysteine, radiolabeled p105 $\beta_1$ integrin was detected in both cell types, as anticipated (Fig. 5). p105 $\beta_1$ integrin disappeared more slowly in the LRP-1-negative MEF-2 cells, compared with the PEA10 cells; more than 70% of the p105 persisted in MEF-2 cells after 5 h. In the PEA10 cells, the mature form of $\beta_1$ integrin (p125) was detected at an earlier time (2 h) and persisted over the 8 h time course as a substantially higher fraction of the total amount of $\beta_1$ integrin (p125+p105). These results were interpreted as supporting the hypothesis that LRP-1 promotes maturation of $\beta_1$ integrin in the secretory pathway.

We assumed that p105 disappeared, during the course of the metabolic labeling, due to conversion into p125; however, in all of our experiments, the amount of p125 generated was less...
than the initial amount of p105 detected. This result may suggest that p125 is degraded during the time course of our experiment, that some p105 is targeted for degradation instead of conversion into p125, or that our antibody is less effective for recovery of p125 by immunoprecipitation. In control experiments, total β1 integrin was assessed in MEF-1 cells by immunoprecipitation or isolated, from the same cells, by immunoprecipitation and then subjected to immunoblot analysis. The fraction of p125 was significantly reduced when the immunoprecipitation step was included (results not shown). Thus, the limited amount of p125 detected in our metabolic labeling experiments is at least partially explained by the function of our antibody in immunoprecipitation.

To test whether LRP-1 alters the stability of cell surface β1 integrin, we cultured LRP-1-expressing MEF-1 cells in the presence of GST-RAP (200 nM) or an equivalent concentration of GST (negative control) for 3 days. RAP binds to the ligand-binding sites in LRP-1, blocking its interaction with all extracellular ligands and reversing its effects on the cell surface binding sites in LRP-1, blocking its interaction with all extracellular ligands and reversing its effects on the cell surface binding sites in LRP-1. RAP did not cause any detectable change in the level of uPAR and APP (24, 35). In three separate experiments, total β1 integrin was assessed in MEF-1 cells by immunoprecipitation or isolated, from the same cells, by immunoprecipitation and then subjected to immunoblot analysis. The fraction of p125 was significantly reduced when the immunoprecipitation step was included (results not shown). Thus, the limited amount of p125 detected in our metabolic labeling experiments is at least partially explained by the function of our antibody in immunoprecipitation.

Adhesion assays were also performed with CHO K1 and CHO 14-2-1 cells since the CHO 14-2-1 cells demonstrated the most profound decrease in cell surface β1 integrin. As shown in Fig. 6B, CHO 14-2-1 cells demonstrated a significant decrease in attachment to 1.0 μg/ml fibronectin (p < 0.05). When the cells were allowed to attach in the presence of 0.5 mM MnCl2, to promote increased integrin activation, the difference related to LRP-1 expression was more profound (p < 0.01); under these conditions, significant differences in cell attachment were observed using multiple fibronectin coating concentrations.

Finally, when CHO K1 and CHO 14-2-1 cells were examined 30 min after addition to fibronectin-coated wells (1.0 μg/ml), the CHO 14-2-1 cells demonstrated substantially decreased spreading (Fig. 6C). This effect was no longer observed when the fibronectin coating density was increased or the time of plating was increased beyond 1 h.

**DISCUSSION**

LRP-1 gene disruption in the mouse is embryonic lethal (36); however, in cell culture model systems, LRP-1 regulates cell signaling and cell physiology by diverse mechanisms. A unique property of LRP-1 is its ability to facilitate endocytosis of other plasma membrane proteins, including uPAR, APP, and tissue factor, and thereby down-regulate cell surface levels of these proteins (21–25). This process probably requires bifunctional ligands or adaptor proteins, such as Fe65, that bridge LRP-1 to other plasma membrane proteins so that the complexes undergo endocytosis as units (18, 21, 37). By decreasing the level of uPAR, LRP-1 indirectly controls cell signaling pathways leading to the activation of Ras, ERK/MAPK, and Rac1 (23, 26). In mice, inactivation of the LRP-1 gene in vascular smooth muscle cells increases cellular levels of PDGF β receptor and its degree of tyrosine phosphorylation (38). Thus, there is evidence that LRP-1 controls the activity of cell signaling receptors in vivo. LRP-1 may also directly regulate cell signaling through interactions involving its intracytoplasmic tail and proteins...
LRP-1 expression regulates β1 integrin in CHO cells. Equal amounts of cellular protein from detergent-soluble cell extracts of CHO K1, CHO 13-5-1, and CHO 14-2-1 cells were subjected to immunoblot analysis for β1 integrin in the three lanes on the left. In the three lanes to the right, cell surface proteins were biotinylated and streptavidin affinity-precipitated prior to immunoblot analysis for β1 integrin. B, densitometry was performed to determine the relative intensities of p125 and p105. The fraction, p125/p125, obtained from five separate replicates, is shown (mean), standardized against that observed in wild type CHO K1 cells.

The possibility that LRP-1 functions to regulate protein trafficking in the secretory pathway was first raised by Pietrzik et al. (41). These investigators demonstrated that LRP-1 stabilizes a 10-kDa proteolytic fragment of APP, which includes its transmembrane domain. The APP fragment was stabilized to a greater extent in CHO 14-2-1 cells, suggesting that LRP-1 in the ER plays a critical role. The results reported here further define the function of LRP-1 as a regulator of plasma membrane protein trafficking in the secretory pathway. We show, for the first time, that LRP-1 expression is associated with a major shift in the degree of maturation and subcellular localization of β1 integrin.

To determine the effects of LRP-1 on β1 distribution, we initially compared β1 integrin expression in LRP-1(+/-) MEF-1 cells, LRP-1(+/-) PEA10 cells, and LRP-1-deficient MEF-2 cells. In each case, LRP-1 expression was associated with an increase in p125, a decrease in p105, and an increase in cell surface β1 integrin, as determined by affinity precipitation of biotinylated proteins. In addition, we were able to prove that a decrease in mature β1 integrin is associated with the absence of LRP-1, by using a gain-of-function model, B41 cells, in which MEF-2 cells are transfected for stable expression of full-length human LRP-1. We confirmed that p105 is localized in the ER, by its susceptibility to Endo-H. Furthermore, we demonstrated that the relationship between LRP-1 expression and β1 integrin maturation is not cell type-specific by analyzing a second model system, the CHO cell. Once again in this cell type, LRP-1 expression was associated with increased p125, decreased p105, and increased cell surface β1 integrin.

The ability of LRP-1 to promote accumulation of β1 integrin at the cell surface represents a novel mechanism by which LRP-1 may regulate cell adhesion, migration, and cell signaling. Our metabolic labeling experiments supported a model, in which LRP-1 promotes trafficking of β1 integrin from the ER to the cell surface; however, the results of the metabolic labeling experiments do not preclude other mechanisms by which LRP-1 may affect the level of cell surface β1. To study whether LRP-1 facilitates β1 integrin endocytosis, we cultured cells for 3 days in the presence of GST-RAP. In previous studies, GST-RAP has been shown to increase cell surface levels of uPAR and APP (23, 35); however, GST-RAP failed to affect levels of p125 or p105. Because the β1 integrin subunit is a component of multiple integrins, our RAP experiments may not be sufficiently sensitive to detect an effect of LRP-1 on one specific integrin. Furthermore, LRP-1-facilitated integrin endocytosis may require specific conditions, such as activation of uPAR in the presence of plasminogen activator inhibitor-1, as previously described (29).

The ability of LRP-1 to promote β1 integrin p125 formation was most profound in cultures that became confluent, suggesting that formation of cell-cell contacts facilitates transfer of β1 integrin to the cell surface in a LRP-1-dependent manner. An exception was the CHO 14-2-1 cell line, which demonstrated a profound defect in β1 integrin transport, coupled with changes...
in cell adhesion and spreading, under all cell culture conditions, despite LRP-1 expression. Because LRP-1 is expressed but retained in the secretory pathway, in CHO 14-2-1 cells, the profound defect in β1 integrin transport may be explained if LRP-1 associates with β1 integrin in the ER, either directly or indirectly, through a bifunctional adaptor protein/chaperone. We were not able to support the hypothesis that β1 integrin associates with LRP-1 by co-immunoprecipitation analysis. Furthermore, when we overexpressed a candidate LRP-1 chaperone, RAP, in CHO K1 cells, CHO 14-2-1 cells, MEF-1 cells, and MEF-2 cells, high levels of RAP expression were demonstrated by immunoblotting; however, p125 and p105 were unchanged (results not shown). Other proteins that could bridge LRP-1 to β1 integrin include calreticulin, Fe65, Hsp90, and ICAP-1 (37, 42–44).

An alternative model to explain the defect in β1 integrin maturation, in CHO 14-2-1 cells, is that both LRP-1 and β1 integrin require the same gene product for proper post-translational trafficking, which is absent in this mutagen-treated cell line. Boca/MESD is a recently described ER chaperone that plays a critical role in the transport of multiple LDL receptor family members through the secretory pathway (45, 46). At this time, it is not known whether Boca/MESD functions in the trafficking of proteins outside the LDL receptor family. The significant decrease in cell surface β1 integrin, in CHO 14-2-1 cells, makes this cell line an attractive model for further study.

β1 integrin associates with multiple α-subunits to form integrin heterodimers that have partially redundant activities in cell adhesion (1). The effects of LRP-1 on individual β1-subunit-containing integrins remains to be determined. Such data may clarify certain results presented here. For example, although the major fibronectin-binding integrins, α5β1 and α6β1, both contain the β1-subunit, we found that adhesion and spreading of CHO 14-2-1 cells was delayed but not blocked. Redundancy in the activity of integrins may be operational here, because α5β1 also serves as a fibronectin receptor (1). Furthermore, although absolute cell surface levels of integrins may affect adhesion and spreading, the state of integrin activation and clustering are also very important (2).

From these studies, a model emerges in which LRP-1 serves to landscape the plasma membrane. It is known that LRP-1 may decrease cell surface levels of membrane proteins by facilitating their endocytosis (22–25). Our results now suggest that LRP-1 also may increase levels of cell surface proteins based on its ability to facilitate membrane protein transport in the secretory pathway. Together, these novel LRP-1 activities may substantially regulate how the cell responds to extracellular stimuli.

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Fig. 6. Adhesion of LRP-1-positive and -negative cells to fibronectin. A, cell culture wells were precoated with the indicated concentrations of fibronectin (FN) or blocked with BSA. PEA10 or MEF-2 cells were allowed to adhere to the wells for 30 min. Adhesion was determined by crystal violet staining. B, CHO K1 and CHO 14-2-1 cells were suspended in medium that included 1 mM MnCl 2 (+Mn 2+ ) or was MnCl 2 -free (−Mn 2+ ). The cells were then plated into wells that were pre-coated with different concentrations of fibronectin (FN) or blocked with BSA, and allowed to adhere for 30 min. Adhesion was determined by crystal violet staining (*, p < 0.05; **, p < 0.01). C, CHO cells were allowed to adhere to 1 μg/ml fibronectin for 30 min and then imaged by phase-contrast microscopy and photographed at equivalent magnification.
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