Abstract. The ability of single subunit chimeric receptors containing various integrin \( \beta \) intracellular domains to mimic and/or inhibit endogenous integrin function was examined. Chimeric receptors consisting of the extracellular and transmembrane domains of the small subunit of the human interleukin-2 receptor connected to either the \( \beta_1 \), \( \beta_3 \), \( \beta_3B \), or \( \beta_1 \) intracellular domain were transiently expressed in normal human fibroblasts. When expressed at relatively low levels, the \( \beta_3 \) and \( \beta_5 \) chimeras mimicked endogenous ligand-occupied integrins and, like the \( \beta_1 \) chimera (LaFlamme, S. E., S. K. Akiyama, and K. M. Yamada. 1992. J. Cell Biol. 117:437), concentrated with endogenous integrins in focal adhesions and sites of fibronectin fibril formation. In contrast, the chimeric receptor containing the \( \beta_{3B} \) intracellular domain (a \( \beta_1 \) intracellular domain modified by alternative splicing) was expressed diffusely on the cell surface, indicating that alternative splicing can regulate integrin receptor distribution by an intracellular mechanism. Furthermore, when expressed at higher levels, the \( \beta_1 \) and \( \beta_3 \) chimeric receptors functioned as dominant negative mutants and inhibited endogenous integrin function in localization to fibronectin fibrils, fibronectin matrix assembly, cell spreading, and cell migration. The \( \beta_3 \) chimera was a less effective inhibitor, and the \( \beta_{3B} \) chimera and the reporter lacking an intracellular domain did not inhibit endogenous integrin function. Comparison of the relative levels of expression of the transfected \( \beta_1 \) chimera and the endogenous \( \beta_1 \) subunit indicated that in 10 to 15 h assays, the \( \beta_1 \) chimera can inhibit cell spreading when expressed at levels approximately equal to the endogenous \( \beta_1 \) subunit. Levels of chimeric receptor expression that inhibited cell spreading also inhibited cell migration, whereas lower levels were able to inhibit \( \alpha_5 \beta_1 \) localization to fibrils and matrix assembly.

Our results indicate that single subunit chimeric integrins can mimic and/or inhibit endogenous integrin receptor function, presumably by interacting with cytoplasmic components critical for endogenous integrin function. Our results also demonstrate that \( \beta \) intracellular domains, expressed in this context, display specificity in their abilities to mimic and inhibit endogenous integrin function. Furthermore, the approach that we have used permits the analysis of intracellular domain function in the processes of cell spreading, migration and extracellular matrix assembly independent of effects due to the rest of integrin dimers. This approach should prove valuable in the further analysis of integrin intracellular domain function in these and other integrin-mediated processes requiring the interaction of integrins with cytoplasmic components.

Integrins comprise the major class of receptors used by cells to interact with the extracellular matrix. Although some integrins are cell-type specific, most integrins are expressed in a variety of cell types, providing cells with the ability to interact with many different extracellular matrix proteins in a variety of cellular processes. Depending upon the type of cell, integrins can function in cell adhesion, cell spreading, and cytoskeletal organization, cell migration, matrix assembly, and signal transduction, thereby playing important roles in embryonic development, wound healing, tumor metastasis, tissue organization, and differentiation (2, 3, 14, 16, 24, 30, 32, 33, 36, 54).

Integrins are \( \alpha/\beta \) heterodimeric transmembrane proteins. The \( \alpha \) and \( \beta \) subunits generally contain short cytoplasmic domains that are believed to interact with the cytoskeleton and other cytoplasmic components to mediate and regulate integrin function in response to the extracellular matrix (56). Molecular genetic approaches are currently being used to define specific functions for individual intracellular do-
mains. Recombinant subunits containing wild-type and mutant intracellular domains have been expressed in other species or in cell lines lacking the expression of the corresponding endogenous subunit in order to study the function of particular intracellular domains in the context of heterodimeric receptors. Roles have been defined in this manner for α intracellular domains in modulating integrin function in cell adhesion, migration, collagen gel contraction (7, 12, 37, 38), and in the regulation of affinity state of the platelet integrin, αIIbβ3 (48, 49). Requirements for β intracellular domain function have similarly been demonstrated for cell adhesion (29, 31), cell spreading (69), integrin localization to focal adhesions (29, 42, 62, 69), and the adhesion-dependent phosphorylation of the focal adhesion kinase pp125FAK (27, 28, 57).

An alternative approach to studying integrin intracellular function is to analyze the function of integrin intracellular domains expressed as separate domains connected to an extracellular reporter and transmembrane domain. Using this approach, we and others have demonstrated that the β intracellular domain can function as a separate domain and can direct the localization of a reporter to focal adhesions (23, 39), presumably by an intracellular mechanism involving an intrinsic ability of the β intracellular domain to bind to cytoskeletal proteins that bind to endogenous integrins concentrated at these adhesion sites. The intrinsic ability of the β intracellular domain to concentrate at focal adhesions appears to be regulated in endogenous integrins by ligand occupancy in a process involving the α intracellular domain (8, 39, 69).

These observations suggest the hypothesis that β intracellular domains, expressed in the absence of their corresponding α intracellular domains, bind to similar cytoplasmic components as ligand-occupied integrins. If this hypothesis were correct, then when expressed at relatively low levels, single subunit chimeric receptors containing β intracellular domains should mimic endogenous integrin function, and at higher levels of expression they should inhibit endogenous integrin function by titrating out required cytoplasmic component(s). In addition, because various β integrin intracellular domains differ in their primary structure and these differences among integrin β subunits are evolutionarily conserved, individual β intracellular domains may differ in the functions that they can affect. We have tested this hypothesis by expressing β intracellular domains connected to a reporter domain and analyzing their abilities to mimic or inhibit various aspects of integrin receptor function. Using this approach, we have compared the ability of β intracellular domains to direct receptor localization to focal adhesions and have defined a role for alternative splicing in regulating this process. Furthermore, we demonstrate that when expressed at relatively high levels, β chimeras can function as dominant negative mutants. Specifically, they can inhibit endogenous integrin function in cell spreading, cell migration, and extracellular matrix assembly, and they show functional specificity dependent upon the cytoplasmic domain present.

Materials and Methods

Construction of Chimeric Receptors

Chimeric receptors were constructed using standard molecular biological techniques (55). DNAs encoding the various β intracellular domains were generated by the polymerase chain reaction, using PCR kits from Perkin Elmer Corp. (Norwalk, CT), and were then inserted as HindIII-XhoI restriction fragments into the previously described plasmid vector encoding the control receptor, consisting of the extracellular and transmembrane domains of the interleukin-2 (IL-2) receptor, in place of its single intracellular lysine residue (39). The integrity of each construct was confirmed by nucleotide sequence analysis. The β5 and β6 intracellular domains were amplified from an human embryonic lung fibroblast CDNA library. The β6 intracellular domain was also generated by the polymerase chain reaction using as template the β6 chimera and a COOH-terminal primer encoding the amino acids modified by alternative splicing. Oligonucleotide primers also encoded either an NH2-terminal HindIII restriction site or a COOH-terminal XhoI restriction site, so that resulting DNAs could easily be inserted as HindIII-XhoI restriction fragments. The oligonucleotide primers for the β6 intracellular domain were as follows: NH2-terminal primer: 5'-GCCCTGCTCATCTGGAAGCTTCTCATACACC-3' COOH-terminal primer: 5'-AGCGACCCTGCAGTTAAGTGCCCACCACGTGATATTGT-3'.

The oligonucleotide primers for the β5 intracellular domain were as follows: NH2-terminal primer: 5'-CTGGAAGCTTCTTTGGTCGATACCCATAGCAGCTT-3' COOH-terminal primer: 5'-GACCTGCGTCAATGTCACCATCCACGAC-3'.

The oligonucleotide primers for β5 intracellular domain were as follows: NH2-terminal primer: 5'-GCTTACCTGGAGAAGCTTCTCATACACC-3' COOH-terminal primer: 5'-AGCGACCCTGCAGTTAAGTGCCCACCACGTGATATTGT-3'.

Cells and Transfections

Normal human foreskin fibroblasts, a generous gift from Dr. Steven Alexander (Bethesda Research Laboratories, Gaithersburg, MD), were cultured in DMEM supplemented with 1 mM glutamine, 50 μg/ml streptomycin, 50 U/ml penicillin, and 10% heat-inactivated FCS. Electrotransfection of these cells was performed as previously described (25, 26) at 170 V and 960 mF with a Bio-Rad Gene Pulser (Bio-Rad Laboratories, Richmond, CA), except 15 to 30 μg of DNA was used per transfection. Lower concentrations of DNA resulted in fewer transfected cells and a lower level of expression compared with higher concentrations of DNA.

Antibodies and Purified Proteins

Laminin was generously provided by Dr. Hynda Kleinman (National Institutes of Health, Bethesda, MD). Collagen I (Vitrogen 100) was purchased from Collagen Corp. (Palo Alto, CA). Human plasma fibronectin was isolated as previously described (44). Vitronectin was generously provided by Drs. Steven Aklyama (National Institutes of Health) and David Cheresh (Scripps Research Institute, La Jolla, CA). The polyclonal antibody R3134 to the small subunit of the IL-2 receptor (59) was a generous gift of Dr. Warren Leonard (National Institutes of Health) and the mouse mAb 4E3, also directed against the small subunit of the IL-2 receptor, was purchased from Boehringer Mannheim Biochemicals (Indianapolis, IN). Mouse mAb 7G7B6 (American Type Culture Collection, Rockville, MD) was purified from ascites by sequential ammonium sulfate precipitation, passage through a DE 52 column (Whatman, Hillsboro, OR) in 30 mM NaCl, 10 mM sodium phosphate, pH 7.4, and then affinity chromatography on protein A-Sepharose (Pharmacia Fine Chemicals, Piscataway, NJ). mAb 13 to the β subunit and goat anti-human fibroblast receptor antibodies directly labeled with FITC were purchased as described (1); mAb 11 to the α5 subunit was generously provided by Dr. Steven Aklyama (National Institutes of Health); mouse mAb to the β1 subunit was purchased from Amac, Inc. (Westbrook, ME). FITC- and rhodamine-labeled second antibodies were purchased from Rockland, Inc. (Gilbertsville, PA) and Tago, Inc. ( Burlingame, CA).

1. Abbreviations used in this paper: AU, arbitrary units; IL-2, interleukin 2.
Immunofluorescence and Interference Reflection Microscopy

Cells were cultured on glass coverslips as indicated, fixed for 30 to 60 min with 4% formaldehyde, 5% sucrose in Dulbecco's PBS, and then washed several times in PBS, permeabilized for 5 min in 0.2% Triton X-100 in PBS, washed several times with PBS, incubated for 30 min in 3% BSA, 0.1% glycine (pH 7.5) to block nonspecific binding, washed once in PBS, incubated for 1 h at ambient temperature with primary antibody in PBS with 1 mg/ml BSA, washed for 30 min in PBS with gentle agitation with several changes of PBS, incubated with the appropriate FITC- or rhodamine-conjugated second antibody, and then washed as above. The coverslips were mounted on microscope slides with 10% glycerol in PBS containing 1 mg/ml 1,4-diaminophenylendiamine (Fluka Chemical Corp., Ronkonkoma, NY) to inhibit photobleaching and then viewed and photographed with a confocal microscope equipped with epifluorescence and interference reflection microscopy. Immunofluorescence was photographed using Kodak TMAX 3200 film. Adhesions on the ventral cell surface were analyzed by interference reflection microscopy (34) by standard methods using an Antiflex Neofluor 63.5/NA 1.25 objective and photographed with Kodak technical pan film processed with Diafine developer (Fuller and dAlbert, Inc., Fairfield, VA).

Flow Cytometry and Fluorescence Activated Cell Sorting (FACS)

To compare the levels of expression of the various chimeras, transfected cells were harvested, washed with PBS, and incubated in a 1/100 dilution of FITC- or rhodamine-conjugated anti-IL-2 receptor antibody 4E3 (Boehringer Mannheim, Sommerville, NJ) at room temperature, washed with PBS, fixed with 2% paraformaldehyde in PBS, and then analyzed using a Becton Dickinson FACScan flow cytometer.

To compare the levels of expression of the transfected β1 chimeras with the endogenous β1 subunit, cells transiently expressing the β1 chimera were harvested, washed with PBS, divided into two aliquots, and then immunostained with either the mouse mAb 4E3 to the IL-2 receptor or the mouse mAb K20 to the β1 subunit (Amac, Inc.). After several washes in PBS, each aliquot was stained with the same FITC-labeled anti-mouse second antibody, washed again with PBS, and then fixed and analyzed by flow cytometry as described above. All antibodies were determined to be at saturating concentrations.

To purify cells expressing the chimeric receptors at specific levels, transfected cells were labeled with FITC- or rhodamine-conjugated mAb 4E3 at a 1/100 dilution in PBS containing 1% BSA. The labeled cells were sorted into low, moderate, or high expressors by FACS under sterile conditions using a Becton Dickinson FACStar Plus fluorescence-activated cell sorter and then assayed as described.

Inhibition of Cell Spreading

To compare the ability of the chimeric receptors to inhibit cell spreading on specific matrix proteins, cells were replated 15 h after transfection onto coverslips that had been previously coated with 20 μg/ml fibronectin, vitronectin, or collagen I and blocked with 1% heat-denatured BSA. After incubation for 1 h at 37°C, the cells were fixed and then stained with mAb 7G7B6 to the IL-2 receptor. Ten groups of 10 randomly selected transfected and nontransfected cells were analyzed by phase contrast microscopy. The percent inhibition for each chimeric receptor on each matrix protein was calculated by subtracting the percentage of nontransfected cells that were spread from the percentage of transfected cells that were spread at this time. To ensure that the differences in inhibition were not due to differences in expression levels, cell surface expression was monitored by flow cytometry. The ability of different chimeric receptors to inhibit cell spreading was also analyzed as a function of their expression level. This was accomplished by two protocols: (a) Cells were transiently transfected with the different chimeric receptors, and 15 h after transfection the cells were plated on collagen I-coated coverslips in 6-well tissue culture dishes for 1.5 h. Although the vast majority of cells from the β3a, β5, and control receptor transfections were attached and spread at this time, many of the cells from the β1 and β2 transfections had not attached. To recover these unattached cells for photometric analysis, the collagen I-coated coverslips were replaced with coverslips coated with 20 μg/ml concanavalin A followed by incubation for an additional hour at 37°C. The cells on both collagen I and concanavalin A-coated coverslips were then fixed with 2% paraformaldehyde and stained with mAb 4E3 to the IL-2 receptor. The level of expression of spread and unspread cells was determined by photometry using a photometer system (Yona Microscope & Instrument Co., Columbia, MD) based on an Oriel photomultiplier (Stratford, CT). Photometer readings from 0 to 1,400 (arbitrary units, AU) above background were obtained for each transfection, although very few cells had readings above 600 AU. Three expression levels were defined: low, from 0-150 AU; moderate, 151-300 AU; and high, 301-600 AU. For each chimeric receptor, five groups of 10 randomly selected cells within each expression level were scored for cell spreading. The percent inhibition was calculated directly with the assumption that untransfected cells were 100% spread. (b) Transiently transfected cells expressing low, moderate, or high levels of specific chimeric receptors were purified by FACS. In one experiment, cells expressing low or high levels of the β1 chimera or the control receptor were purified and then incubated on unblocked plastic tissue culture dishes in serum-containing medium. After 15 h, the ability of these cells to spread was assessed by phase contrast microscopy. In a different experiment, cells expressing low or moderate levels of either the mouse mAb 4E3 to the IL-2 receptor or rat mAb 11 to the a5 subunit with a rhodamine-labeled goat anti-rat second antibody were dual stained and then incubated in serum-containing medium. After 15 h, these cells were replated in 96-well tissue culture dishes in which the wells had been previously coated with either 20 μg/ml fibronectin or 20 μg/ml collagen I and blocked with 1% heat-denatured BSA. Cell spreading was again assessed by phase contrast microscopy. Expression levels of chimeric receptors on the sorted cells were also analyzed by photometry.

Cell Migration

Normal human fibroblasts were transfected with either the β1 chimera, the control receptor or mock transfected. 15 h after transfection, cells expressing high levels of the transfected receptors were isolated by fluorescence activated cell sorting and then replated in serum-containing medium at low density (~2 x 10⁴ cells) in 35 mm plastic tissue culture dishes (Costar Corp., Cambridge, MA), previously coated with 10 μg/ml of human plasma fibronectin. The cells were maintained in growth medium in recording chambers at 37°C supplemented with CO₂. Cell migration was recorded at one frame/15 min using either the ICM 405 or Opton inverted microscope (Carl Zeiss, Oberkochen, Germany) equipped with Newvicon Model C2400 video cameras (Hamamatsu Photonics, Hamamatsu City, Japan) as previously described (65). For each experiment, the cells to be compared were recorded simultaneously. Images were stored on optical discs (Panasonic models TQ 2028F or TQ5031F; Matsushita Electronic Corp., Secaucus, NJ) for later analysis.

The migration rates (μm/h) of all cells that remained in the field of view for 10 h were digitized using the Track Points feature of Image 1 software (Universal Imaging Corp., West Chester, PA). A stage micrometer was used with the same microscopes, magnification, recording equipment, and monitor to standardize the scanning software to the scale of the images. Migration rates were confirmed independently by tracing the movement of individual cells on acetate sheets in direct contact with the video monitor. The tracings were then digitized with Sigma Scan software (Jandel Scientific, Corte Madera, CA).

Inhibition of α5β1 Localization to Fibronectin Fibers and Matrix Assembly

Normal human fibroblasts were transiently transfected with the various chimeric receptors. After 24 h, the cells were replated on 22 x 22 mm coverslips in six-well tissue culture dishes at ~1 x 10⁶ cells per well and then incubated an additional 15 h at 37°C. The cells were then fixed and the distribution of α5 and/or β1 fibronectin was analyzed on the cell surface of individual nonpermeabilized transfected and nontransfected cells by double-label immunofluorescence using either: (a) FITC-labeled mouse mAb 4E3 to the IL-2 receptor and rat mAb 11 to the α5 subunit with a rhodamine-labeled goat anti-rat second antibody preabsorbed with mouse IgG; or (b) mouse mAb 7G7B6 to the IL-2 receptor with a rhodamine-labeled goat anti-mouse second antibody and FITC-labeled goat polyclonal antibodies to fibronectin (1). The localization of endogenous α5β1 was scored on individual cells as inhibited if it was expressed diffusely on the surface of the cell. Similarly, matrix assembly was scored on individual cells as inhibited if fibronectin was not observed in fibers or stitches on the cell surface. For each transfection, 10 groups of 10 randomly selected transfected and nontransfected cells were scored as described above. To calculate the percent inhibition, the percentage of nonpermeabilized transfected cells with the inhibited phenotype was subtracted from the percentage of transfected cells with the inhibited phenotype. These experiments were repeated several times, each
Figure 1. Chimeric integrin receptors. Chimeric receptors contained cDNA sequences of the gp55 subunit of the IL-2 receptor from the NH2 terminus through Trp 259 at the end of the transmembrane domain (41) connected to one of the following: (a) the B intracellular domain (5), (b) the B intracellular domain (21), (c) the alternatively spliced B intracellular domain, B (66), or (d) the B intracellular domain (43, 51). The amino acids of the B intracellular domain modified by alternative splicing are indicated in bold.

Table I: Localization of Chimeric Receptors

| Chimeric receptor | FN | VN | LM | COLI | Serum* |
|------------------|----|----|----|------|--------|
| B                | +  | +  | +  | +    | +      |
| (39/50)          |    |    |    |      |        |
| B                | +  | +  | +  | +    | +      |
| (33/50)          |    |    |    |      |        |
| B                 | –  | –  | –  | –    | –      |
| (0/50)           |    |    |    |      |        |
| B                 | +  | +  | +  | +    | +      |
| (25/50)          |    |    |    |      |        |

Transiently transfected cells were plated for 1–1.5 h on coverglasses coated overnight at 4°C with the indicated matrix protein and then blocked for 1 h at ambient temperature with 1% heat-denatured BSA. FN, fibronectin; VN, vitronectin; LM, laminin; COLI, type I collagen. * Transiently transfected cells were also plated on a tissue culture substrate for 15 h in the presence of serum. + or – indicates the ability or inability of the chimeric receptor to localize at focal adhesions. The number of positively expressing cells with focal adhesions that have the chimeric receptor in focal adhesions is given in parentheses for cells spread on FN, COLI, or plated on an unblocked coverslip in the presence of serum.

Results

Chimeric Receptors as Mimics of Endogenous Integrins: Localization to Focal Adhesions

Chimeric receptors (Fig. 1) were constructed containing the small subunit of the IL-2 receptor as an extracellular reporter and transmembrane domain connected to either the B, B, or a B intracellular domain modified by alternative splicing (66), designated B. A similar chimeric receptor containing the B intracellular domain was previously described (39). To analyze and compare the ability of integrin β intracellular domains to direct receptor localization, normal human fibroblasts were transiently transfected with the various chimeric receptors. Transfected cells were either plated on unblocked coverslips in serum-containing medium for 15 h, or on the specific matrix proteins fibronectin, collagen I, laminin, and vitronectin for 1–1.5 h. Since fibroblasts do not normally express the IL-2 receptor, the distribution of the chimeric receptors on the cell surface was analyzed by immunofluorescence using mAb 4E3 to the IL-2 receptor portion of the chimeric receptors. The presence or absence of chimeric receptors in focal adhesions was confirmed by interference reflection microscopy, where focal adhesions appear black (34).

The B and B chimeric receptors concentrated at focal adhesions in cells cultured under all these conditions (Table I; Fig. 2, A and C), indicating that the B and B intracellular domains, like the B intracellular domain, also contain sufficient information to target a reporter domain to focal adhesions formed by endogenous receptors. However, the B intracellular domain appeared to have a lesser propensity to do so compared to the B and B intracellular domains (Table I). In contrast, the B chimera was expressed...
diffusely on the cell surface and did not concentrate in focal adhesions under any condition, indicating that the distribution of the $\beta_{35}$ chimera was also not affected by the identity of the substrate (Table I; Fig. 2 B). These results suggest that similar cytoplasmic interactions are involved in the localization of the $\beta_1$, $\beta_3$, and $\beta_5$ chimeras and their corresponding integrin receptors to focal adhesions. These results further suggest that alternative splicing may provide a means of regulating receptor distribution, perhaps by altering the ability of receptors to interact with the cytoskeleton. As documented previously (39), the control receptor lacking an intracellular domain was always diffuse in distribution, as was the original full-length IL-2 receptor subunit (data not shown).

Localization to Fibronectin Fibrils

When fibroblasts are plated for several hours in serum-containing medium, their $\alpha_\beta_1$ fibronectin receptors become concentrated at sites where fibronectin fibrils associate with the plasma membrane (e.g., see reference 61). The cytoskeletal proteins $\alpha$-actinin, talin, and vinculin that are present in focal adhesions (reviewed in reference 11) can also be found colocalized with fibronectin fibrils; however, the relative amounts of these proteins and their apposition to the plasma membrane may vary (9, 10, 13, 60). These cytoskeletal similarities suggest that perhaps the interactions between integrins and the cytoskeleton are similar at focal adhesions and sites where fibronectin fibrils associate with the plasma membrane. If this notion were correct, the $\beta_5$ and $\beta_3$ intracellular domains, like the $\beta_1$ intracellular domain, should also target a reporter domain to sites of fibronectin fibril association with the plasma membrane. To explore this possibility, normal human fibroblasts were again transiently transfected with the various chimeric receptors, plated in serum-containing medium for 15 h, and then double immunostained with polyclonal antibodies to the IL-2 receptor portion of the chimeras (A and B) and mAb 11 to the $\alpha_5$ subunit of the $\alpha_\beta_5$ fibronectin receptor (C and D). Arrows indicate examples where the chimeric receptors colocalize with the endogenous $\alpha_5$ subunit. Bar, 10 $\mu$m.

Figure 3. Colocalization of chimeric receptors with $\alpha_\beta_1$ at fibronectin fibrils. Normal human fibroblasts transfected with the $\beta_3$ chimera (A and C) or the $\beta_5$ chimera (B and D) were plated in serum-containing medium for 15 h and then double-immunostained with polyclonal antibodies to the IL-2 receptor portion of the chimeras (A and B) and mAb 11 to the $\alpha_5$ subunit of the $\alpha_\beta_5$ fibronectin receptor (C and D). Arrows indicate examples where the chimeric receptors colocalize with the endogenous $\alpha_5$ subunit. Bar, 10 $\mu$m.
Chimeric Receptors as Dominant Negative Mutants

We next tested the possibility that high levels of expression of these chimeric receptors might function as dominant negative mutants and inhibit endogenous integrin receptor function by competing with endogenous receptors for the binding of the cytoplasmic proteins required for their function. We tested this hypothesis by analyzing the ability of various chimeric receptors to inhibit endogenous integrin receptor function in receptor localization to fibronectin fibrils, matrix assembly, cell spreading, and cell migration.

To determine the relative levels of β chimera available to serve as a potential dominant negative inhibitor, we first compared the level of expression of the transfected and endogenous receptors by flow cytometry as described in Materials and Methods. Transfected cells expressed the β, chimera at levels from 1/10 that of the endogenous β, subunit to levels 10 times higher than the endogenous subunit (Fig. 4). Very similar results were also obtained using combinations of either mouse or rat mAbs to the IL-2 receptor and to the β, subunit, and both types of analysis showed that over-expression of the β, chimera does not alter cell surface expression of endogenous β, integrins (not shown).

Inhibition of Integrin Receptor Function in Cell Spreading

We first tested the ability of the β, chimera, when expressed at high levels, to function as a dominant negative mutant and inhibit endogenous integrin function in cell spreading. Normal fibroblasts were transfected with either the β, chimera or the control receptor lacking an intracellular domain. After 24 h, positively expressing cells were sorted by FACS into two populations: low expressors and high expressors (Fig. 5, A-F). These different populations of sorted cells were plated separately for 15 h on unblocked tissue culture substrates in serum-containing medium. After this period of time, there was no evidence of cell surface expression of the FITC-labeled antibody originally used in the sorting protocol. Cells expressing either high or low levels of the control receptor spread and formed focal adhesions soon after plating (Fig. 5, G and H). Although cells expressing low levels of the β, chimera attached, spread (Fig. 5 I),
and formed focal contacts (not shown), the majority of the high expressors remained round (Fig. 5 J). This marked inhibition of cell spreading in high expressors was reversible. After further incubation for an additional 24 h (during which time the level of expression of the transiently transfected receptor was decreased approximately tenfold according to flow cytometry), the cells expressing high levels of the β1 chimera that were previously inhibited in cell spreading were now able to spread (Fig. 5 K). Similar results were also obtained when cells were transfected with the β3 chimera (not shown). High expressors were apparently not immediately lost after transfection because the transfected cells attached and formed focal adhesions prior to the cell surface expression of the transfected chimeric receptor. It is likely that the assembly of integrin transmembrane linkages is more easily inhibited than the maintenance of linkages already formed.

We then compared the ability of the β1, β3, β5, and β3B chimeras to inhibit cell spreading in short term assays on the specific matrix proteins, fibronectin, collagen I, and vitronectin. In this experiment, cells transiently expressing the various chimeric receptors were not purified by FACS, but were directly incubated on these substrates in serum-free medium for 1-1.5 h. The cells were then fixed and immunostained with antibodies to the IL-2 receptor, and the percentage of cells expressing each chimeric receptor that were inhibited in spreading at this time was determined as described.

![Figure 6](image_url)

Figure 6. Inhibition of cell spreading on various substrate ligands. Normal human fibroblasts transiently transfected with the β1, β3, β3B, or the β5 chimeric receptor or the control receptor, C, lacking an intracellular domain were plated in serum-free medium for 1 h on 20 μg/ml fibronectin (A), vitronectin (B), or collagen I (C). Cells were fixed and then immunostained with mAb 7G7B6 to the IL-2 receptor. The percent inhibition was calculated as described in Materials and Methods.

![Figure 7](image_url)

Figure 7. Inhibition of cell spreading as a function of expression level. (A) Comparison of the ability of various chimeric receptors to inhibit cell spreading on collagen I as a function of their expression level. Cells transfected with either the β1, β3, β3B, or β5 chimera or the control receptor, C, lacking an intracellular domain were plated on collagen I for 1.5 h. The level of expression of spread and unspread cells was determined by photometry and is given in arbitrary units (AU). The ability of the various chimeric receptors to inhibit cell spreading when expressed at low levels (0-150 AU), moderate levels (151-300 AU), or high levels (301-600 AU) was determined as described in Materials and Methods. (B) Comparison of the ability of transfected cells purified by FACS expressing either moderate (med) or low levels of the β3 or β3B chimera to inhibit cell spreading on collagen I and fibronectin. When analyzed by photometry, cells purified by FACS with low levels of expression had a mean fluorescence of 122 AU and cells with moderate levels of expression had a mean fluorescence of 280 AU.
Figure 8. Inhibition of cell migration. Cells expressing high levels of either the control receptor (A) or the β1 chimera (B) were purified by fluorescence-activated cell sorting and plated on a fibronectin substrate. The migration of individual transfected cells was followed by video microscopy for a 10-h period. Tracings of individual cells and their paths of migration are shown.

In Materials and Methods. As quantitated in Fig. 6, we found that expression of either the β1 or the β1 chimera receptor inhibited cell spreading on all these matrix proteins, whereas the β3 chimera was a poor inhibitor, and the β3b chimera and the control receptor lacking an intracellular domain did not function as inhibitors of cell spreading. The intrinsic ability of cells to spread, however, was not inhibited, since cells transfected by the β1 chimera were still able to spread if the substrate was concanavalin A (data not shown).

We next examined the relationship between the level of expression of the different chimeric receptors and their ability to inhibit endogenous function in short-term cell spreading assays on a defined substrate. Again, cells transiently expressing the various chimeric receptors were not purified by FACS but were directly incubated on collagen I for 1.5 h as described above. The cells were then immunostained using the mAb 4E3 to the IL-2 receptor and the level of expression of each chimeric receptor was analyzed in spread and non-spread cells by photometry as described in Materials and Methods. The ability of each chimeric receptor to inhibit cell spreading as a function of its level of expression is quantitated in Fig. 7A. These results confirm our previous results and furthermore demonstrate that the β3 chimera can also inhibit endogenous integrin function but requires higher levels of expression as compared with the β1 and β3 chimeras.

Differences in the abilities of low and moderate levels of expression of the β1 and β3b chimera to inhibit cell spreading was also demonstrated in transfected cells purified by FACS. These results are quantitated in Fig. 7B and again show that the β3 chimera does not act as an inhibitor of endogenous integrin function in cell spreading. In contrast, at low levels of expression, the β3 chimera is a poor inhibitor and at higher levels of expression effectively inhibits endogenous integrin function in cell spreading both on fibronectin and collagen I substrates.

Inhibition of Cell Migration

The ability of high levels of expression of the β1 chimera to inhibit cell migration was also examined. Cells expressing high levels of either the β1 chimera or the control receptor purified by FACS as in Fig. 5 were also analyzed by time-lapse video microscopy. Cells expressing high levels of the control receptor were observed to spread and migrate (Fig. 8A), whereas cells expressing high levels of the β1 chimera remained round and did not migrate (Fig. 8B). Rates of cell migration were digitized, and they confirmed that expression of the chimera containing the β1 cytoplasmic domain caused a large inhibition of migration: cells expressing high levels of the control receptor lacking any cytoplasmic domain had a migration rate of 3.2 ± 2.8 μm/h, whereas transfectants expressing high levels of the β1 domain migrated at only 0.068 ± 0.09 μm/h, (n = 29 and 28, respectively; P < .0001); the average migration rate was decreased by 50-fold in the presence of the β1 cytoplasmic domain. Therefore, high levels of expression of the β1 chimera not only inhibited cell spreading but also cell migration.

Inhibition of αβ1 Fibronectin Receptor Localization to Fibrils and Function in Matrix Assembly

Since the αβ1 fibronectin receptor has been shown to be involved in matrix assembly in culture (1, 22, 45, 53, 68, 70), and at relatively low levels of expression, the β1, β3, and β3 chimeric receptors colocalized with endogenous αβ3 at fibrils (Fig. 3), we also tested the possibility that at higher levels of expression, these chimeric receptors might inhibit
Inhibition of matrix assembly in transfected cells purified by FACS. Normal human fibroblasts were transiently transfected with various chimeric receptors and then were sorted into low or moderate expressors by FACS. Cells expressing either low levels of the \( \beta_3 \) chimera (A) or moderate levels of either the \( \beta_3 \) chimera (B), the \( \beta_{3B} \) chimera (C), or the \( \beta_5 \) chimera (D) were plated on coverslips in serum-containing medium for 15 h and then immunostained with FITC-conjugated polyclonal antibodies to human fibronectin. When the sorted cells expressing low and moderate levels of the transfected receptors were analyzed by photometry, the low expressors had a mean fluorescence of 122 AU and the moderate expressors had a mean fluorescence of 280 AU. Bar, 10 \( \mu \)m.

Discussion

Integrin intracellular domains are an important link in the pathway by which cells respond to their extracellular matrix. Understanding how the extracellular matrix can affect cell behavior requires the definition and comparison of integrin intracellular domain function, as well as the determination of how this function is regulated. To this end, we have expressed various \( \beta \) intracellular domains as single-subunit chimeric receptors and tested the ability of these chimeras to mimic or inhibit specific aspects of integrin receptor function. Using this approach, we have compared the ability of different \( \beta \) intracellular domains to direct receptor localization and have defined a role for alternative splicing in regulat-
ing this process. The ability of specific β chimeras to mimic the localization of ligand-occupied integrins suggested that their β intracellular domains can interact with cytoplasmic complexes in a ligand-independent or a constitutively "active" manner. If the hypothesis were correct, this ability should also allow these chimeric receptors to function as dominant negative mutants and inhibit endogenous integrin function; in fact, we found that single-subunit chimeric receptors could inhibit endogenous integrin localization and integrin functions in cell spreading, cell migration, and fibronectin matrix assembly. Furthermore, we have also demonstrated that individual β intracellular domains display specificity in their ability to mimic and inhibit these aspects of integrin function.

We found that the β1 and β3 chimeras, like the β1 chimera, colocalized with endogenous integrins at focal adhesions and at sites where fibronectin fibrils associate with the plasma membrane, indicating that the β1 and β3 intracellular domains can also direct receptor localization. The ability of the β3 chimera to concentrate at focal adhesions is consistent with previous reports demonstrating the ability of endogenous β3 integrins to localize at these sites (18, 20, 61), as well as the observation that the function of the β1 and β3 intracellular domains can be interchangeable in this process (63). There have been, however, contrasting reports regarding the ability of endogenous β5 integrins to concentrate at focal adhesions (15, 50, 67; also see Stuiver, I., and J. W. Smith. 1993. Mol. Cell. Biol. 4[Suppl.] 285a). Our results with the β3 chimera are consistent with the notion that the β3 intracellular domain can also direct receptor localization to focal adhesions, but may differ in its ability to do so compared with the β1 and β3 intracellular domains.

The ability of the β1 and β3 chimeras to colocalize with endogenous α5β1 at sites where fibronectin fibrils associate with the plasma membrane suggests that the β1 and β3 intracellular domains, like the β1 intracellular domain, can bind to similar cytoplasmic components as α5β1 at these sites, although the β3 intracellular domain appears to have a lower affinity for these interactions. The apparent lack of colocalization of endogenous β1 and β3 integrins with β1 integrins at fibronectin fibrils may be due to functional differences in their extracellular domains, especially since there is no evidence as yet that any integrins other than members of the β1 subfamily can function in fibronectin matrix assembly (1, 22, 45, 53, 68, 70).

In contrast to the other β chimeras, the β1β3 chimera was expressed diffusely on the cell surface and did not concentrate in focal adhesions or at sites of association of fibronectin with the plasma membrane, indicating that alternative splicing can regulate integrin distribution by altering intracellular interactions. A protein corresponding to the β1β3 variant mRNA has not yet been characterized, so the effect of this variant intracellular domain on heterodimer function is not yet known. However, our results would lead us to predict that heterodimers containing this variant intracellular domain will not concentrate at focal adhesions due to their inability to interact with the appropriate cytoskeletal proteins. Interestingly, a similar splicing variant has been described for the β1 intracellular domain (4), and heterodimers containing a recombinant form of this β1 variant do not concentrate in focal adhesions (6).

Our results also demonstrate that the β1 and β3 chimeras can function as dominant negative mutants and inhibit endogenous integrin localization and function. When we compared the levels of expression of the β1 chimera with the endogenous β1 subunit, we found that in 15 h assays, the β1 and β3 chimeras could inhibit cell spreading and cell migration when expressed at 1-10 times the level of the endogenous β1 subunit. Lower levels of expression of the β1 and β3 chimeras were required to inhibit matrix assembly. Furthermore, lower levels of expression of the chimeric receptors were required to inhibit cell spreading in 1-1.5 h assays as compared to 15 h assays.

We also found that β intracellular domains differ in their ability to inhibit endogenous integrin function. Higher levels of expression of the β3 chimera were required to inhibit cell spreading as compared to the β1 and β3 chimeras. This finding is consistent with reported differences in the abilities of endogenous β1 and β3 integrins to function in cell spreading (40). Furthermore, the β1β3 chimera did not inhibit endogenous integrin function at any of the expression levels examined.

The ability of the β1 chimera to inhibit matrix assembly suggests that there is a requirement for the integrin β1, but not the α5 intracellular domain in matrix assembly in agreement with previous reports (17, 68). In addition, the β1 and β3 chimeras always inhibited matrix assembly to a lesser extent than αββ1 localization to fibrils. This difference may be because the redistribution of αββ1 and/or its concentration at fibrils requires a stable interaction between the αββ1 integrin and the cytoskeleton, whereas matrix assembly may require only more transient interactions. In contrast to the β1 and β3 chimeras, the β3 chimera was observed to colocalize with αββ1 at fibronectin fibrils, but not to inhibit αββ1 localization or function, suggesting that the β3 chimera can bind to cytoplasmic components already concentrated at these sites, but not with sufficient affinity to compete successfully with the β1 intracellular domain for their binding.

The mechanism of inhibition by the chimeric receptors in these processes is not yet known. Inhibition may involve competition with specific integrin–cytoskeletal interactions. However, signal transduction events might also be required to trigger cell spreading, cell migration and matrix assembly. If these events involve integrins, they could also be targets for inhibition. In addition, it is important to note that the proportions of total endogenous integrin receptors that are actually directly involved in any particular biological process such as cell spreading or matrix assembly are likely to be relatively low at any particular time (e.g., 10–20%, as recently reported for interactions of β1 integrins with the dense extracellular matrix of chick embryo fibroblasts; 19). Consequently, the ratio of chimeric molecules to ligand-occupied integrin molecules is probably considerably higher than to total integrin molecules. Furthermore integrin–cytoskeletal interactions required in these processes may be specific for ligand-occupied integrins. Therefore, chimeric receptors may be in excess and competitively inhibiting ligand-occupied integrins rather than the whole population of integrins.

The specific intracellular interactions involved in these processes have not yet been defined. Detailed mutational analysis of the β1 intracellular domain has defined three regions of the β1 intracellular domain, designated cyto 1,
cyto 2, and cyto 3 that are important for β₁ integrin localization in focal adhesions (52), although mechanisms responsible for the effects of these mutations are not yet understood. Nonetheless, in vitro binding sites for α-actinin overlap with these regions (47) and a synthetic peptide overlapping cyto 2 can inhibit the in vitro binding of talin to integrins (64). These results suggest that talin and/or α-actinin function in receptor localization to focal adhesions consistent with the observations that α-actinin and talin colocalize with integrins at these sites in tissue culture (reviewed in reference 11). Whether interactions of integrins with α-actinin and talin are involved in cell spreading, matrix assembly, or cell migration is not yet known. However, microinjection of antibodies against talin have been found to inhibit both cell spreading and cell migration, implying a role for talin in these two processes (46). Our results also suggest that similar interactions are required for both cell migration and cell spreading, although it is possible that intracellular interactions in addition to those involved in cell spreading are required for cell migration (e.g., see reference 58).

In summary, we found that relatively high levels of expression of the β₁ and β₂ chimeras could effectively inhibit endogenous integrin function. We also found that some processes were more easily inhibited than others and some β chimeras were better inhibitors than others. If inhibition occurs by the competition of the β chimera for cytoskeletal or cytoplasmic proteins required for function, it is reasonable that some processes would be inhibited more easily than others depending on the intracellular concentration, and possible local concentration, of these components. β intracellular domains will likely contain overlapping binding sites for several cytoplasmic proteins, each involved in specific integrin-mediated processes. The identity of these proteins, their sites of interaction with β intracellular domains and how these interactions are regulated to mediate specific processes are central to understanding integrin intracellular function. Our results define a new approach that allows analysis of integrin intracellular function without complications due to other aspects of heterodimer function. Furthermore, the ability of the different intracellular domains to cross-inhibit each other's function to differing degrees will allow comparisons of integrin intracellular functions.

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References

1. Akiyama, S. K., S. S. Yamada, W.-T. Chen, and K. M. Yamada. 1989. Analysis of fibronectin receptor function with monoclonal antibodies: Roles in cell adhesion, migration, matrix assembly, and cytoskeletal organization. J. Cell Biol. 109:863-875.
2. Akiyama, S. K., K. Nagata, and K. M. Yamada. 1990. Cell surface receptors for extracellular matrix components. Biochim. Biophys. Acta. 1031:91-110.
3. Albeeld, S. M., and C. A. Buck. 1990. Integrins and other cell adhesion molecules. FASEB (Fed. Am. Soc. Exp. Biol.) J. 4:2868-2880.
4. Altruda, F., P. Cervella, G. Tarone, C. Botta, F. Balzac, G. Stefanutto, and L. Silengo. 1990. A human integrin β₁ subunit with a unique cytoplasmic domain generated by alternative mRNA processing. Gene. 95:251-266.
5. Argraves, W. S., S. Suzuki, H. Arai, K. Thompson, M. D. Pierschbacher, and E. Ruoslabi. 1987. Amino acid sequence of the human fibronectin receptor. J. Cell Biol. 105:1183-1190.
6. Balzac, F., A. M. Belkin, V. E. Kotelnikov, Y. V. Balabanov, F. Altruda, L. Silengo, and G. Tarone. 1993. Expression and functional analysis of a cytoplasmic domain variant of the β₁ integrin subunit. J. Cell Biol. 121:171-178.
7. Bauer, J. S. J. Varner, C. Scheiner, L. Kornberg, R. Nicholas, and R. L. Juliano. 1993. Functional role of the cytoplasmic domain of the integrin αs subunit. J. Cell Biol. 122:209-221.
8. Brizewitz, R., A. Korn, and E. N. Marcantonio. 1993. Ligand-dependent and -independent integrin focal contacts: localization of the role of the α chain cytoplasmic domain. Mol. Biol. Cell. 4:593-604.
9. Burridge, K. J., and R. F. Faraonico. 1980. Microinjection and localization of a 105kD protein in living fibroblasts: a relationship to actin and fibronectin. Cell. 19:587-595.
10. Burridge, K., and L. Connell. 1983. Talin: a cytoskeletal component concentrated in adhesion plaques and other sites of actin-membrane interaction. Cell Motil. 3:405-417.
11. Burridge, K., K. Fath, T. Kelly, G. Nuckolls, and C. Turner. 1988. Focal adhesions: Transmembrane junctions between the extracellular matrix and the cytoskeleton. Annu. Rev. Cell Biol. 4:487-525.
12. Chan, B. M., P. D. Kassner, J. A. Schiro, H. R. Byers, T. S. Kupper, and M. E. Hemler. 1992. Dynamic cellular functions expressed by different VLA integrin β₁ subunit cytoplasmic domains. Cell. 68:1051-1060.
13. Chen, W.-T., and S. J. Singer. 1982. Immunoelectron microscopic studies of the sites of cell-substratum and cell-cell contacts in cultured fibroblasts. J. Cell Biol. 95:205-222.
14. Cheres, D. A. 1992. Structural and biologic properties of integrin-mediated cell adhesion. Clin. Lab. Med. 12:217-236.
15. Conforti, G., M. Calza, and A. Beltran-Nunez. 1994. α₂β₁ integrin is localized at focal contacts by HT-1080 fibrosarcoma cells and human skin fibroblasts attached to vitronectin. Cell Adh. Commun. 1:279-293.
16. Damsky, C. H., and Z. Werb. 1992. Signal transduction by integrin receptors for extracellular matrix: cooperative processing of extracellular information. Curr. Opin. Cell Biol. 4:772-781.
17. Darribere, T., K. Guida, H. Larjava, K. E. Johnson, K. M. Yamada, J. P. Thiery, and J.-C. Boucaut. 1990. In vivo analyses of integrin β₁ subunit function in fibronectin matrix assembly. J. Cell Biol. 110:1813-1823.
18. Dejana, E., S. Cotella, G. Conforti, M. Abbabdi, M. Guboli, and P. C. Marchisio. 1988. Fibronectin and vitronectin regulate the organization of their respective Arg-Gly-Asp adhesive receptors in cultured human endothelial cells. J. Cell Biol. 107:1215-1223.
19. Enomoto-Iwamoto, M., A. S. Menko, N. Philp, and D. Boettiger. 1993. Evaluation of integrin molecules involved in substrate adhesion. Cell Adh. Commun. 1:191-202.
20. Fath, K. R., C.-J. Edgell, and K. Burridge. 1989. The distribution of distinct integrins in focal contacts is determined by the substratum composition. J. Cell Sci. 92:67-75.
21. Fitzgerald, L. A., B. Steiner, S. C. Ral Jr., S.-S. Lo, and D. R. Phillips. 1987. Protein sequence of endothelial glycoprotein IIIa derived from a cDNA clone. J. Biol. Chem. 262:3926-3931.
22. Fogerty, F. J., S. K. Akiyama, K. M. Yamada, and D. F. Mosher. 1990. Inhibition of binding of fibronectin to matrix assembly sites by anti-integrin (αβ) antibodies. J. Cell Biol. 111:699-708.
23. Geiger, B., D. Salomon, M. Takeichi, and R. G. Wirtz. 1992. A chimeric N-cadherin/β₁-integrin receptor which localizes to both cell-cell and cell-matrix adhesions. J. Cell Sci. 103:943-951.
24. Ginsberg, M. H., X. Du, and E. F. Plow. 1992. Inside-out integrin signaling. Curr. Opin. Cell Biol. 4:766-771.
25. Giordano, T., T. H. Howard, J. Coleman, K. Sakamoto, and B. H. Howard. 1991. Isolation of a population of transiently transfected quiescent and senescent cells by magnetic affinity cell sorting. Exp. Cell Res. 192:193-197.
26. Goldstein, S., C. M. Fordis, and B. H. Howard. 1989. Enhanced transfection efficiency and improved cell survival after electroporation of G2/M-synchronized cells and treatment with sodium butyrate. Nucleic Acids Res. 17:3593-3571.
27. Gnan, J. L., J. E. Trevithick, and R. O. Hynes. 1991. Fibronectin/integrin interaction induces tyrosine phosphorylation of a 120-kD protein. Cell Regul. 2:951-964.
28. Hanks, S. K., M. B. Calalb, M. C. Harper, and S. K. Patel. 1992. Focal adhesion protein tyrosine kinase phosphorylated in response to cell attachment to fibronectin. Proc. Natl. Acad. Sci. USA. 89:8487-8491.
29. Hayashi, Y., B. Haimovich, A. Rezza, D. Boettiger, and A. Horwitz. 1992. Expression and function of chicken integrin β₁ subunit and its cytoplasmic domain mutants in mouse 3T3 cells. J. Cell Biol. 110:175-184.
30. Hemler, M. E. 1990. VLA proteins in the integrin family: structures, functions and their role on leukocytes. Annu. Rev. Immunol. 8:365-400.
31. Hibbs, M. L., H. Xu, S. A. Slacker, and T. A. Springer. 1991. Regulation
of adhesion ICAM-1 by the cytoplasmic domain of LFA-1 integrin β subunit. Science (Washington, DC). 251:1611–1613.

32. Hogg, N. 1991. An integrin overview. Chem. Immunol. 50:1–12.

33. Hyne, R. O. 1992. Integrins: versatility, modulation, and signaling in cell adhesion. Curr. Opin. Cell Biol. 4:11–15.

34. Izard, C. S., and L. R. Lochner. 1976. Cell-to-substrate contacts in living fibroblasts: an interference reflection study with an evaluation of the technique. J. Cell Sci. 21:129–159.

35. Johnson, G. D., and G. M. Nogueira Araujo. 1981. A simple method of reducing the fading of immunofluorescence during microscopy. J. Immunol. Methods. 43:349–350.

36. Juliano, R. L., and S. Haskill. 1993. Signal transduction from the extracellular matrix. J. Cell Biol. 120:571–577.

37. Kassner, P. D., and M. E. Hemler. 1993. Interchangeable alpha chain cytoplasmic domains play a positive role in control of cell adhesion mediated by VLA-4, a β integrin. J. Exp. Med. 178:649–660.

38. Kawaguchi, S., and M. E. Hemler. 1993. Role of the α subunit cytoplasmic domain in regulation of adhesion activity mediated by the integrin VLA-2. J. Biol. Chem. 268:16279–16285.

39. LaFlamme, S. E., S. K. Akiyama, and K. M. Yamada. 1992. Regulation of fibronectin receptor redistribution. J. Cell Biol. 117:437–447.

40. Leavesley, D. I., G. D. Ferguson, E. A. Wayner, and D. A. Cheresh. 1992. Requirement of the integrin β subunit for carcinoma cell spreading or migration on vitronectin and fibronectin. J. Cell Biol. 117:1101–1107.

41. Leonard, W. J., J. M. Depper, G. R. Crabtree, S. Rudikoff, J. Pumphrey, R. J. Robb, M. Kronke, P. B. Svetlik, N. J. Peffer, T. A. Waldmann, and W. C. Greene. 1984. Molecular cloning and expression of cDNAs for the human IL-2 receptor. Nature (London). 311:626–631.

42. Marcantonio, E. E., J.-L. Guan, J. E. Trevithick, and R. O. Hynes. 1990. Mapping of the functional determinants of the integrin β cytoplasmic domain by site-directed mutagenesis. Cell Regul. 1:597–604.

43. McLean, J. W., D. J. Vestal, D. A. Cheresh, and S. C. Bodary. 1990. cDNA sequence of the human integrin ββ subunit. J. Biol. Chem. 265:17126–17131.

44. Miekka, S. I., K. C. Ingham, and D. Menache. 1982. Rapid methods for isolation of human plasma fibronectin. Cell Regul. 3:1561–1568.

45. Mosher, D. F., J. Sottile, C. Wu, and J. A. McDonald. 1992. Assembly of extracellular matrix. Curr. Opin. Cell Biol. 4:810–818.

46. Nuckolls, G. H., L. H. Romer, and K. Burridge. 1992. Microinjection of antibodies against talin inhibits the spreading and migration of fibroblasts. J. Cell Sci. 102:753–762.

47. Oet, C. A., G. B. Vasquez, K. Burridge, and B. W. Erickson. 1993. Mapping of the α in binding site within the β integrin cytoplasmic domain. J. Biol. Chem. 268:21193–21197.

48. O'Toole, T. E., D. Mandelman, J. Forsyth, S. J. Shattil, E. F. Plow, and M. H. Ginsberg. 1991. Modulation of the affinity of integrin αIIbβ3 (GPIIb-IIIa) by the cytoplasmic domain of αIIb. Science (Washington, DC). 254:845–847.

49. O'Toole, T. E., Y. Katagiri, R. J. Faull, K. Peter, R. Tamura, V. Quaranta, J. C. Lofthus, S. J. Shattil, and M. H. Ginsberg. 1994. Integrin cytoplasmic domains mediate inside-out signal transduction. J. Cell Biol. 124:1047–1059.

50. Pasquale, R., and M. Hemler. 1994. Contrasting roles for integrin β1 and β2 cytoplasmic domains in celluclular localization, cell proliferation, and cell migration. J. Cell Biol. 125:447–460.

51. Ramaswamy, H., and M. E. Hemler. 1990. Cloning, primary structure and properties of a novel human integrin β subunit. EMBO (Eur. Mol. Biol. Organ.) J. 9:1561–1568.

52. Reszka, A. A., Y. Hayashi, and A. F. Horwitz. 1992. Identification of amino acid sequences in the integrin β, cytoplasmic domain implicated in cytoskeletal association. J. Cell Biol. 117:1231–1330.

53. Roman, J., R. M. LaChance, T. J. Broekelmann, C. J. Kennedy, E. A. Wayner, W. G. Carter, and J. A. McDonald. 1989. The fibronectin receptor is organized by extracellular matrix fibronectin: implications for oncogenic transformation and for cell recognition of fibronectin matrices. J. Cell Biol. 108:2529–2543.