Regulation of Cell Adhesion Receptors by Transforming Growth Factor-β

CONCOMITANT REGULATION OF INTEGRINS THAT SHARE A COMMON β1 SUBUNIT

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Cell adhesion to extracellular matrices is mediated by a set of heterodimeric cell surface receptors called integrins that might be the subject of regulation by growth and differentiation factors. We have examined the effect of transforming growth factor-β1 (TGF-β1) on the expression of the very late antigens or αβ1 group of integrins in human cell lines. The six known members of this family share a common β1 subunit but have distinct α subunits that confer selective affinity toward type I collagen, fibronectin, laminin, and other as yet unknown cell adhesion proteins. Using a panel of specific antibodies and cDNA probes, we show that in WI-38 lung fibroblasts TGF-β1 elevates concomitantly the expression of α1, α2, α3, α5, and β1 integrin subunits at the protein and/or mRNA level, their assembly into the corresponding αβ1 complexes, and their exposure on the cell surface. The rate of synthesis of total α subunits relative to β1 subunit is higher in TGF-β1-treated cells than in control cells. The characteristically slow (t1/2 ~ 10 h) rate of β1 conversion from precursor form to mature glycoprotein in untreated cells increases markedly (to t1/2 ~ 3 h) in response to TGF-β1. The results suggest that in WI-38 fibroblasts the β1 subunit is synthesized in excess over α subunits, and assembly of β1 subunits with rate-limiting α subunits is required for transit through the Golgi and exposure of αβ1 complex on the cell surface. TGF-β1 does not induce the synthesis of integrin subunits that are not expressed in unstimulated cells, such as α2 and α6 subunits in WI-38 fibroblasts. However, α1 and α6 subunits can be regulated by TGF-β in those cells that express them. The results suggest that TGF-β regulates the expression of individual integrin subunits by parallel but independent mechanisms. By modifying the balance of individual αβ integrins, TGF-β1 might modulate those aspects of cell migration, positioning, and development that are guided by adhesion to extracellular matrices.

Integrins are a family of membrane spanning glycoproteins that mediate cell adhesion to extracellular matrix and basement membranes, to other cells, and to plasma proteins (reviewed in Refs. 1 and 2). The cytoplasmic domains of these cell adhesion receptors may interact with actin filament connecting proteins, suggesting that integrins link extracellular matrix to cytoskeleton. The basic structure of integrins is a heterodimeric complex of one 130–200-kDa β subunit linked noncovalently to one 90–130-kDa α subunit. Three different β subunits have been identified. They are structurally related to each other, with about 44–46% amino acid sequence identity (3–6), and they define three families of integrins (1).

The prototype integrin is the fibronectin receptor originally purified from human placenta (7) and from human osteosarcoma cells (8). It recognizes the Arg-Gly-Asp sequence present in the fibronectin molecule or in synthetic peptides (9). Its α subunit is divided in two fragments that derive from proteolytic processing of the translation product but remain disulfide-bonded to one another in the mature polypeptide (4).

Independent studies on T-lymphocyte surface membrane proteins have revealed a family of heterodimeric cell adhesion receptors originally termed very late antigens (VLAs) (10–12). The VLAs share one common subunit, now known as the β1 integrin subunit. The VLA or αβ1 integrin family consists of at least six different members (12). At least four of the members of this family mediate adhesion of cells to extracellular matrix proteins. αβ integrin (VLA-1) was first described in activated peripheral blood lymphocytes. However, it is absent from most other hematopoietic cells, while it is expressed by several other cell types, predominantly fibroblasts. αβ integrin appears to be a cell adhesion receptor, but its ligand is unknown (10, 12, 13). αβ integrin can bind type I collagen and αβ integrin mediates attachment to laminin, collagen, and fibronectin (14, 15). αβ (VLA-4) is widely expressed in leukocytes, but it is usually absent from most adherent cell types (16). The αβ integrin was independently identified as a VLA-5, but amino acid sequence and immunological evidence have indicated that it is the same as the classical fibronectin receptor, initially called also αβ (17). αβ integrin (VLA-6) has been recently identified as a novel integrin in human platelets (18). The function of αβ integrins is still unknown. A large body of evidence indicates that the αβ integrins play an important role as mediators of cell adhesion and migration, and their function influences cell proliferation and differentiation (for reviews see Refs. 1 and 2). Recent studies have shown that the normal function of αβ integrins is critical in certain differentiation and developmental processes. For example, disruption of fibronectin receptor function by addition of specific antibodies to chick embryo myoblasts alters myogenic differentiation of

1The abbreviations used are: VLA, very late antigen; TGF-β1, transforming growth factor-β1; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; EGTA, [ethylenebis(oxyethylenenitrilo)] tetracetic acid.
these cells (19). Furthermore, mutations that disrupt the β integrin subunit structural gene in Drosophila lead to a phenotype characterized by a lethal failure to assemble muscular tissue during the fly’s embryogenesis (20).

In previous studies, we have shown that expression of fibronectin receptors in fibroblasts and epithelial cells from human, rat, and mouse origin is regulated by transforming growth factors-β1 and -β2 (TGF-β1, TGF-β2). Increases in the level of fibronectin receptor β1 subunit mRNA and β1 subunit protein caused by TGFs-β lead to a higher level of fibronectin receptor on the cell surface, higher fibronectin binding capacity and elevated adhesion of cells to fibronectin matrices (21). It has also been shown that fibroblasts treated with TGF-β1 retain higher levels of fibronectin receptor α5 subunit mRNA (22). TGFs-β are representative of a large family of hormonally active polypeptides that control growth, differentiation, and morphogenesis in cultured cells and organisms from insects to mammals (reviewed in Ref. 23). This family includes members implicated in developmental processes such as embryogenic dorsoventral patterning and imaginal disk development in Drosophila (24) and morphogenesis in Xenopus early embryo (25). TGFs-β themselves can act as inducers of mesodermal mysogeneses markers in Xenopus embryos (26, 27). In addition to their ability to regulate expression of fibronectin receptors, TGFs-β are potent stimulators of the expression of extracellular matrix proteins including fibronectin (28), various types of collagen (28, 29), matrix proteoglycans (30), as well as inhibitors of matrix-degrading proteases (31, 32). The extensive changes induced by TGFs-β in extracellular matrix proteins are regarded as events through which these factors may control expression of specific phenotypes in vitro and may influence tissue formation and repair in vivo (21, 23, 29).

We have explored the possibility that TGFs-β might regulate other members of the integrin family in addition to the fibronectin receptor. This report focuses on the large group of integrins that share the β1 subunit. A companion paper (33) describes the regulation by TGF-β of two other families of integrins, those defined by the presence of β2 and β3 subunits and involved in cell-cell and cell-vitronectin adhesion. It is not clear to what extent TGFs-β influence the expression of several coexisting αβ integrins. The results of this study also provide information on the process of assembly and maturation of mammalian αβ integrins.

**EXPERIMENTAL PROCEDURES**

**Antibodies**—Polyclonal anti-β integrin subunit antiserum was raised in rabbits by immunization with human placental fibronectin receptor affinity-purified according to the method of Pytel et al. (34). The following monoclonal antibodies all against human proteins were used: A-1A5 specific for mature β1 subunit (10), TS2/7 specific for αβ subunit (13), 12F1 specific for αβ subunit (16), J143 specific for α5 subunit (11, 35), B5G10 specific for α5 subunit (36), and GOH3 specific for α5 subunit (18). In the immunoprecipitation experiments some of the monoclonal antibodies were used with a secondary antibody: either with rat anti-mouse κ chain (mAb 185.1) (37) or with mouse anti-rat κ chain (R7G9) (38).

**Cell Culture**—WI-38 human lung fibroblasts (American Type Culture Collection) were grown in minimum essential medium supplemented with 10% fetal bovine calf serum. For biosynthetic labeling experiments, cells were grown to confluence and labeled with 50 μCi/ml of [35S]methionine (Amersham Corp., 200 Ci/mmol) or 20 μCi/ml of [35S]cysteine (Amersham Corp.; 1000 Ci/mmol) in methionine- or cysteine-free minimum essential medium, respectively.

Cell surface iodinations were carried out with cells in suspension. Cells were detached by incubation for 15 min at 37 °C with a solution containing 125 mM NaCl, 5 mM KCl, 10 mM Tris, 1 mM EDTA, pH 7.4, and recovered by centrifugation at 500 x g for 5 min. The samples were adjusted to contain equal numbers of cells in 0.5 ml of a buffer consisting of 128 mM NaCl, 5 mM KCl, 1.2 mM CaCl2, and 28 mM HEPES, pH 7.4. Iodination was carried out on ice by addition of 0.5 mCi of Na125I (Amersham Corp.; 16 mCi/μg), 0.1 mg of lactoperoxidase, and 0.005% H2O2 with periodic mixing for 10 min. Cells were washed four times in the same buffer before solubilization and immunoprecipitation.

**Immunoprecipitation**—Biosynthetically labeled cell monolayers were washed on ice with a buffer containing 150 mM NaCl, 1 mM CaCl2, 1 mM MgCl2, and 25 mM Tris-HCl, pH 7.4, followed by detachment of the cells by scraping. Cell pellets obtained by centrifugation at 500 x g for 5 min were solubilized in 0.2 ml of the same buffer containing 100 mM N-ethyl-m-d-glucoperoxidase (Sigma) on ice for 10 min with occasional vortexing. The insoluble material was removed by centrifugation at 13,000 x g for 4 min at 4 °C. Triton X-100 (0.5 v/v %) and bovine serum albumin (0.5 mg/ml) were added to the supernatants, which were then preclared by incubation with 20 μl of packed protein A-Sepharose beads (Pharmacia LKB Biotechnology Inc.). The resulting supernatants were immunoprecipitated with antibodies for 12 h at 4 °C. Conditions for quantitative immunoprecipitation of the corresponding cellular components were established for each antibody preparation in preliminary experiments. Rabbit nonimmune serum and monoclonal rat anti-mouse immunoglobulin κ chain antibodies were used as negative controls. Immune complexes were recovered by binding to protein A-Sepharose, washing the beads four times with Tris-buffered saline containing 0.1% Triton X-100 and 1 mg/ml bovine serum albumin, and once with 150 mM NaCl and 25 mM Tris-HCl, pH 7.4. The immunoprecipitates were resolved by electrophoresis on polyacrylamide-dodecyl sulfate gels followed by fluorography using Enlightening (DuPont-New England Nuclear). Gel electrophoresis was performed in the absence of reductant unless otherwise indicated.

**Western Immunoblot**—WI-38 fibroblasts were detached as described above, counted using a Coulter Counter, lysed into electrophoresis sample buffer, and subjected to dodecyl sulfate-polyacrylamide gel electrophoresis. Proteins were then transferred electrophoretically onto nitrocellulose membranes for 12 h at 250 mA and at 4 °C. Membranes were incubated for 2 h with 30 μg/ml of bovine serum albumin in 0.15 M NaCl, 25 mM Tris, pH 7.4, and then overnight with the same buffer containing a polyclonal anti-fibronectin receptor antiserum at 1:100 dilution. The membranes were washed with the same buffer, incubated for 2 h with [32P]labeled protein A-Sepharose (2–10 μCi/μg, DuPont-New England Nuclear) in the same buffer, washed, and subjected to autoradiography.

**Endoglycosidase H Treatment**—For endoglycosidase H (endo-β-N-acetylglucosaminidase, EC 3.2.1.96; Boehringer Mannheim) digestion, immunoprecipitates bound to protein A-Sepharose were mixed with 300 μl of 0.2 M sodium citrate buffer, pH 5.5, containing 5 millilitres of enzyme, and incubated for 20 h at 37 °C. After incubation, Sepharose beads were precipitated by centrifugation heated in sample buffer, and analyzed by electrophoresis.

**Northern Blot Hybridization**—Northern hybridization analysis of integrin mRNA was done using total cellular RNA prepared by the guanidine isothiocyanate, CsCl centrifugation procedure (39). The RNA was fractioned on 1% agarose gels containing formaldehyde, transferred onto nitrocellulose, and hybridized to cDNA probes specific for αβ integrin subunit (40), or β1 subunit (4). A cDNA probe specific for glyceraldehyde-3-phosphate dehydrogenase (41) was used as a control. The cDNA probes were labeled with [32P] using the Multiprime DNA labeling system (Amersham Corp.).

**RESULTS**

**Identification of αβ Integrin Subunits and Their Biosynthetic Precursors in WI-38 Cells**—The human fibronectin receptor consists of one αβ subunit and one β1 subunit held together via noncovalent interactions that are stabilized by divalent cations (1, 2). The β1 subunit is present in at least five additional human integrins that contain distinct α subunits (1, 12). To raise antibodies that would immunoprecipitate all these integrins, we immunized rabbits with human placenta fibronectin receptor affinity-purified by affinity chromatography. The resulting polyclonal antibodies recognized β1 subunit but not α5 subunit in purified fibronectin receptor preparations as determined by immunoblot analysis (Fig. 1A).

2 R. Hynes and V. Patel, personal communication.
Polyclonal antibodies immunoprecipitated $\alpha_5$ and $\beta_1$ subunits under conditions that preserve integrin complex stability, but they precipitated only $\beta_1$ subunit if the calcium chelator EGTA or the ionic detergent sodium dodecyl sulfate were present during immunoprecipitation (results not shown). These observations indicated that the polyclonal antibodies recognized predominantly epitopes in the $\beta_1$ subunit. The ability of these antibodies to precipitate $\alpha_5$ and other $\alpha$ subunits under conditions that stabilize integrin complexes (see below) could be due to either recognition of conformational epitopes in assembled $\alpha$ subunits or, most likely, coprecipitation of $\alpha$ subunits that were assembled with $\beta_1$ subunit. These polyclonal antibodies were highly specific as they recognized only $\beta_1$ subunits or their biosynthetic precursors in immunoblots of crude extracts from WI-38 human fibroblasts (Fig. 1A).

Immunoprecipitation of detergent extracts from $[^{35}S]$methionine-labeled WI-38 cells using this polyclonal antiserum yielded labeled products of 105, 115, 130, 150, and 190 kDa, respectively (Fig. 1B). Only the 130- and 190-kDa products could be detected by precipitation of extracts from WI-38 cells surface-labeled with $[^{35}S]$methionine (Fig. 1B). Based on their properties, these products were tentatively identified as biosynthetic precursors of the $\beta_1$ integrin subunit (the 105- and 115-kDa products), mature $\beta_1$ subunit exposed in part on the cell surface (the 130-kDa product), and at least two $\alpha$ subunits (150- and 190-kDa products) coprecipitated with $\beta_1$ subunits.

The identity of these products was confirmed in part by pulse-chase metabolic labeling experiments using $[^{35}S]$cysteine to label selectively the cysteine-rich $\beta_1$ subunit (3, 4). The 105–115-kDa precursors were progressively converted into the 130-kDa mature $\beta_1$ subunit (Fig. 2A; see Fig. 6 below).
for longer chase time points). The β1 subunit biosynthetic precursor(s) migrated in sodium dodecyl sulfate-polyacrylamide gels as a broad species that in some experiments resolved well into two bands. This heterogeneity was not eliminated by treatment of immunoprecipitated samples with endoglycosidase H, an enzyme that can remove immature (endoplasmic reticulum-type) N-linked oligosaccharide side chains from glycoproteins (Fig. 2B). However, the heterogeneous appearance of the β1 subunit precursors was eliminated by reduction of the samples before electrophoresis (Fig. 2C). Under reducing conditions, all of the labeled β1 subunit precursor material migrated as a tight 115-kDa band, suggesting that the heterogeneity was due to the presence of different forms corresponding to progressive stages of disulfide bond formation in the cysteine-rich β1 polypeptide chain and progressive acquisition of molecular compactness due to interchain disulfide bonding. The decrease in electrophoretic mobility of mature integrin β subunits upon reduction has been consistently noted in previous reports (1, 2).

To assess further the nature of the products precipitated by the polyclonal antibody, samples of metabolically labeled WI-38 cells were precipitated with monoclonal antibody directed against the mature human β1 subunit. This antibody precipitated the mature β1 subunit but not its biosynthetic precursors (Fig. 1C). More importantly, this antibody also precipitated the 150- and 190-kDa α subunits. These results indicated that WI-38 human fibroblasts contain multiple α integrin subunits that are assembled with β1 subunits as functional integrin complexes and can be coprecipitated with the β1 subunit by anti-β1 subunit antibodies.

**Integrin Subunits Regulated by TGF-β—** Treatment of WI-38 cells with TGF-β1 increased the rate of synthesis of β1 subunit and the level of this subunit on the cell surface. Importantly, TGF-β1 treatment also led to a marked increase in the levels of precipitable α subunits (Fig. 1B). The higher level of α subunits in samples immunoprecipitated from TGF-β1-treated WI-38 fibroblasts could derive from increased coprecipitation due to higher availability of β1 subunit. Alternatively, the increase in α subunits could be due to a direct effect of TGF-β1 on their expression. To distinguish between these two possibilities, extracts from [35S]methionine-labeled cells were precipitated with a panel of anti-integrin monoclonal antibodies, including antibodies against α1, α2, α3, α4, and α6 subunits in addition to monoclonal and polyclonal antibodies against β1 subunit. Unstimulated WI-38 cells expressed 190-kDa α1 subunits, 150-kDa α2 subunits, and 155-kDa α3 subunits as detected by precipitation with the corresponding antibodies. Furthermore, treatment of cells with TGF-β1 markedly increased the level of newly synthesized α1, α2, and α3 subunits (Fig. 3). Expression of α4 subunit could not be detected in either control or TGF-β1-treated WI-38 cells after cell labeling with [35S]methionine (Fig. 3) or [3H]cysteine (not shown). Anti-α5 subunit antibodies precipitated a 165-kDa product whose level was slightly increased by TGF-β1 (Fig. 3). This product is larger than the 140–150-kDa α6 subunits previously identified in other cell types (18). The relationship of this 165-kDa product to α5 subunit remains to be clarified.

The amount of mature β1 subunit coprecipitated with α1, α2, and α5 subunits added together was close to the amount of mature β1 subunit precipitated with its corresponding monoclonal or polyclonal antibodies (Fig. 3) suggesting that the contribution of other α subunits (e.g., α5) to the total pool of α subunits assembled with β1 subunit in WI-38 cells was relatively small. Reciprocally, the amount of α subunits precipitated with their antibodies was similar to the amount precipitated with anti-β1 subunit monoclonal antibodies (Fig. 3). Thus, the use of this panel of antibodies allowed a close quantitation of assembled integrin complexes in WI-38 cells. Assuming a 1:1 stoichiometry of α and β subunits in integrin complexes and given the methionine content of α and β subunits deduced from the corresponding cDNA sequences (4), the results shown in Fig. 3 suggest that unstimulated cells synthesized β1 subunits at a faster rate than α subunits. Antibodies against α5 subunits were not available for these studies, but Northern blot analysis of WI-38 RNA using α5 subunit and β1 subunit cDNA probes indicated that treatment of WI-38 cells with TGF-β1 increased the steady-state level of α5 subunit mRNA in parallel with the increase in β1 subunit mRNA (Fig. 4). The time of this effect was between 4–8 h.

The effect of TGF-β1 on the biosynthesis and cell surface expression of multiple integrin subunits occurred in the absence of a general increase in protein synthesis for at least 24 h (Fig. 5). We note that treatment of WI-38 cells with TGF-β1 did not always increase the levels of all integrin subunits in parallel. Fig. 5 illustrates one case in which the strong

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Unpublished results.
elevation of \(\alpha_1\) subunit expression by TGF-\(\beta 1\) occurred in parallel with a weak response in \(\beta_1\) subunit synthesis and no significant effect in \(\alpha_2/\alpha_5\) (150-kDa \(\alpha\)) subunit synthesis. TGF-\(\beta 1\) increased the synthesis of \(\alpha_1\), \(\alpha_4\), and \(\alpha_5\) integrin subunits in 10 out of 10, 7 out of 9, and 6 out of 8 experiments, respectively. The average-fold increase induced by TGF-\(\beta 1\) was 10-fold for the \(\alpha_1\) subunit, 2.5-fold for the \(\alpha_2\) subunit, and 3-fold for the \(\alpha_5\) subunit, relative to control cells. Although the variables that influence the responsiveness of individual integrin subunits to TGF-\(\beta 1\) in WI-38 fibroblasts have not been defined yet, these results suggested that expression of individual integrin subunits might be regulated by independent mechanisms.

Modulation of Integrin Complex Assembly by TGF-\(\beta 1\): Role of \(\alpha\) Subunit Availability—The biosynthetic maturation and assembly of \(\alpha\) and \(\beta\) integrin subunits and their modulation by TGF-\(\beta 1\) were examined in more detail. Parallel pulse-chase metabolic labeling assays were performed in control and TGF-\(\beta 1\)-treated WI-38 cells, followed by precipitation with anti-\(\alpha_1\) subunit antibody and anti-\(\beta_1\) subunit antibody. The amount of \(\beta_1\) subunit precursor synthesized during a 1-h pulse with [\(^{35}\)S]methionine was slightly higher in TGF-\(\beta 1\)-treated cells compared to control cells (Fig. 6A). More important, the fate of this newly synthesized material differed markedly between control and TGF-\(\beta 1\)-treated cells. In control cells, the \(\beta_1\) subunit precursors matured at a very slow rate (\(t_m\), approximately 10 h) characteristic of \(\beta_1\) subunit maturation in other fibroblasts (21, 42). Furthermore, a large portion of the \(\beta_1\) subunit precursor pool in control cells turned over before it reached maturation (Fig. 6A). The accumulation of mature \(\beta_1\) subunit in TGF-\(\beta 1\)-treated cells was faster (\(t_m\), approximately 3 h) than in control cells, and the precursor was converted into mature subunit with little premature degradation (Fig. 6A). The appearance of mature \(\beta_1\) subunits in both control and TGF-\(\beta 1\)-treated cells correlated with the assembly with \(\alpha_1\) and \(\alpha_2/\alpha_5\) subunits as indicated by the coprecipitation of these subunits with anti-\(\beta_1\) subunit antibodies (Fig. 6A).

Precipitation of \(\alpha_1\) subunit with its corresponding antibody showed that TGF-\(\beta 1\) caused the characteristic strong elevation in the rate of synthesis of this subunit (Fig. 6B). The rate of conversion of the 180-kDa \(\alpha_1\) subunit precursor into the 190-kDa mature subunit was rapid compared to that of \(\beta_1\). subunits and was not markedly altered by TGF-\(\beta 1\) even though this treatment increased \(\alpha_1\) subunit synthesis rate (Fig. 6B). Like the mature \(\alpha_1\) subunit, the precursor could be coprecipitated with \(\beta_1\) subunits as seen at short chase time points (1 h, Fig. 6A). The \(\beta_1\) subunit coprecipitated with \(\alpha_1\) subunit was mostly in the mature form (Fig. 6B), but a small amount of \(\beta_1\) subunit precursor could be detected at the 1-h chase time point after prolonged autoradiography of the gel shown in Fig. 6B (not shown).

The ratio of newly synthesized \(\alpha\) subunits to \(\beta_1\) subunits was markedly higher in TGF-\(\beta 1\)-treated cells than in control cells (Figs. 1, 3, and 6). These results together with the unusual kinetics of \(\beta_1\) subunit maturation suggested the possibility that availability of \(\alpha\) subunits in WI-38 cells might determine the rate of assembly of integrin complexes. To obtain further support for this possibility, we examined the biosynthesis of integrins in A431 human epidermoid carcinoma cells whose basal rate of \(\alpha_1\) subunit relative to \(\beta_1\) subunit (Fig. 7A) was higher than in WI-38 cells. In A431 cells, the precursor and mature forms of the \(\beta_1\) subunit migrated closely on electrophoresis gels, but could be distinguished from each other by the sensitivity of the precursor form but not the mature form to endoglycosidase H (Fig 7A). The rate of \(\beta_1\) subunit maturation and complex formation was considerably faster in A431 cells than in WI-38 cells. Conversely, 3T3-L1 mouse fibroblasts that had a ratio of \(\alpha\) to \(\beta_1\) subunit synthesis even lower than WI-38 fibroblasts showed the slowest rate of \(\beta_1\) subunit maturation (Fig. 7B). Thus, an inverse relationship exists between the availability of newly synthesized \(\alpha\) subunits and the rate of \(\beta_1\) subunit maturation.

Regulation of \(\alpha_\beta_i\) Integrins in Other Cell Lines—Table I summarizes the results of a survey of \(\alpha_\beta_i\) integrin responses to TGF-\(\beta 1\) in eight human cell lines in addition to WI-38 fibroblasts. Each of the six \(\alpha\) subunits that belong to the \(\alpha_\beta_i\) integrin family was expressed in at least one of the cell lines examined. The expression of each of the six \(\alpha\) subunits increased in response to TGF-\(\beta 1\) in most but not all cell lines. Like in WI-38 fibroblasts, TGF-\(\beta 1\) did not induce the expression of integrin subunits that were not expressed in unstim-
ulated cells. In the case of MG-63 osteosarcoma cells, elevated synthesis of \( \alpha_2 \) and \( \beta_1 \) subunits in response to TGF-\( \beta_1 \) correlated in the same experiments with an 80% reduction in the rate of \( \alpha_6 \) subunit synthesis. These results provide further evidence for the ability of TGF-\( \beta_1 \) to regulate the expression of all members of the \( \alpha_\beta_1 \) integrin family via parallel but independent mechanisms.

**Discussion**

Using a panel of polyclonal and monoclonal antibodies, we have shown in the present studies that WI-38 human lung fibroblasts express simultaneously various \( \alpha\beta_1 \) integrins. These integrins include \( \alpha_\beta_1 \), whose exact function is not yet known. \( \alpha_\beta_1 \) subunits that functions as a receptor for type I collagen and \( \alpha_\beta_1 \) subunits that functions as a broad specificity fibronectin receptor that also binds laminin and type I collagen (14, 15). In addition, the presence of \( \alpha_6 \) integrin subunit mRNA suggests that the \( \alpha_6\beta_1 \) fibronectin receptor is also expressed in these cells. Like other adherent cell types (16), WI-38 fibroblasts do not show detectable expression of \( \alpha_4 \). A 165-kDa product recognized by anti-\( \alpha_6 \) subunit antibody is present in WI-38 cells, but its relationship to the 140–150-kDa \( \alpha_6 \) subunit form described in other cell types (18) is not clear yet.

Coexpression of multiple integrins that share the same type of \( \beta_1 \) subunit is not unique to WI-38 fibroblasts but is a general phenomenon. The biosynthesis and assembly of \( \alpha\beta_1 \) integrins is a multifactorial process in which distinct \( \alpha \) subunits may have to compete for a limited supply of \( \beta_1 \) subunit, or \( \beta_1 \) subunit may be made in excess to support the process of assembly with \( \alpha \) subunits. The latter is the situation found in WI-38 and other fibroblasts. Information on the basic features of the biosynthesis of \( \alpha\beta_1 \) integrins is needed before the regulation of \( \alpha\beta_1 \) integrin expression by TGF-\( \beta_1 \) can be properly analyzed and interpreted. The general characteristics of \( \alpha\beta_1 \) integrin biosynthesis and assembly in the WI-38 human cell model system are as follows.

**Biosynthesis of Integrins That Share a Common \( \beta_1 \) Subunit**—The amino acid sequence and electrophoretic mobility of \( \beta_1 \) integrin subunit have indicated that the extracellular domain of this protein is rich in cysteine residues, many of which are in the oxidized state forming intrachain disulfide bonds (3, 4). In WI-38 human lung fibroblasts, the cysteine-rich \( \beta_1 \) subunit is synthesized as a 115-kDa precursor that is slowly converted to a 105-kDa form. The 105-kDa form can be converted to a 115-kDa form by reduction in vitro, suggesting that it is generated from the 115-kDa precursor by progressive formation of disulfide bonds. The process of disulfide bond formation occurs while the \( \beta_1 \) subunit is still in precursor form, i.e. when it is probably located in the endoplasmic reticulum. The sensitivity of the 105-kDa \( \beta_1 \) form to deglycosylation by endoglycosidase H supports this conclusion. The shift to the mature molecular mass of 130 kDa occurs with generation of a \( \alpha_1 \) subunit form whose N-linked glycan chains are no longer sensitive to endoglycosidase H. By analogy with the biosynthetic process of other membrane glycoproteins, these changes most likely mark the passage of the \( \beta_1 \) subunit through the Golgi complex where the N-linked carbohydrate chains undergo final conversion and extension. Thereafter, the \( \beta_1 \) subunit moves to the cell surface where it can be identified by surface labeling.

The biosynthetic steps of \( \beta_1 \) integrin subunit pathway are typical of other cell surface glycoproteins. However, the kinetics of maturation of \( \beta_1 \) subunit in WI-38 cells and in other cell types including 3T3-L1 and NIH3T3 fibroblasts (21, 42) are exceptionally slow. The results suggest that this phenomenon might be due to limited availability of \( \alpha \) subunits and is

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**Table I**

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<th>Cell line</th>
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*Only mRNA level was analyzed; 0, not expressed in this cell line; N.D., not determined.
consistent with a model in which assembly of $\beta_1$ subunits with $\alpha$ subunits is necessary for transit of integrins through the Golgi complex where they mature as glycoproteins. Several lines of evidence support this model. First, the results show that the time of retention of the $\beta_1$ subunit as an immature precursor presumably located in the endoplasmic reticulum is inversely proportional to the rate of $\alpha$ subunit synthesis. Second, the results (Figs. 3, 6, and 7) suggest that in the basal state, WI-38 and 3T3 fibroblasts synthesize $\beta_1$ subunits in excess over $\alpha$ subunits. Third, the various $\alpha$ subunits expressed by WI-38 cells mature rapidly from their biosynthetic precursors (possibly assembling with a preexisting pool of unlabeled $\beta_1$ subunit precursors) while the $\beta_1$ subunit matures slowly. Fourth, the results of coprecipitation experiments with anti-$\beta_1$ antibodies show that the $\alpha$ subunits can assemble with $\beta_1$ subunits when they are still in the precursor form. Labeled $\beta_1$ subunit precursors cannot be readily detected by coprecipitation with anti-$\alpha$ subunit antibodies probably due to dilution of the biosynthetically labeled $\beta_1$ subunit precursors into a larger pool of unlabeled precursors that preexists in the cell. Taken together, these results suggest that integrin complexes are assembled when both subunits are still in precursor form.

The retention of unassembled $\beta_1$ subunit precursor in a locus proximal to the endoplasmic reticulum as suggested by our results is analogous to the retention of unassembled and/or incorrectly folded immunoglobulin heavy chains (43) or influenza virus hemagglutinin subunits (44). Folding and assembly of subunits precedes the release of multisubunit complexes from the endoplasmic reticulum, at least in the case of influenza virus hemagglutinin (45). Immunoglobulin heavy chains produced by pre-B lymphocytes that do not express light chains appear to be recognized by an active retention mechanism in the endoplasmic reticulum (43). A similar mechanism is involved in the retention of influenza virus hemagglutinin subunits that are unable to assemble into trimeric complexes (44). These mechanisms may relate to the one that recognizes fibroblast integrin $\beta_1$ subunit precursors unable to assemble into dimeric integrins due to limited availability of $\alpha$ subunits. However, we have not detected association of $\beta_1$ subunit precursor with the BiP/gp 78 protein, the 78-kDa endoplasmic reticulum resident protein that is associated with unfolded immunoglobulin chains and viral hemagglutinin subunits and perhaps intervenes in subunit folding and the oligomer assembly process (43, 44, 46, 47). $\beta_1$ integrin subunits synthesized in excess in WI-38 fibroblasts are directly degraded when they are still in the precursor form, as shown by the results (Fig. 6). This degradation might occur by a mechanism similar to that recently reported for the elimination of excess T-cell receptor subunits in a nonsylosomal locus proximal to the endoplasmic reticulum (48).

Our model proposing that the availability of $\alpha$ subunits determines the rate of assembly and maturation of $\beta_1$ subunits is in contrast to that suggested by Akiyama and Yamada (42) who reported that the kinetics of maturation of $\beta_1$ integrin subunits are slow in mouse NIH3T3 and chick embryo fibroblasts noted by Akiyama and Yamada (42) could be due to the high sensitivity of the higher level of $\alpha$ subunits available for assembly of integrin complexes. However, it is possible that in addition to this effect, TGF-β1 might control directly the mechanism for intracellular retention of unassembled subunits.

In addition to its effect on the levels of integrin $\alpha$ and $\beta$ subunits, TGF-β1 elevates the expression of fibronectin, type I collagen and chondroitin/dermatan sulfate proteoglycan with similar kinetics in all cases (this report, Refs. 28 and 30, and results not shown). The effect of TGFs-β on these cell adhesion proteins and receptors contrasts with the lack of a detectable effect on the expression of basement membrane proteins including laminin and type IV collagen. The parallel effect of TGFs-β on multiple members of the integrin family and other matrix components suggests the presence of com-

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1. J. Heino and J. Massagué, unpublished results obtained with the anti-BiP monoclonal antibody described in Ref. 46 and generously supplied by J. Kearney.
mon regulatory elements that control the expression of these proteins. TGFs-β increase the mRNA levels of multiple cell adhesion proteins and the available evidence suggests the involvement of transcriptional as well as posttranscriptional events in this response (30, 32, 49–52). A nuclear factor 1 (NF1) binding site present in the mouse α1 (I) collagen gene promoter region has been implicated in the transcriptional activation of this gene by TGF-β (50). Putative NF1 binding sites may also be present in other genes regulated by TGF-β, but in one such case, the human fibronectin gene, removal of a NF1 binding site does not block transcriptional activation by TGF-β (52). Progress in defining transcription control sequences in TGF-β-regulated genes as well as posttranscriptional control sites may lead to the identification of the molecular basis for parallel regulation of these genes by TGF-β. However, the results of the present study indicate that cell-specific and other as yet undefined variables determine the degree of responsiveness of individual integrin subunits to TGF-β. In at least one case (MG-63 osteosarcoma cells), the expression of one α integrin subunit decreases in response to TGF-β while the expression of other α and β integrin subunits increases. Changes of opposite sign also take place in the levels of α1 and α2 integrin subunits in human fibroblasts when they become quiescent due to serum deprivation (53). The evidence accumulated thus far suggests that regulation of individual integrin subunits and extracellular matrix proteins by TGF-β and other factors is accomplished via overlapping but independent mechanisms.

The present results provide an explanation for the previously observed enhancement of the ability of TGF-β-treated cells to incorporate type I collagen into their extracellular matrix (21, 28). Two integrins, αβ1 and αβ6, that are up-regulated in response to TGF-β1 mediate cell attachment to type I collagen (14, 15). The involvement of the αβ1 fibronectin receptor in the enhancement of cell adhesion to fibronectin in response to TGF-β1 and TGF-β2 has also been described (21). The functional implications of the marked increase in αβ1, αβ6, and αβ1 integrin expression in response to TGF-β shall become clear once the ligands for these integrins are identified.

The coexistence of multiple α subunits that can assemble with a common β subunit to form a repertoire of integrin complexes with distinct adhesive specificities, and the fashion in which these complexes are assembled have important implications in the regulation of cell adhesion in vivo. Alterations in the expression of individual integrin subunits by regulatory factors like TGF-β are likely to change the balance of functional integrin complexes expressed on the surface of the cell, modifying as a result the adhesive interactions that determine cell homing and positioning in tissues as well as the expression of cell phenotype during development and morphogenesis.

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TGF-β Regulates β₃ Integrins