Cross-talk between integrin-mediated adhesion and growth factors has been described in many recent studies; however, the underlying mechanisms remain incompletely understood. We report here that detachment of cells from the extracellular matrix induces a decrease in both the autophosphorylation and protein levels of the platelet-derived growth factor receptor β (PDGF-R β), which was completely reversed upon replating cells on fibronectin. The effect occurred in all cells examined but to a greater extent in primary fibroblasts compared with established cell lines. Decreased PDGF-R levels in suspended cells correlated with ubiquitination of the PDGF-R and was blocked by treatment with inhibitors of the proteasome pathway. Unlike PDGF-induced down-regulation, detachment-induced degradation did not require receptor autophosphorylation, internalization, or tyrosine kinase activity. We conclude that cell detachment results in cellular desensitization to PDGF that is mediated by degradation of the PDGF-R via a novel ubiquitin-dependent pathway.

Cell adhesion to the extracellular matrix induces a wide array of intracellular signals mediated primarily by members of the integrin family of transmembrane receptors. Integrins act coordinate with growth factors to regulate migration, growth or differentiation, and survival (reviewed in Ref. 1). For instance, several recent studies reported a synergistic effect of PDGFα1 and cell adhesion on autophosphorylation of the PDGF-R and activation of the mitogen-activated protein kinase cascade (2, 3). Moreover, PDGF induces the association of its phosphorylated receptor with integrin α5β1 in fibroblasts plated on vitronectin, which correlates with enhanced PDGF-induced cell proliferation (4). These effects appear to contribute to the anchorage dependence of growth (1).

PDGF-Rs are transmembrane tyrosine kinases that mediate the effects of PDGF on growth and motility in cell types including fibroblasts and smooth muscle cells (5–7). Two structurally related receptors, α and β, possess different affinities for the A and B PDGF isoforms and generate similar though not identical cellular responses. Thus, both the α and β receptors stimulate cell proliferation, membrane ruffling, and loss of actin stress fibers, but only the β receptor promotes chemotaxis (5, 6). Binding of PDGF to its receptor induces receptor dimerization and autophosphorylation on several tyrosine residues, which provide docking sites for SH2-containing molecules, thereby initiating a variety of signaling pathways.

e-Cbl, one of the numerous proteins that bind to the PDGF-R (8), has recently been identified as a proteasomal E3-like ubiquitin ligase that is involved in down-regulation of tyrosine kinase receptors (9–11). PDGF-induced down-regulation is a complex process that involves receptor internalization through clathrin-coated pits and requires the receptor tyrosine kinase activity (12, 13). The 100-kDa GTPase dynam is responsible for pinching off of the vesicles, which results in the delivery of receptors to lysosomes, where both the ligand and the extracellular domain of the receptor are degraded (reviewed in Ref. 14). C-Cbl-mediated ubiquitination is also reported to target receptors for degradation by the proteasome (15). Hence, cells become less sensitive to PDGF following down-regulation of the PDGF-R.

In the present study, we provide evidence for degradation of the PDGF-R in cells detached from the substratum. Receptor degradation was preceded by ubiquitination and was blocked by proteasome inhibitors. However, detachment-induced degradation is distinct from ligand-induced down-regulation because the former did not require the presence of PDGF and was independent of receptor tyrosine kinase activity and receptor internalization. These results may therefore provide a novel mechanism by which integrins potentiate the cellular effects of PDGF.

**EXPERIMENTAL PROCEDURES**

Reagents—PDGF-BB was from Life Technologies, Inc. Calpain inhibitor II and AG1296 were from Calbiochem. Clostridium perfringens β-lactacystin was from Biomol Research Laboratories (Plymouth, PA). Chloroquine, soybean trypsin inhibitor, and bovine serum albumin were purchased from Sigma. Fibronectin was prepared from human plasma as previously described (16). GRGDS and GRGESP peptides were obtained from Peninsula Laboratories (Belmont, CA).

Cell Culture—Unless otherwise noted, experiments were performed using primary fibroblasts derived from p53−/− mouse embryos (murine embryonic fibroblast cells) cultured in Dulbecco's modified Eagle's medium (low glucose) containing 10% fetal bovine serum at 37 °C in 5% CO2. Cells were plated in 60-mm dishes until confluence and starved for 2 h in serum-free medium supplemented with 0.2% bovine serum albumin. Other cell lines were used where indicated. They were grown in similar conditions, except for the human primary fibroblasts, which were grown in 15% serum. For transfection, cells were seeded into 60-mm dishes at 300,000 cells/dish. The next day, cells were transfected with 1 μg of cDNA/dish using the Effecten transfection reagent (Qiagen) according to the manufacturer's instructions. Transfection efficiency was routinely 20–30% of cells. Cells were used 48 h after transfection.

**Viral Infection**—Recombinant adenoviruses expressing dynamin-2 K44A (a dominant-negative form of dynamin) and a tetracycline-sensitive transactivator were used to co-infect cells for 2 h with 200 plaque-forming units/cell in phosphate-buffered saline (17). They were then washed twice in phosphate-buffered saline, and complete medium was added with or without 20 ng/ml tetracycline. After 24 h the cells were...
starved for 4 h before use in suspension experiments.

Cell Suspension—Cells were treated with trypsin-EDTA for 3 min at room temperature and then placed on ice. Ice-cold medium containing 0.2% bovine serum albumin and 250 μg/ml soybean trypsin inhibitor was added to stop trypsinization, and cells were centrifuged at 2,000 rpm for 2 min and washed once. They were finally resuspended in 3 ml of the same medium and maintained in suspension at 37 °C in the incubator for the indicated periods of time, with occasional agitation to prevent clumping. Attached or suspended cells were chilled on ice and washed twice with ice-cold buffer consisting of 50 mM Hepes, pH 7.6, 150 mM NaCl, 10 mM EDTA, 10 mM Na2HPO4, 200 μM vanadate. Cells were solubilized in the same buffer also containing 1% Triton X-100, 0.5% deoxycholate, 0.1% SDS, 2 mM phenylmethylsulfonyl fluoride, 10 μg/ml aprotonin, and 2 μg/ml leupeptin. After 20 min, lysates were clarified by centrifugation at 13,000 rpm for 15 min, and the protein concentration was determined using the BCA protein assay reagent (Pierce).

Samples containing 200 μg of cellular protein were subjected to immunoprecipitation using 4 μg of anti-PDGF-R antibodies (Upstate Biotechnology, Lake Placid, NY), which were preadsorbed on protein A-agarose (Pierce). Immunoprecipitations were conducted at 4 °C for 4 h, and precipitates were washed three times in 50 mM Hepes, pH 7.6, 150 mM NaCl, 0.1% Triton X-100 and reaccessed in Laemmli sample buffer. Additionally, whole cell lysates (30 μg of protein) were denatured in Laemmli sample buffer.

Western Blots and Antibodies—Samples were subjected to SDS-polyacrylamide gel electrophoresis and transferred to Hybond-C nitrocellulose (Amersham Pharmacia Biotech). Immunoblots were blocked with 5% fat-free milk and incubated with the following primary antibodies: 0.05 μg/ml polyclonal anti-PDGF-R β (sc-432), 0.2 μg/ml monoclonal anti-ubiquitin (sc-8017), 0.2 μg/ml monoclonal anti-FAK antibodies. For quantitation of receptor amounts, values represent the percent relative to untreated attached cells. Data are means ± S.E. from three experiments.

were incubated with peroxidase-conjugated goat antibodies against mouse or rabbit IgG at 0.3 μg/ml (Biosource International, Camarillo, CA). Immunoblots were developed using luminol reagent (Santa Cruz Biotechnology). Stripping of membranes was performed at 65 °C for 1 h in 0.2 M glycine, pH 2.5, 0.5 M NaCl, 1% SDS, 0.5% Tween 20.

RESULTS

Effects of Cell Adhesion on PDGF-R Levels—To detect possible interactions between cell adhesion and PDGF-R function, primary murine embryo fibroblasts were maintained in serum-free medium for 2 h either attached or in suspension. The β isoform of the PDGF-R was then analyzed in response to increasing concentrations of PDGF-BB, which specifically binds to this receptor. Western blotting of whole cell lysates with anti-phosphotyrosine revealed a major protein of 185 kDa corresponding to the size of the PDGF receptor that was induced by PDGF (Fig. 1). As described previously (2), the phosphorylation of the PDGF-R was dramatically decreased in suspended cells compared with attached cells. As expected, a band of approximately 120 kDa that showed dramatic dephosphorylation in suspended cells but only a slight response to PDGF was most likely represents focal adhesion kinase (FAK) (18–20). At 10 ng/ml PDGF, autophosphorylation of PDGF-R in suspended cells was 27 ± 8% (n = 3) of the response in adherent cells. Probing with antibodies to the PDGF-R revealed an unexpected decrease in total receptor levels as well. Because the level of FAK was somewhat higher in suspended cells, we conclude that both autophosphorylation and levels of the PDGF receptor were specifically decreased in suspended cells.

Cell Type Specificity—To test whether this effect is cell type-specific, other cell lines were examined. Primary murine embryonic fibroblasts, NIH 3T3, Swiss 3T3, 10T1/2, BHK, and normal diploid human fibroblasts all showed some decrease in PDGF-R levels and autophosphorylation in suspended cells, but the extent of the effect varied (Table I). Primary fibroblasts were the most sensitive, with phosphorylation and receptor levels in suspended cells that were less than 25% compared with attached cells. By contrast, immortalized cell lines still showed substantial receptor autophosphorylation when suspended. It should be noted, however, that the changes in receptor phosphorylation did not precisely correlate with receptor levels in all of the cell lines, suggesting that additional mechanisms may regulate receptor phosphorylation. These results are consistent with past studies of these cell lines demonstrating that growth factors still induced a subset of normal signaling events in suspended cells (3, 21, 22).

Reversibility—The reversibility of these effects was tested by replating the cells onto dishes coated with fibronectin. Replating induced an increase of PDGF-induced receptor phosphorylation, which was detectable at 30 min of replating and was optimal after 3 h (Fig. 2). Interestingly, receptor phosphoryla-

### Table I

Comparison of cell lines

| Cell type     | PDGF-induced autophosphorylation (%) | PDGF-R amount |
|--------------|-------------------------------------|---------------|
|              | Att | Sus | Att | Sus | Sus + PDGF |
| MEF cells    | 100 | 45  | 100 | 80 ± 6 | 28 ± 7 | 14 ± 5 |
| human fibroblasts | 100 | 14 ± 3 | 100 | 67  | 26 ± 1 | 28 ± 5 |
| BHK          | 100 | 49 ± 21 | 100 | 103 ± 14 | 43 ± 21 | 42 ± 21 |
| Swiss 3T3    | 100 | 78 ± 33 | 100 | 95 ± 8 | 50 ± 20 | 55 ± 20 |
| 10T1/2       | 100 | 57 ± 36 | 100 | 42 ± 10 | 41 ± 17 | 31 ± 15 |
| NIH 3T3      | 100 | 59  | 100 | 74  | 24  | 15  |
tion was higher 3 and 4 h after replating on fibronectin than in stably attached cells. A corresponding recovery of receptor levels was also observed. These data demonstrate that loss of PDGF-R was completely reversible and are consistent with published studies demonstrating that replating cells on extracellular matrix proteins can potentiate growth factor responses more effectively than stable cell adhesion (23).

**Ubiquitination of the PDGF-R**—To detect events that precede degradation, cells were detached and kept in serum-free medium for 5, 15, 60, or 120 min, including stimulation with 5 ng/ml PDGF during the last 5 min. Immunoblotting of anti-PDGF-R immunoprecipitates with anti-phosphotyrosine antibodies revealed that PDGF-R phosphorylation was not impaired after 5 or 15 min but was substantially diminished after 1 or 2 h (Fig. 3). Interestingly, in addition to the 185-kDa receptor, a higher molecular weight band was immunoprecipitated by the anti-PDGF-R antibody after 5 and 15 min in suspension, indicative of a covalent modification of the receptor. A similar shift due to ubiquitination of the receptor has been described after exposure of cells to PDGF. Although smearing due to polyubiquitination has been reported for many proteins, it has been previously reported that much of the ubiquitinated PDGF-R runs as a sharp band at a similar molecular weight to what we observed (24). Reprobing with an antibody against ubiquitin revealed a reactive band in cells that had been in suspension for 5 and 15 min that co-migrated with the upper band in the anti-phosphotyrosine blots. Ubiquitination is known to be involved in the degradation of the PDGF-R (25). Thus, the decrease in receptor amounts induced by detachment of cells from the extracellular matrix is likely to result from ubiquitin-dependent receptor degradation.

To test whether detachment-induced receptor ubiquitination and degradation required PDGF, cells were detached and maintained in suspension in the absence of PDGF for increasing periods. Immunoblotting of PDGF-R immunoprecipitates with anti-receptor antibodies showed that degradation occurred rapidly in the absence of added PDGF (Fig. 4A). However, ubiquitination was difficult to detect consistently under these experimental conditions. To increase the sensitivity of the assay, cells were transfected with a plasmid encoding a His<sub>6</sub> tagged-ubiquitin. When probed with an antibody to the epitope tag, ubiquitination of the unstimulated receptor was readily detected (Fig. 4B). Ubiquitination occurred as soon as

![Fig. 2. Replating onto fibronectin.](Image)

**FIG. 2. Replating onto fibronectin.** Cells were either left attached (A) or suspended for 2 h at 37 °C (S). Suspended cells were replated on dishes coated with fibronectin (25 μg/ml) and blocked with 10 mg/ml heat-denatured bovine serum albumin. PDGF-BB was added or not for 5 min at 37 °C as indicated. Whole cell lysates were analyzed by Western blotting using anti-PDGF-R or anti-phosphotyrosine antibodies. Membranes were stripped and reprobed using antibodies to FAK.

![Fig. 3. PDGF-R ubiquitination.](Image)

**FIG. 3. PDGF-R ubiquitination.** Cells were suspended in serum-free medium for the indicated periods. Attached (Att) and suspended cells were treated with PDGF (5 ng/ml) for 5 min. PDGF-Rβ immunoprecipitates were analyzed by Western blotting using anti-phosphotyrosine antibodies. Membranes were stripped and reprobed with monoclonal antibodies to ubiquitin. Two experiments gave similar results.

![Fig. 4. Ligand-independent degradation and ubiquitination of the PDGF-R.](Image)

**FIG. 4. Ligand-independent degradation and ubiquitination of the PDGF-R.** A, cells were suspended in serum-free medium for increasing periods of time or left attached (Att). PDGF-R immunoprecipitations were analyzed by Western blotting with anti-receptor antibodies. Total lysates were analyzed in parallel by Western blotting with antibodies to FAK. Results are representative of six experiments. B, cells transfected with a cDNA coding for His-tagged ubiquitin were suspended for various periods of time or left attached. PDGF-Rs were immunoprecipitated, and ubiquitination was detected by probing blots with an antibody to the His tag. C, adherent cells or cells that had been in suspension for 2 h were treated or not with PDGF (10 ng/ml) for 1 h. Lysates were analyzed by Western blotting with anti-receptor antibodies, and results were quantitated as a percent relative to non-stimulated attached cells (means ± S.E. from seven experiments). **IP**, immunoprecipitation.
cells were detached, reached a maximum level between 5 and 30 min, and decreased after 2 h, concomitant with loss of the receptor. In some experiments, blots were stripped and reprobed with antibodies to phosphotyrosine, which confirmed that the receptor was not phosphorylated under these conditions (data not shown). We conclude that ubiquitination and degradation of the PDGF receptor constitute a ligand-independent process induced by loss of cell adhesion. However, ligand binding to the receptor accelerated and further increased the rate of degradation (Fig. 4C).

**Fig. 5. Suspension without trypsin.** A, serum-deprived cells were left attached or gently scraped off the plate and maintained in suspension for 15 or 120 min. Attached cells were stimulated or not with 10 ng/ml PDGF-BB for 15 min as indicated. PDGF-R immunoprecipitates were analyzed by Western blotting with antibodies to the receptor. Membranes were stripped and reprobed with antibodies to ubiquitin. B, cells were treated with RGD- or RGE-containing peptides (800 μg/ml) in medium with additional Hepes pH 7.6 for the indicated periods. PDGF-R immunoprecipitates were analyzed by Western blotting using antibodies to the receptor. Membranes were stripped and reprobed with antibodies to ubiquitin. Whole cell lysates were analyzed in parallel by Western blotting with anti-receptor antibodies. Results are representative of three experiments. Susp, suspended; IP, immunoprecipitation.

cells were detached, reached a maximum level between 5 and 30 min, and decreased after 2 h, concomitant with loss of the receptor. In some experiments, blots were stripped and reprobed with antibodies to phosphotyrosine, which confirmed that the receptor was not phosphorylated under these conditions (data not shown). We conclude that ubiquitination and degradation of the PDGF receptor constitute a ligand-independent process induced by loss of cell adhesion. However, ligand binding to the receptor accelerated and further increased the rate of degradation (Fig. 4C).

**Detachment without Trypsin**—To ensure that degradation of the PDGF-R was not due to trypsinization, cells were detached without trypsin by gently scraping with a rubber policeman. Detachment via this method induced ubiquitination at 15 min, followed by a decrease in PDGF receptor levels at 2 h (Fig. 5A), similar to trypsinization. As before, no increase in tyrosine phosphorylation of the receptor was detected (data not shown). We also examined cells treated with an RGD-containing peptide, which inhibits integrin binding to their ligands and thus induces cell detachment. RGE peptide was used as a negative control. After 20 min of RGD treatment, cells started to round up and were usually detached after 60 min (data not shown). Ubiquitination of the receptor was clearly observed between 20 and 30 min (Fig. 5B), and degradation occurred slightly later, with about 50% of the receptor being lost after 45 min of RGD

**Fig. 6. Effect of tyrphostin AG 1296.** Cells were treated with or without AG1296 (20 μM) for 30 min before detachment, and the inhibitor was maintained during suspension. Attached cells were incubated with or without the inhibitor for 30 min prior to PDGF stimulation (10 ng/ml) for 1 h. Lysates were analyzed by Western blotting using antibodies to the PDGF-R. Quantitation is expressed as a percent relative to untreated attached cells (means ± S.E. from four independent experiments). Susp, suspended.

**Fig. 7. Effect of proteasomal and lysosomal inhibitors.** A, attached cells were incubated or not with calpain inhibitor-II (CI, 50 μM) or clasto-lactacystin β-lactone (βL, 20 μM) for 30 min prior to PDGF addition (10 ng/ml) for 1 h. Suspended cells were incubated with the inhibitors for 30 min before suspension for 2 h. Lysates were analyzed by immunoblotting with anti-receptor antibodies. Results are expressed as a percent relative to untreated attached cells. Means ± S.E. from four experiments (calpain inhibitor-II) and two experiments (clasto-lactacystin β-lactone) are shown. B, the experiment was performed as described above except that the lysosomal inhibitor chloroquine was used (20 μM). Quantitation shows values ± S.E. from three experiments.
treatment. These data indicate that PDGF-R degradation is a consequence of loss of integrin-mediated interactions and is not due to trypsinization.

Degradation Is Independent of PDGF-R Internalization and Tyrosine Kinase Activity—The observation that PDGF binding to the receptor was not required for detachment-induced degradation indicated that it could be independent of the receptor tyrosine kinase. Thus, we examined the specific inhibitor AG1296, which completely blocked PDGF-R kinase activity under these experimental conditions (data not shown). As a control, attached cells were stimulated with PDGF for 60 min to induce down-regulation of the receptor. As shown in Fig. 6, tyrphostin AG1296 inhibited ligand-induced down-regulation but had no effect on suspension-induced degradation. Thus, detachment-induced degradation does not require receptor tyrosine kinase activity.

Ligand-induced down-regulation of tyrosine kinase receptors involves receptor internalization and sorting to the lysosomes for degradation (12). In addition, ubiquitination targets the receptor to the proteasome (15, 25). To assess the contributions of these pathways, we used a variety of inhibitors. Two inhibitors of the proteasome, calpain inhibitor II (50 μM) and clasto-lactacystin β-lactone (20 μM), prevented degradation of the PDGF-R in suspended cells but had little effect on ligand-induced down-regulation (Fig. 7A). Although calpain inhibitor II has cross-reactivity with calpain and possibly with lysosomal enzymes, the clasto-lactacystin β-lactone is highly specific for the proteasome.

In contrast, chloroquine, which specifically inhibits lysosomal degradation, had no effect on loss of PDGF-R in suspended cells but blocked PDGF-induced receptor degradation (Fig. 7B). This result indicates that loss of the PDGF-R in suspended cells is independent of the lysosomal pathway but is strongly dependent on proteasome activity.

To determine whether receptor internalization is required for suspension-induced degradation, cells were incubated at 4°C to decrease membrane trafficking. As expected, low temperature prevented PDGF-induced down-regulation of the receptor (Fig. 8A). Remarkably, low temperature did not prevent receptor degradation following suspension. To further test the requirement for internalization, we expressed dynamin-2 mutated at lysine 44, which abolishes the GTPase activity of the protein. This construct functions as a dominant negative to prevent endocytosis of coated-pit vesicles, which instead accumulate at the cell surface (26). Cells were co-infected with recombinant adenoviruses expressing dynamin-2 K44A and a tetracyclin-sensitive transactivator (17). In the presence of tetracycline, the mutant dynamin was not expressed, and ligand-dependent as well as suspension-induced degradation of the receptor was observed (Fig. 8B). In the absence of tetracycline, when the HA-dynamin mutant was expressed, ligand-induced PDGF-R down-regulation was blocked, whereas receptor degradation still occurred in suspended cells. These results indicate that internalization is not required for PDGF-R degradation in suspended cells.

**DISCUSSION**

Integrins and PDGF act synergistically to modulate cell proliferation and migration. One of the first steps in the collaborative pathway is the enhancement of PDGF-R tyrosine kinase activity induced by cell adhesion (27). Our data show that disruption of cell adhesion resulted in a dramatic decrease of PDGF-R autophosphorylation and a similar decrease of receptor levels due to degradation. Although this effect occurred in all of the cell lines tested, the extent showed significant variations, with primary or diploid fibroblasts being more sensitive than immortalized cell lines.

Several lines of evidence demonstrate that the mechanism of suspension-induced degradation of the PDGF-R is distinct from that of ligand-induced down-regulation. Thus, PDGF-stimulated receptor down-regulation requires receptor tyrosine kinase activity and autophosphorylation. By contrast, degradation in suspended cells was independent of the receptor kinase and was not correlated with its phosphorylation. Moreover, ligand-induced down-regulation involves receptor internalization (12, 13) and is sensitive to the lysosomal inhibitor chloroquine (25), whereas in degradation suspended cells were not blocked by treatments that inhibit internalization or lysosomal degradation.

On the other hand, both pathways share a dependence on ubiquitination (Ref. 25 and this study). Ubiquitin is a highly conserved polypeptide of 76 amino acids that is conjugated via its carboxyl-terminal carboxyl group to the amino-terminal group of a lysine in the acceptor protein (reviewed in Ref. 28). Ubiquitin is first activated by the ubiquitin-activating enzyme E1. It is then transferred to the ubiquitin-conjugating enzyme E2, which promotes its binding to a protein together with the ubiquitin-ligase E3. It is now accepted that single and poly-ubiquitins act as functionally distinct signals. Thus, poly-ubiquitin chains of at least four molecules (29) target the protein to a large protease complex called the 26 S proteasome. The molecular mechanism of targeting involves a dramatic increase in affinity, with Ub 4 being the shortest chain that binds with high affinity to the proteasome (30). In contrast, a single ubiquitin is only a weak signal for degradation by the proteasome but serves to trigger internalization of cell surface proteins into the endocytic pathway (reviewed in Ref. 31). Indeed, resolution
of the three-dimensional structure of folded ubiquitin has recently revealed an internalization signal that is sufficient to promote endocytosis of proteins to which it is linked (32).

Upon ligand binding, the PDGF-R is modified with multiple ubiquitin molecules (24). However, because the cytoplasmic domain of the receptor contains several lysine residues, it is unknown whether the modified receptor carries only long ubiquitin chains or includes mono-ubiquitins on different lysines. The result that PDGF triggers ubiquitin-dependent receptor degradation via both proteasomes and lysosomes (25) suggests that under these conditions the PDGF-R contains mixtures of poly- and mono-ubiquitins.

Graphical determinations of the sizes of the PDGF-R and the Ub-PDGF-R resulted in apparent molecular masses of 190,000 and 220,000–230,000 kDa, respectively. This gain of 30,000–40,000 kDa corresponds to the addition of at least four ubiquitin molecules. Because it appears as a sharp band instead of a smear, we think it likely that a single poly-ubiquitin chain is appended to the PDGF-R in suspended cells. As discussed above, the addition of four ubiquitins would allow targeting of the receptor to the proteasome but would not trigger internalization consistent with our observations.

It has been proposed that poly-ubiquitin linked to the cytoplasmic domain of membrane-bound proteins could recruit the 26 S proteasome to the membrane compartment (discussed in Ref. 33). The proteasome might also extract the protein from the membrane, with the ATPase of the regulatory subunits providing the required energy. Therefore, whereas ligand-induced down-regulation includes both lysosomal and proteasomal degradation pathways and occurs during the transit of the receptor through the endosomal/lysosomal compartments, we hypothesize that in suspended cells receptors are degraded by the proteasome primarily at the plasma membrane.

Ligand-stimulated ubiquitination of the PDGF receptor is mediated by the phosphorylation-dependent recruitment of the SH2 domain-containing protein Cbl (15). Once associated with the phosphorylated receptor, Cbl triggers the transfer of ubiquitin from an E2 ubiquitin-conjugating enzyme to the receptor and therefore functions as a proteasomal E3-like ubiquitin ligase (9–11). However, receptor phosphorylation was not observed in suspended cells, and the tyrosine kinase activity of the PDGF-R was not required for degradation. Thus, Cbl is not a likely mediator of degradation in suspended cells.

The mechanism by which integrins target the PDGF-R for degradation via the proteasome system is currently unknown. Loss of cell adhesion is usually thought to trigger termination of cell signal and protein activities. For instance, suspended cells show low signals of GTPases, such as Ras, Rac, and Cdc42, mitogen-activated protein kinases, and the protein tyrosine kinases FAK and Src (34–36). According to this view, it would appear unlikely that detachment sends a signal to activate degradation. Instead, integrins may normally prevent the PDGF-R from constitutive degradation by inhibiting its targeting to the proteasome. However, there is evidence that unoccupied integrins can in fact transmit signals that inhibit cell proliferation (36–38). Degradation of PDGF receptors could therefore be actively triggered by unoccupied integrins following loss of contact with extracellular matrix proteins.

Many recent studies have identified various mechanisms of cross-talk between integrins and growth factor receptors that result in the control of cell proliferation, differentiation, or survival. It is now well known that integrins stimulate many signaling cascades that are shared by growth factor receptors, for example, the phosphatidylinositol 3-kinase pathway and the mitogen-activated protein kinase cascade (34, 35, 39). Concomitant activation of these pathways would lead to a critical level of activation needed to induce proliferation or differentiation. In addition, integrins appear to associate with and modulate the activity of tyrosine kinase receptors, leading to enhanced growth factor signals (1, 40). This work therefore identifies a new mode of cross-talk in which integrins modulate the very first step of PDGF signaling by altering the number of responsive PDGF receptors at the cell surface. These effects very likely all contribute to the anchorage dependence of cell survival and growth, mechanisms that are essential to the integrity of multicellular organisms (34, 35, 40).

Acknowledgments—The cDNA encoding His6-tagged ubiquitin was generously provided by Dr. Alfred Goldberg (Harvard Medical School, Boston, MA). Recombinant adenoviruses were kindly provided by Hanae Damke, Kenneth N. Fish, and Sandra L. Schmidt (Department of Cell Biology, Scripps Research Institute, La Jolla, CA).

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