Contrasting Roles for Integrin β_1 and β_5 Cytoplasmic Domains in Subcellular Localization, Cell Proliferation, and Cell Migration

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Abstract. To carry out a detailed comparison of the roles of integrin β_1 and β_5 cytoplasmic domains, we expressed both wild type β_1 and chimeric $\beta_{1/5}$ constructs in CHO cells. In the latter, the cytoplasmic domain of β_1 was replaced with that of β_5. The human β_1 and $\beta_{1/5}$ constructs appeared at similar levels at the cell surface (mostly as $\alpha^5\beta_1$ heterodimers) and contributed equally to CHO cell adhesion to fibronectin. However, β_1 but not $\beta_{1/5}$ localized to focal adhesion-like structures when CHO cells were spread on fibronectin. Furthermore, only the β_1-CHO cells showed increased proliferation in response to fibronectin plus an integrin-activating anti-β_1 antibody, and showed increased appearance of ^{32}P-labeled protein (p90) that correlated with proliferation. In sharp contrast, the $\beta_{1/5}$-CHO cells were notably more migratory than β_1-CHO cells in a transwell haptotactic migration assay. These results indicate that the β_1 and β_5 integrin subunit cytoplasmic domains can translate similar adhesive information into highly contrasting subsequent events. Thus, we have established that "inside-out" and "outside-in" integrin signaling pathways are regulated by fundamentally distinct mechanisms. In addition, we suggest that the same properties of the β_1 cytoplasmic domain that promote recruitment to visible focal adhesion-like structures may also be conducive to cell proliferation. Conversely, the properties of the β_5 tail that make it less likely to localize into focal adhesion-like structures may contribute to enhanced cell migration.

THE 21 transmembrane $\alpha\beta$ heterodimers in the integrin family of cell adhesion receptors (26, 34) play key roles in two-way signaling across the membrane. The diverse cytoplasmic domain sequences within the various integrin α and β subunits are particularly important in this regard.

In a process known as "inside-out signaling," constitutive cellular signals or agonist-triggered signals act through integrin cytoplasmic domains to regulate the ligand-binding properties of the extracellular domains (23, 76). Experiments involving deletions and point mutations within the cytoplasmic domains of β_1 (25), β_2 (31, 32), and β_3 (12) have focused attention on a few conserved residues in each of these subunits as being most critical for control of cell adhesion.

Conversely, external ligand binding may cause changes in cytoplasmic domains (outside-in signaling) associated with a diversity of postligand binding events, such as cell spreading, migration, differentiation, focal adhesion formation, and gene induction (9, 13, 34, 43). Thus far, despite differences in β subunit cytoplasmic domain sequences, many integrin-mediated signaling events appear to be rather nonspecific. For example, several different integrins are able to cause calcium flux (37, 58, 60, 65, 70, 86), a rise in intracellular pH (36, 45, 71, 72), and activation of p125^{FAK} (24, 42, 47). For some postligand binding events, resulting from outside-in signaling through integrins, it is clear that cytoplasmic domains do play specific roles. For example, chimeric α chain constructs were used to demonstrate that the α^2 and α^5 cytoplasmic domains differed from the α^4 cytoplasmic domain with respect to collagen gel contraction and cell migration mediated by $\alpha^2\beta_1$ (VLA-2) (9).

An obvious assumption has been that the subcellular localization of integrins plays a key role in determining their functions. For example, integrin clustering may make an important contribution to cell adhesion (30). In highly spread cells, a very prominent type of integrin cluster called a focal adhesion complex has been studied in great detail. A variety of studies involving deletions (25, 49, 64, 75), chimeras (21, 43), alternatively spliced β_1 (4), and point mutations (64) have established that the β_1 cytoplasmic domain controls β_1 integrin localization to focal adhesions. Many of the cytoplasmic domain residues critical for focal adhesion localization (64) are shared by several other β subunits, thus explaining why the cytoplasmic domains of β_1 and β_3 have similar capability to localize to focal adhesions (74). Despite this abundance of background information, the functional consequences of having integrins that differ in their ability to localize to focal adhesions has only begun to be explored (68).

Among the integrin β_1, β_2, β_3, β_7, and β_5 cytoplasmic do-

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mains, the latter has the most distinctive primary amino acid sequence (61, 88). Perhaps related to this, the $\alpha^v\beta_5$ and $\alpha^v\beta_3$ integrins appear to differ in terms of localization to focal adhesions and contribution to cell migration (44, 82). However, these reported functional differences between $\alpha^v\beta_5$ and $\alpha^v\beta_3$ have not yet been mapped to specific domains; (e.g., β chain cytoplasmic domains).

Here, we have expressed in CHO cells cDNA constructs coding for human β_1 and a chimeric $\beta_{1/5}$ subunit, in which the cytoplasmic domain of β_1 was replaced with that of β_5. These have enabled us to carry out highly controlled and detailed comparative studies showing that (*a*) the β_1 and β_5 cytoplasmic domains differed dramatically in their control of integrin subcellular localization, and (*b*) that differences in subcellular localization were associated with differences in integrin-dependent proliferation and migration but not cell adhesion. Thus, mechanisms for cytoplasmic tail control of cell adhesion (inside-out signaling) are fundamentally different from mechanisms for control of diverse postadhesion events (outside-in signaling).

Materials and Methods

Cells, Transfections, and Cell Culture

CHO cells negative for the dihydrofolate reductase gene ($dhfr^-$)[1] were grown in MEM α^+ medium with 10% FCS, and then switched to MEM α^- with 10% dialyzed FCS (JRH Biosciences, Lenexa, KS) after transfection. The $dhfr^+$ p901 vector (40) was kindly provided by Dr. M. Rosa, Biogen Co. (Cambridge, MA). The pECE vector containing the β_1 insert has been previously described (22). A fragment corresponding to the β_5 cytoplasmic domain was obtained from mRNA (from the CCL-228 carcinoma line) by reverse transcription PCR. In one of the oligonucleotide primers used for PCR, a single mismatch was incorporated to create a HindIII site at the carboxy-terminal side of the transmembrane region. At this site, coding for the amino acids Lys and Leu, AAGCTG was converted to AAGCTT (a silent mutation). The entire PCR-derived β_5 insert was subcloned into pBluescript (Stratagene, La Jolla, CA) and sequenced. The β_1 cytoplasmic domain was removed from the pECE vector using HindIII/XbaI sites (just after the transmembrane and in the 3′ polylinker region, respectively) and replaced with the β_5 cytoplasmic domain also excised (from pBluescript) using HindIII and XbaI sites. The resulting $\beta_{1/5}$ chimera had the carboxy-terminal sequence . . . GLALLAIWKLLVTIHDRREFAKFQSERSRARYEMASNPLYRKPISTHTVDFTFNKFNKGTVD with the underlined portion derived from β_5, and the rest of the molecule derived from β_1.

For transfection, CHO cells were washed once in PBS/EDTA, twice in MEM α^+, and then resuspended in serum-free MEM α^+ media with two different purified plasmids (1 μg p901 $dhfr^+$ and 10 μg β_1-pECE or $\beta_{1/5}$-pECE). After pulsing with a gene pulser (Bio-Rad Laboratories, Richmond, CA) (set at 280 V and 960 μF), cells were resuspended in MEM α^+ medium, with 10% FCS. After 5 d, the cells were grown in MEM-α^-, and after an additional 8–10 d, the cells were stained with the anti-β_1 monoclonal antibody A-1A5, and then sorted using a flow cytometer (Epics; Coulter Corp., Hialeah, FL) to select the 5% most positive population. These cells were grown in the presence of methotrexate (Aldrich Chemical Co., Milwaukee, WI) as described (22) to amplify β_1 and $\beta_{1/5}$ expression, and several additional rounds of amplification and sorting were carried out until human β_1 and $\beta_{1/5}$ expression levels were ≥10 times higher than hamster β_1. Control cell lines include CHO (untransfected) and CHO-MTX. The latter was transfected with p901 $dhfr^+$ plasmid plus β_5 cDNA in the pRc/CMV vector (Invitrogen, San Diego, CA), but during methotrexate (MTX) amplification (in parallel with β_1 and $\beta_{1/5}$ amplification), the β_5 expression was lost.

Abbreviations used in this paper: dhfr, dihydrofolate reductase gene; FN, fibronectin; MTT, 3-[4,5-dimethylthazol-2-yl]-2,5-diphenyltetrazolium; MTX, methotrexate.

Antibodies and Extracellular Matrix (ECM) Proteins

The monoclonal antibodies used were: A-1A5 (28), TS2/16 (27), and 4B4 (52), all anti-human β_1; PB1, anti-hamster $\alpha^5\beta_1$ (7); 7E2, anti-hamster β_1 (18); B1E5, anti-α^5 cross-reactive with human and hamster (83); and 1G8, anti-β_5 cross-reactive with human and hamster (59). Rabbit polyclonal sera to β_5 (61) and α^5 (35) cytoplasmic domain peptides were previously described. Both fibronectin and vitronectin were purchased from Telios Pharmaceuticals (La Jolla, CA), and pronectin, a synthetic Arg-Gly-Asp–containing matrix protein, was obtained from Promega Corp. (Madison, WI). A mouse monoclonal antibody to the cytoskeletal protein talin was obtained from Sigma Immunochemicals (St. Louis, MO), and the mouse antiphosphotyrosine antibody 4G10 (16) was a gift from Dr. Brian Druker (Dana-Farber Cancer Institute).

Flow Cytometry

Cells were incubated in PBS containing 1% BSA (Sigma Immunochemicals) and 5% human serum (Gibco Laboratories, Grand Island, NY) for 30 min on ice, then washed three times in the same solution. Subsequently, cells ($2–3 \times 10^5$) were incubated with purified antibodies (1–10 μg/ml) or ascites (diluted 1:100) for 45 min, and then washed three times in PBS containing 1% BSA, and treated with goat anti-mouse IgG coupled to fluorescein (Cappel Laboratories, Cochranville, PA) for 45 min on ice. After three washes, cells were resuspended in PBS and analyzed using a flow cytometer (FACScan®; Becton Dickinson Immunocytometry Systems, Mountain View, CA).

Radiolabeling and Immunoprecipitation

Cells were surface labeled with ^{125}I using lactoperoxidase or metabolically labeled using [^{35}S]methionine and cysteine, and then lysed in the presence of 1% NP-40 (Calbiochem Corp., La Jolla, CA) and protease inhibitors (PMSF, aprotinin, leupeptin were all from Sigma Immunochemicals). Immunoprecipitations were performed using protein A–Sepharose beads (Pharmacia Fine Chemicals, Piscataway, NJ) previously incubated with unlabeled cell extract to prevent nonspecific binding of radiolabeled material. Labeled extracts were precleared by incubation with normal mouse or rabbit serum, followed by *Staphylococcus aureus* Cowan 1 (Pansorbin; Calbiochem Corp.). Immunoprecipitations were performed using monoclonal antibodies at 2 μg/ml (purified) or diluted 1:100 (ascites), with 3×10^6 cpm of radiolabeled cell extract per sample. Rat anti-mouse IgG mAb 187.1 (87) was added to optimize binding to protein A–Sepharose. Incubations lasted from 30 min to overnight, and then samples were washed three times in 1% NP-40 and analyzed by SDS-PAGE using 6–8% acrylamide gels under nonreducing conditions, unless otherwise stated.

Adhesion and Migration Assays

Cell adhesion assays were performed in 96-well microtiter plates (Flow Laboratories Inc., McLean, VA) coated overnight at 4°C with extracellular matrix protein ligands, followed by 0.1% BSA (for 30 min at 37°C) added to block nonspecific adhesion. Cells were labeled by incubation with the fluorescent dye BCECF (Molecular Probes, Inc., Eugene, OR) for 20 min. After washing two times with PBS, 5×10^4 of the labeled cells were added in triplicate to plates in the presence or absence of antibodies (1 μg/ml or 50 μl of hybridoma supernatant) and incubated for 20 min at 37°C, followed by three washes with RPMI medium containing 0.1% BSA. Cells remaining attached to the plate were analyzed using a fluorescence concentration analyzer (IDEXX Co., Portland, ME). Results are reported as the mean ± 1 SD.

Haptotactic migration assays were performed as described (44), using Boyden chambers (Transwell; Costar Corp., Cambridge, MA), containing membranes with 6.5 mm diameter, 10 μm thickness, and 8 μm pore size. Lower faces of the membranes were coated overnight with fibronectin, vitronectin, or pronectin (Promega Corp.) at 3 μg/ml in serum-free medium. Cells were labeled with [^{35}S]methionine for 3 h, washed, and then incubated for 10–15 min on ice with mAb PB1 to block hamster $\alpha^5\beta_1$ function. In some experiments, cells were also preincubated with TS2/16 (1 μg/ml). For migration assays, the upper and lower faces of the membrane were equilibrated in α^- MEM with 2% FCS, and then the cells were placed in the upper compartment (50,000/chamber), and incubated at 37°C for 1–12 h. After extensive washing, membranes were removed and analyzed by liquid scintillation counting, and the mean of triplicate values for ^{35}S cpm representing each experimental condition was calculated. No radioactivity above background was detected in the upper part of the chambers after washing.

Immunofluorescence

Cells were detached from tissue culture dishes using 2.5 mM EDTA in PBS, washed in serum-free α^- MEM, and resuspended in the same medium containing 1% FCS. Then cells were plated on fibronectin-coated slides and incubated for 6 or 12 h. After adhesion and spreading, nonadherent cells were removed and the adherent cells were fixed for 15 min on ice with 0.5% paraformaldehyde. After three washes with PBS, methanol was added for 2 min, followed by four more washes. Fixed cells were then incubated for 30–40 min with anti-β_1 monoclonal antibody A-1A5 (1:400), antitalin monoclonal antibody (1:500 dilution of ascites; Sigma Immunochemicals), anti-hamster $\alpha^5\beta_1$ PB1 (1 μg/ml), or anti-P-Tyr (1 μg/ml), then washed and incubated with FITC-labeled goat anti-mouse antibody (1:40 dilution; Calbiochem Corp.) for 35 min. After staining, slides were washed with PBS four times and mounted.

Proliferation Assays

Cells were serum deprived by culturing in α^- medium containing 1% FCS for 24 h, and then 50,000 cells were placed in the same solution, in 96-well plates coated with or without different ECM proteins. Also, in some experiments, cells were preincubated with various antibodies for 15 min before they were added to the 96-well plates.

Proliferation was measured by pulsing the cells with 1 μCi [^{3}H]thymidine/well during the last 8 h of 36-h culture. As a positive control, cells were plated in the presence of 10% FCS. A PHD cell harvester (Cambridge Technology, Inc., Cambridge, MA) was used to transfer ^{3}H-labeled DNA to glass fiber filters for analysis by liquid scintillation counting.

Alternatively, cell proliferation was assessed using the MTT dye method, which detects mitochondrial enzymes (79). Briefly, during the last 4 h of 36-h culture at 37°C, MTT (3-[4,5-dimethylthazol-2-yl]-2,5-diphenyltetrazolium bromide) was added to each well to give 0.5 mg/ml. Then, after the cells were solubilized in 0.01 N HCl containing 10% SDS for 8–12 h at 37°C, OD at 650 minus 590 nm was determined using a microplate reader (VMAx; Molecular Devices Co.).

Results

Expression of Human β_1 and $\beta_{1/5}$ cDNA in CHO Cells

The expression of human β_1 and $\beta_{1/5}$ cDNA in CHO cells was evaluated by flow cytometry as shown in Fig. 1. After selection in nucleoside-free medium, human β_1 and $\beta_{1/5}$ were expressed on CHO cells at comparable levels (column 3) that were at least as high as the level of endogenous hamster β_1 (column 2) and greater than the level of staining with a negative control antibody (column 1). After amplification in methotrexate followed by cell sorting, human β_1 and $\beta_{1/5}$ were homogeneously expressed at high levels (column 4) 10-fold greater than endogenous hamster β_1 in the same cells (column 2). Mock-transfected CHO cells (*top row*) expressed endogenous hamster β_1, but showed no cross-reactivity with the anti-human β_1 antibody. Levels of hamster β_5 did not change (column 5), but hamster α^5 was expressed at equivalently higher levels on β_1-CHO and $\beta_{1/5}$-CHO cells compared to control CHO cells (column 6). Consistent with this, others have noted that β_1 on CHO cells is predominantly associated with the α^5 subunit (69).

To avoid selection for anomalous functional traits, no efforts were made to establish clonal cell lines. Instead, bulk cell populations amplified for β_1 or $\beta_{1/5}$ expression were used for all subsequent experiments, along with untransfected or mock-transfected CHO cells.

The human β_1 and $\beta_{1/5}$ subunits expressed at the surface of CHO cells were further analyzed by immunoprecipitation from nonionic detergent cell extracts of ^{125}I-labeled cells. Polyclonal serum to the β_5 cytoplasmic domain immunoprecipitated the 90-kD hamster β_5 subunit (*lower arrow*), presumably associated with hamster α^v from β_1-CHO, $\beta_{1/5}$-CHO and CHO-MTX cells (Fig. 2 *A*, lanes *a–c*). An additional band corresponding to the 100-kD chimeric $\beta_{1/5}$ chain was seen only from $\beta_{1/5}$-transfected cells (lane *b*, *upper arrow*). As expected, an antibody to the human β_1 ectodomain (A-1A5) coimmunoprecipitated associated β and α subunits from β_1 CHO (lane *d*) and $\beta_{1/5}$-CHO cells (lane *e*), but not from mock-transfected (lane *f*) or untransfected CHO cells (not shown). The pattern of bands recognized by the anti-human β_1 antibody (lanes *d* and *e*) looked very similar to the α^5 and β_1 bands immunoprecipitated from β_1-CHO (lane *g*), $\beta_{1/5}$-CHO (lane *h*), and CHO-MTX (lane *i*) by an antibody recognizing the cytoplasmic domain of hamster α^5. These results are consistent with α^5 being the predominant subunit associated with human or hamster β_1 on these cells.

Immunoprecipitation from ^{35}S-metabolically labeled $\beta_{1/5}$-CHO cells confirmed the presence of the $\beta_{1/5}$ protein (Fig. 2 *B*). Antiserum to the β_5 carboxy terminus recognized an extra band migrating similar to β_1 (lane *b*, *arrow*) that was

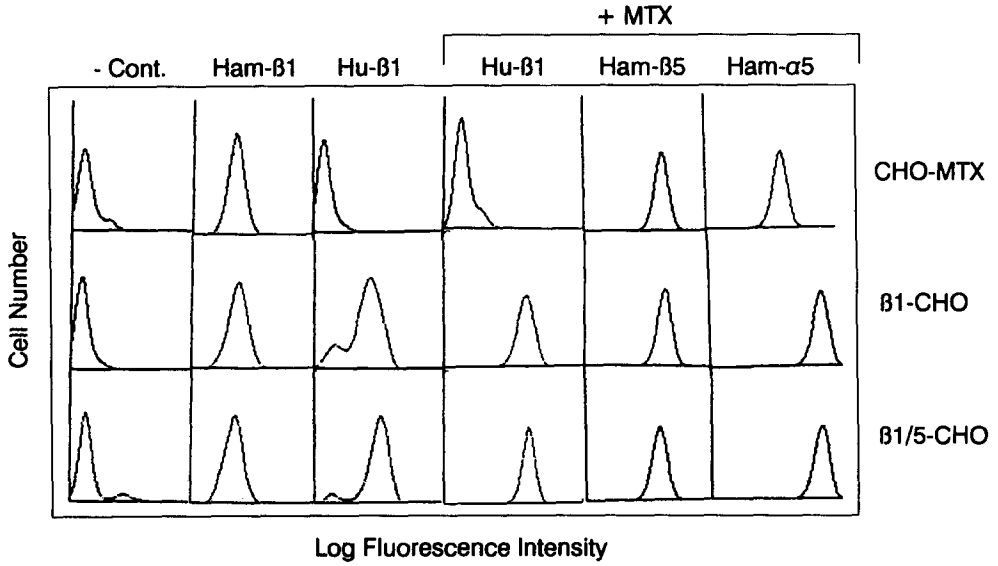


Figure 1. Flow cytometric analysis of CHO-MTX, β_1-CHO, and $\beta_{1/5}$-CHO cells. Cells were stained with a negative control antibody (P3, column 1), anti-hamster β_1 (7E2, column 2), anti-human β_1 (TS2/16, columns 3 and 4), anti-hamster β_5 (1G8, column 5), or with anti-hamster α^5 (B1E5, column 6). Cells in columns 1-3 were stained after selection for ~6 wk in α^- medium, but before methotrexate amplification. Cells in columns 4-6 were analyzed after three cycles of methotrexate amplification.

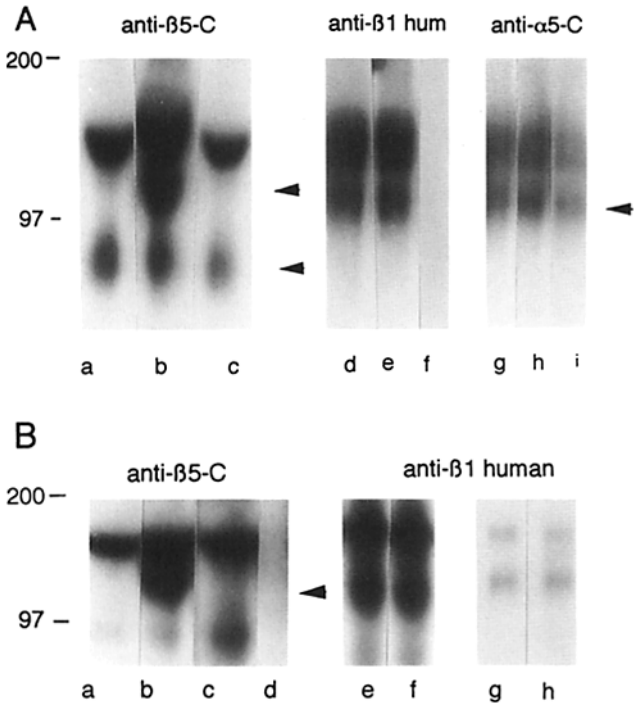


Figure 2. Immunoprecipitation of human β_1 and $\beta_{1/5}$ subunits from CHO cells. (*A*) Extracts from ^{125}I-labeled cells were immunoprecipitated using anti-β^5 COOH-terminal polyclonal antiserum (lanes *a*, *b*, and *c*), anti-human β_1 and mAb A-1A5 (lanes *d*, *e*, and *f*), and anti-α_5 COOH-terminal polyclonal serum (lanes *g*, *h*, and *i*), and the resulting proteins were analyzed using nonreducing conditions. Cells were β_1-CHO (lanes *a*, *d*, and *g*), $\beta_{1/5}$-CHO (lanes *b*, *e*, and *h*), and CHO-MTX (lanes *c*, *f*, and *i*). (*B*) Anti-β_5 COOH-terminal polyclonal antiserum was used to immunoprecipitate ^{35}S-labeled β_1-CHO cells (lane *a*), $\beta_{1/5}$-CHO cells (lane *b*), or CHO-MTX cells (lane *c*). The mAb P3 was used for a negative control precipitation from CHO-MTX cells (lane *d*). Also, in two separate experiments, anti-human β_1 mAb A-1A5 was used to precipitate integrins from $\beta_{1/5}$-CHO (lanes *e* and *g*) and β_1-CHO (lanes *f* and *h*). Immunoprecipitated proteins were analyzed using reducing (lanes *a–d*) or nonreducing conditions (lanes *e–h*).

not seen in β_1-CHO (lane *a*) or CHO-MTX cells (lane *c*). There was no evidence for α^1, α^2, α^4, or α^6 subunits. If present, these would have been seen as distinct bands under the reducing conditions used for lanes *a–d*. Also, two separate experiments showed that ^{35}S-labeled β_1- and $\beta_{1/5}$-CHO cells yielded very similar patterns of human β and hamster α subunits (compare lanes *e* and *f*, *g* and *h*). These results are consistent with the similar human β_1 and $\beta_{1/5}$ expression levels seen in Fig. 1.

Adhesive Properties of β_1-CHO and $\beta_{1/5}$-CHO Cells

The adhesion of β_1 and $\beta_{1/5}$ CHO cells to fibronectin (Fig. 3 *A*) was carried out both in the absence (parts *a* and *b*) and presence (parts *c* and *d*) of the PB1 antibody added to block background adhesion mediated by hamster $\alpha^5\beta_1$. In either case, adhesion by β_1 and $\beta_{1/5}$ CHO cells was greater than that seen by control (CHO-MTX) cells (parts *a* and *c*), although the difference was more obvious when PB1 was present (part *c*). When the anti-human β_1 antibody 4B4 was added, adhesion by β_1 and $\beta_{1/5}$ CHO cells was no longer elevated compared to the CHO-MTX cells (parts *b* and *d*). The inhibitory effect of 4B4 was especially obvious when PB1 was also present (part *d*). Thus, the substantial contributions of human β_1 and $\beta_{1/5}$ subunits to fibronectin adhesion were most obvious when the background contribution of hamster $\alpha^5\beta_1$ was blocked.

In contrast to fibronectin, collagen IV and laminin supported only low levels of adhesion by the various CHO cells, and the adhesion by β_1- and $\beta_{1/5}$-CHO cells was not significantly greater than adhesion by CHO-MTX cells (Fig. 3 *B*, parts *e* and *f*). These results are consistent with β_1 being mostly associated with the hamster α^5 subunit. Also, adhesion to vitronectin was essentially unchanged among CHO, β_1-CHO, and $\beta_{1/5}$-CHO cells (not shown), indicating that the presence of the $\beta_{1/5}$ chimera had no obvious effect on the adhesive function of hamster $\alpha^v\beta_5$.

In the presence of PB1 (anti-hamster $\alpha^5\beta_1$), both β_1-CHO and $\beta_{1/5}$-CHO cells bound equally well to fibronectin in a dose-dependent fashion, markedly above the level of adhesion seen for mock-transfected (CHO-MTX) cells (Fig. 3 *B*). In the presence of the stimulatory anti-β_1 antibody TS2/16, adhesion of both β_1- and $\beta_{1/5}$-CHO cells was upregulated to the same extent, especially at the lower doses of fibronectin. In addition, titration of Mg^{2+} (from 0.005 to 50 mM) revealed no significant differences between β_1- and $\beta_{1/5}$-dependent adhesion (Fig. 3 C). In summary, the results in Fig. 3 show that the β_1 and $\beta_{1/5}$ subunits have a similar level of constitutive activity, are equally responsive to stimulation by the anti-β_1 antibody TS2/16, and show equivalent use of Mg^{2+} to support adhesion.

Subcellular Localization of $\beta_{1/5}$ and β_1 in CHO Cells

To compare the subcellular distribution patterns of the β_1 and $\beta_{1/5}$ subunits, β_1- and $\beta_{1/5}$-CHO cells were plated for 6–12 h on fibronectin. Then, after fixing and staining of permeabilized cells with the anti-β_1 antibody A-1A5 (specific to the extracellular domain), a very distinct distribution pattern was observed for the two cell lines (Fig. 4). Wild type human β_1 (Fig. 4, *A*, *C*, *E*, and *G*) consistently showed a punctate distribution pattern with many focal adhesion-like structures present. In marked contrast, $\beta_{1/5}$ was diffusely distributed (Fig. 4, *B*, *D*, *F*, and *H*) and not localized to focal adhesion-like structures in these and numerous other experiments. Control experiments (Fig. 5) showed that both β_1-CHO cells (*A*, *C*, and *E*) and $\beta_{1/5}$-CHO cells (*B*, *D*, and *F*), had equal ability to form focal adhesions containing hamster $\alpha^5\beta_1$ (weakly stained; *A* and *B*), talin (strongly stained; *C* and *D*) or phosphotyrosine (*E* and *F*). Antibody staining of nonpermeabilized cells or preincubation with antibody before permeabilization also yielded striking differences in the localization patterns of β_1 and $\beta_{1/5}$, with the latter being much more diffuse (not shown). These control experiments indicate that the diffuse staining seen for $\beta_{1/5}$ was not simply caused by selective accumulation of that protein within intracellular pools. Also, endogenous wild type hamster β_5 remained diffusely localized in both β_1- and $\beta_{1/5}$-CHO cells, consistent with the diffuse localization of $\beta_{1/5}$.

Contribution of β_1 and $\beta_{1/5}$ to CHO Cell Proliferation

There have been several reports that integrins can deliver

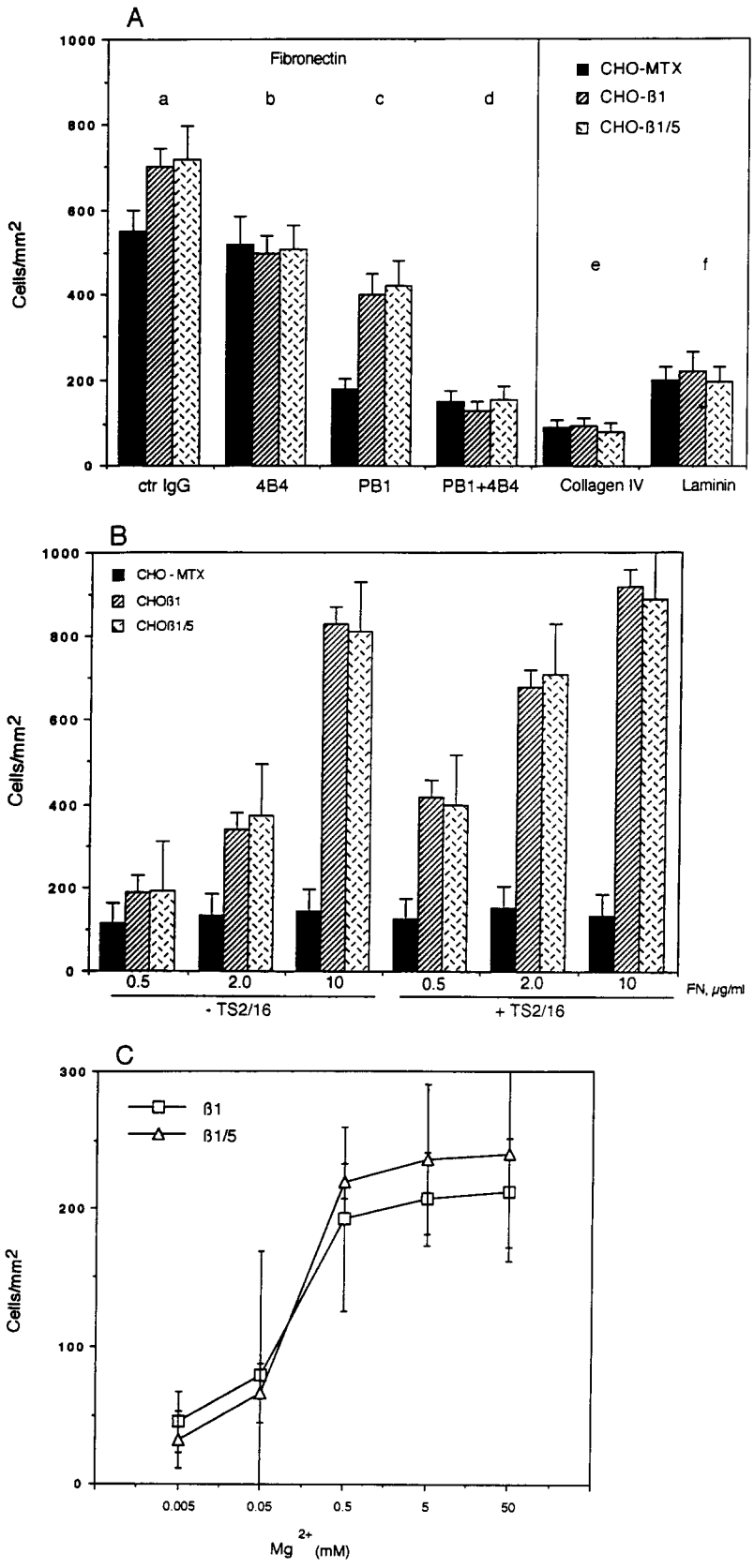


Figure 3. Comparison of adhesive properties of β_1-CHO, $\beta_{1/5}$-CHO, and CHO-MTX cells. (*A*) Plastic surfaces were coated with fibronectin, collagen IV, or laminin (each at 5 µg/ml), and then cell adhesion assays were carried out as described in Materials and Methods. The fibronectin adhesion assays were carried out in the presence of control antibody, anti-human β_1 (4B4), anti-hamster $\alpha^5\beta_1$ (PB1), or both 4B4 and PBl as indicated. (*B*) Adhesion to fibronectin (coated at 0.5, 2.0, and 10 µg/ml) was carried out in the presence of 2 µg/ml purified PBl (to block hamster $\alpha^5\beta_1$ function). As indicated, 1 µg/ml of stimulatory anti-human β_1 mAb TS2/16 was added for some assays. (*C*) Adhesion to fibronectin (coated at 5 µg/ml) was carried out in the presence of varying concentrations of Mg^{2+}, and a constant level of PBl (2 µg/ml).

costimulatory (15, 50) or direct stimulatory (53, 80) signals leading to cell proliferation. We hypothesized that the striking differences in the subcellular localization properties of β_1 and $\beta_{1/5}$ might correlate with differences in their contributions towards proliferation. Thus, conditions were set up to examine the contribution of integrins towards proliferation of CHO cells, exploiting the window of time (20–36 h) in which the CHO cells could maintain viability while be-

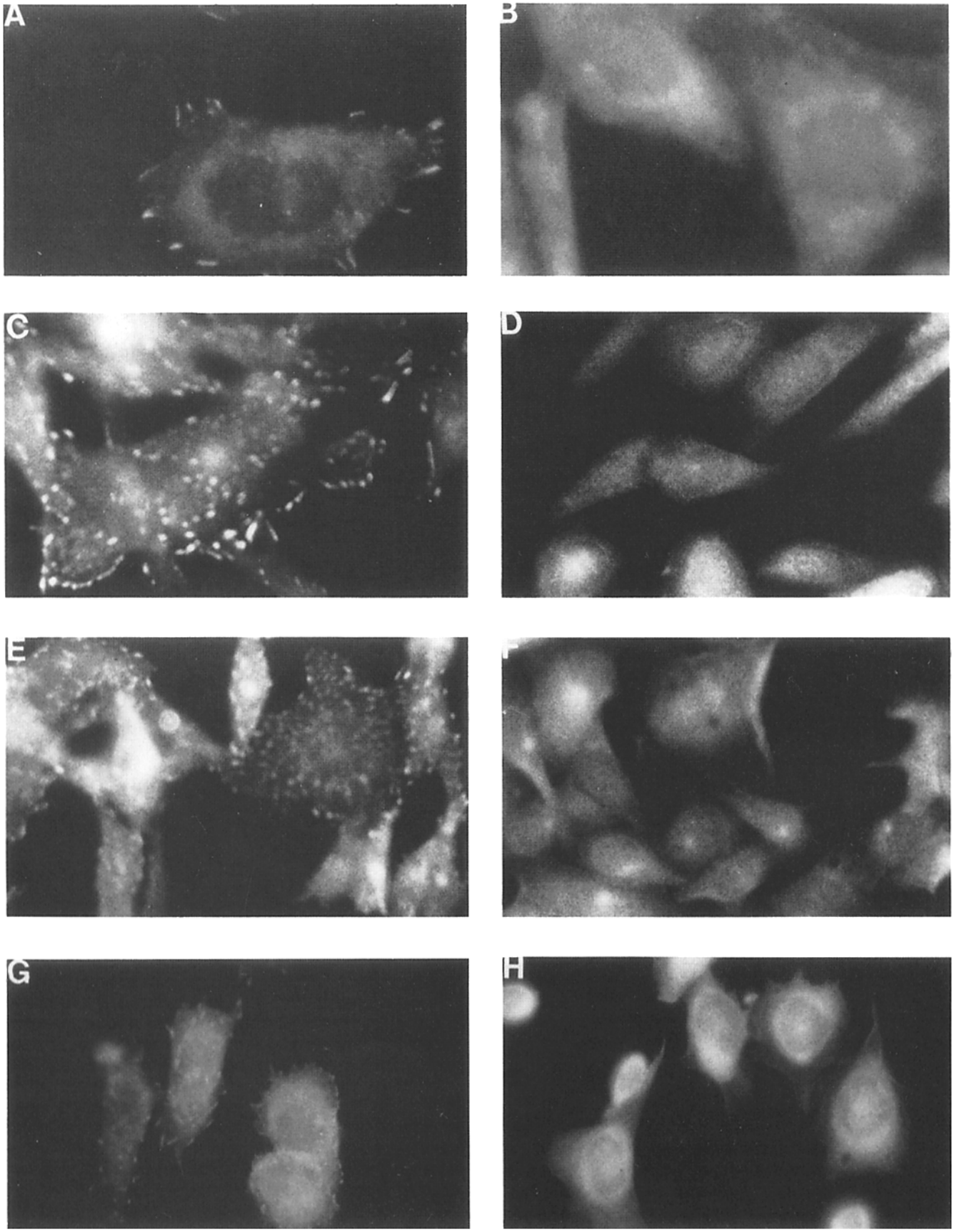


Figure 4. Immunofluorescent localization of human β_1 and $\beta_{1/5}$ in CHO cells. The β_1-CHO (*left panels*) and $\beta_{1/5}$-CHO cells (*right panels*) were allowed to attach and spread on fibronectin-coated slides for 6 h (*A–D*) or 12 h (*E–H*), and then were stained with anti-human β_1 mAb A-1A5 as described in Materials and Methods. ×900 (*A* and *B*), ×630 (*C–F*), or ×400 (*G* and *H*).

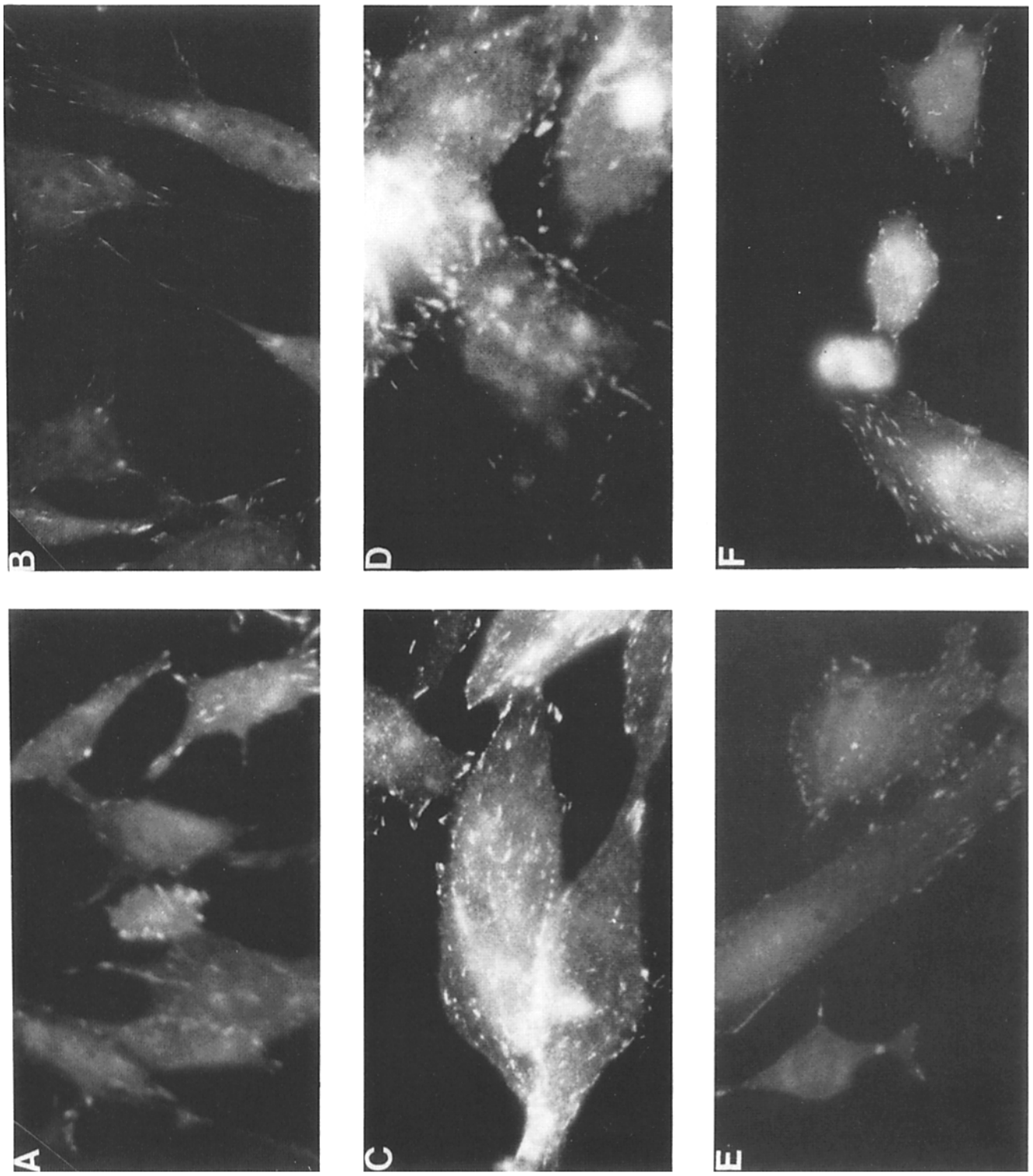


Figure 5. Immunofluorescent localization of hamster $\alpha^5\beta_1$, talin, and phosphotyrosine in β_1- and $\beta_{1/5}$-CHO cells. The β_1-CHO (*left panels*) and $\beta_{1/5}$-CHO cells (*right panels*) were allowed to attach and spread on fibronectin-coated slides for 6 h, and then were stained with anti-hamster $\alpha^5\beta_1$ mAb PB1 (*A* and *B*), antitalin polyclonal antiserum (*C* and *D*), or anti-phosphotyrosine antibody (*E* and *F*) as described in Materials and Methods. ×630 (*A–D*) and ×400 (*E* and *F*).

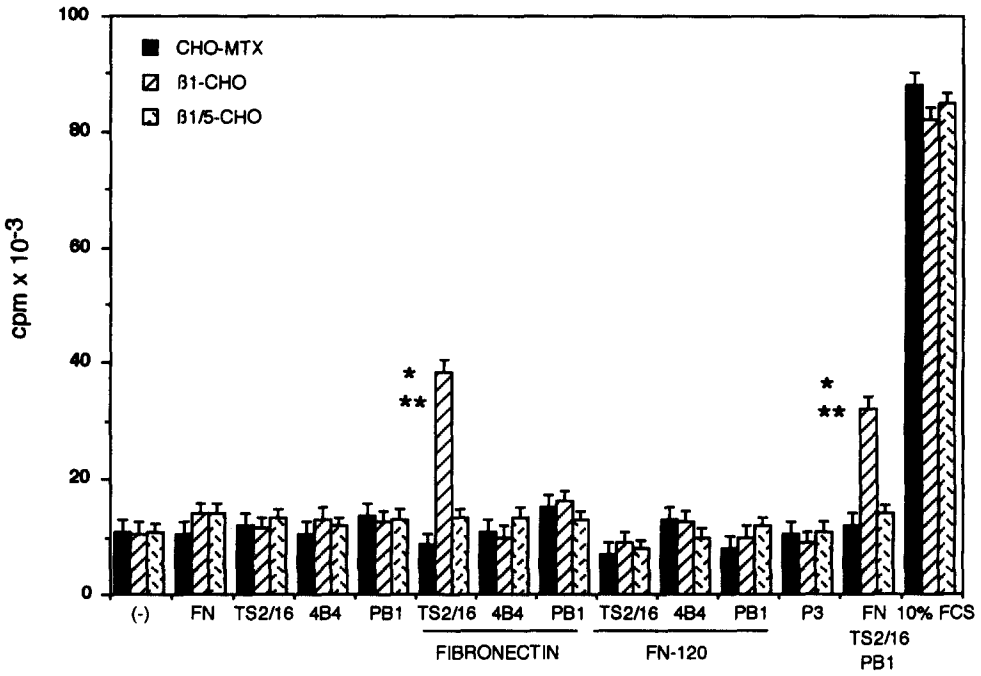


Figure 6. Comparison of proliferative responses of β_1-CHO and $\beta_{1/5}$-CHO cells. Serum-deprived cells were cultured in the presence of different ECM proteins (*first row under figure*) and different anti-integrin antibodies (*second row under figure*), and then proliferation was determined by [^{3}H]thymidine uptake measurements as described in Materials and Methods. In a positive control experiment, 10% FCS was added back to serum-starved cells. * Proliferation by β_1-CHO cells is statistically greater than $\beta_{1/5}$-CHO and CHO-MTX cells ($P < 0.002$, Student's unpaired t test). ** Proliferation by β_1-CHO cells in the presence of FN and TS2/16 is statistically greater compared to all of the other listed conditions ($P < 0.001$, Student's unpaired t test).

ing deprived of serum. As shown in Fig. 6, the combination of immobilized fibronectin plus soluble TS2/16 caused a marked proliferation of serum-deprived β_1-CHO cells, but not $\beta_{1/5}$-CHO or control CHO-MTX cells, as measured by uptake of [^{3}H]thymidine. Notably, neither fibronectin nor TS2/16 alone was sufficient. Also, the proliferative response to TS2/16 and fibronectin was not appreciably blocked by anti-hamster $\alpha^5\beta_1$ antibody PB1, indicating that fibronectin, like TS2/16, was acting through the human β_1 subunit. In control experiments, a nonstimulatory anti-human β_1 antibody (4B4), the hamster $\alpha^5\beta_1$ (PB1), and the control antibody P3 had no stimulatory effect on proliferation, either alone or together with fibronectin. In the presence of 10% serum, transfected and control CHO cells all proliferated at a high level, with no detectable difference among them.

Interestingly, intact fibronectin could not be replaced by the 120-kD fibronectin fragment, even though it contains both the central $\alpha^5\beta_1$ binding site (the RGD region), as well as a second synergizing site of interaction with $\alpha^5\beta_1$ (54). The requirement for intact fibronectin was not unique to CHO cells; a human B cell line (JY) and a myeloid cell line (KG1) also showed a similar β_1-dependent proliferation to intact fibronectin, but not to FN-40 or to FN-120 fragments (not shown).

To further emphasize the proliferation difference between β_1-CHO and $\beta_{1/5}$-CHO cells, the results of six different [^{3}H]thymidine uptake experiments are shown in Table I. In each experiment, the proliferation of β_1-CHO cells was markedly greater than $\beta_{1/5}$-CHO cells in response to a combination of FN plus TS2/16. Such a difference was not obvious if the cells were not stimulated or stimulated with FN alone. To complement the [^{3}H]thymidine uptake experiments, we also carried out proliferation experiments by measuring cell viability using the MTT dye that detects mitochondrial enzymes (79). As shown in Table II in three separate experiments, β_1-CHO cells but not $\beta_{1/5}$-CHO cells proliferated in response to a combination of FN plus TS2/16. Consistent with results in Table I and Fig. 6, elevated proliferation by β_1-CHO cells was not obvious if the cells were not stimulated or stimulated with FN alone (Table II). In general, MTT staining of CHO and $\beta_{1/5}$-CHO cells was not diminished as a result of FN plus TS2/16 treatment, and β_1-CHO and $\beta_{1/5}$-CHO cells showed no loss of viability when those cells were monitored by MTT staining before,

Table I. Proliferation of β_1- and $\beta_{1/5}$-CHO Cells Monitored by [^{3}H]Thymidine

Experiment	No stimuli‡	FN‡	FN + TS2/16‡	CHO-MTX
1. β_1-CHO	−500	3,000	27,500	11,000*
$\beta_{1/5}$-CHO	−200	3,200	2,400	"
2. β_1-CHO	−140	2,500	30,600	10,500
$\beta_{1/5}$-CHO	−180	2,000	2,800	"
3. β_1-CHO	1,500	4,600	10,000	13,700
$\beta_{1/5}$-CHO	2,400	3,200	5,500	"
4. β_1-CHO	−200	3,800	25,000	12,000
$\beta_{1/5}$-CHO	−5,800	1,400	1,600	"
5. β_1-CHO	1,100	1,600	19,600	10,150
$\beta_{1/5}$-CHO	−3,400	2,600	−1,800	"
6. β_1-CHO	1,100	2,300	7,500	8,700
$\beta_{1/5}$-CHO	1,600	1,200	2,900	"

* All numbers represent cpm of [^{3}H]thymidine and are the mean of six determinations.

‡ Background values for [^{3}H]thymidine uptake by mock-transfected CHO-MTX cells (last column) are subtracted from all values shown in the first three columns. Serum-deprived cells were grown in the presence of immobilized fibronectin and in the presence or absence of soluble TS2/16, and proliferation was determined as described in the Materials and Methods.

Table II. Proliferation of β_1- and $\beta_{1/5}$-CHO Cells Monitored by MTT Staining

Experiment*	No stimuli‡	FN‡	FN + TS2/16‡
1. CHO-MTX	0.24	0.13	0.29
β_1-CHO	0.19	0.27	1.11
$\beta_{1/5}$-CHO	0.27	0.21	0.24
2. CHO-MTX	0.38	0.40	0.34
β_1-CHO	0.41	0.29	1.27
$\beta_{1/5}$-CHO	0.31	0.39	0.18
3. CHO-MTX	0.34	0.39	0.23
β_1-CHO	0.31	0.35	0.98
$\beta_{1/5}$-CHO	0.39	0.26	0.24

* In each experiment, cells were serum-deprived for 24 h and then cultured for 26 h in the presence or absence of immobilized FN and soluble TS2/16 as described (see Materials and Methods).
‡ All numbers represent $OD_{570-660}$ values indicative of MTT staining of viable cells (see Materials and Methods), and they are the mean of triplicate determinations.

during, and after a 32-h interval of serum deprivation (not shown). Thus, the proliferation differences observed in Fig. 6 and Tables I and II were not caused by selective apoptosis.

Specific Phosphorylation of a 90-kD Protein in β_1 CHO Cells

The same conditions that caused a difference in proliferation between β_1-CHO and $\beta_{1/5}$-CHO cells also yielded an obvious biochemical difference. The β_1-CHO and $\beta_{1/5}$-CHO cells were stimulated with fibronectin and TS2/16 while in the presence of [^{32}P]orthophosphate, and then whole cell extracts were prepared in the presence of phosphatase inhibitors and analyzed by SDS-PAGE. Whereas several phosphoproteins were present in both β_1-CHO and $\beta_{1/5}$-CHO cell extracts, a 90-kD phosphoprotein band was present only in the extract from β_1-CHO cells (Fig. 7). Using a common 95-kD phosphoprotein as an internal standard, gel scanning confirmed that the p90 was prominent in β_1-CHO cell extracts, but almost undetectable in extracts from $\beta_{1/5}$ cells (Fig. 7), CHO cells, or CHO-MTX cells (not shown).

In several additional experiments, the specific appearance of the p90 phosphoprotein showed a strong correlation with cell proliferation (Table III). Under conditions of serum deprivation, the p90/p95 ratio was elevated for β_1-CHO but not $\beta_{1/5}$-CHO cells, and only when both fibronectin and TS2/16 were present at the same time. In contrast to TS2/16, an anti-β_1 antibody not able to stimulate cell adhesion (4B4) did not costimulate (with fibronectin) the appearance of either p90 phosphoprotein or proliferation. Under normal growth conditions (10% serum), both proliferation and elevated p90 expression were observed. In summary, the appearance of p90 is closely associated with cell proliferation and provides an independent readout consistent with the selective involvement of β_1 but not $\beta_{1/5}$ in cell proliferation.

Migration on Fibronectin

The $\alpha^5\beta_1$ integrin has a well-established role in cell migration on fibronectin. Thus, to further investigate the functional properties of the β_1- and $\beta_{1/5}$-CHO cells, we used modified Boyden chambers containing 8.0 μm porous membranes to analyze cell migration. In the presence of PB1 (added to block the adhesion of endogenous hamster $\alpha^5\beta_1$)

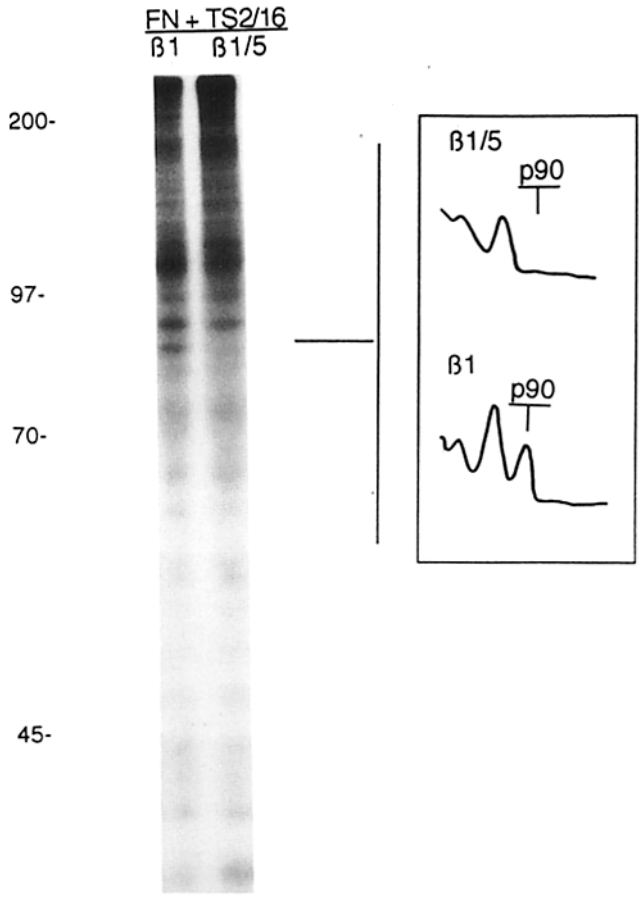
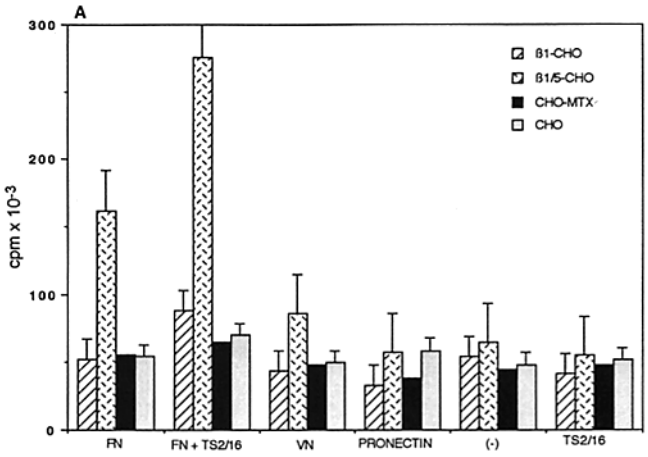


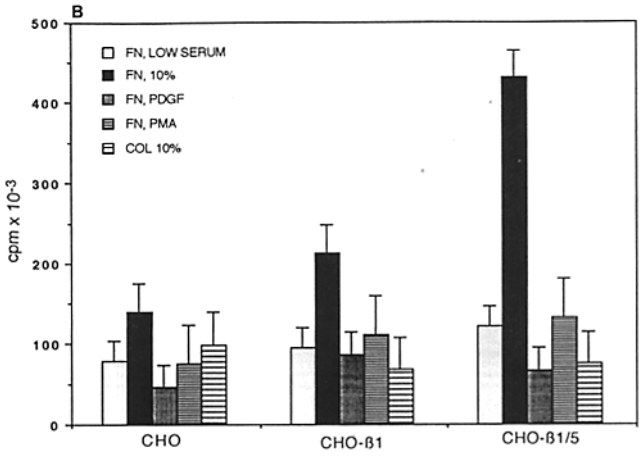
Figure 7. Induction of phosphorylation of a 90-kD protein in β_1-CHO cells. β_1-CHO or $\beta_{1/5}$-CHO cells were plated on fibronectin (coated at 10 μg/ml) for 3 h, in the presence of 1 μg/ml TS2/16, and 1 mCi [^{32}P]orthophosphate (200 μCi/ml, carrier free; New England Nuclear) per 100-mm dish in phosphate-free DME medium. Cell extracts were prepared in the presence of 200 mM sodium vanadate and fractionated by SDS-PAGE, as shown in the left panel. Also, the lanes were scanned using a densitometer as shown in the right panel.

Table III. Correlation between p90 Phosphorylation and β_1- CHO Proliferation

Growth Condition	Cells	p90/p95 ratio	Proliferation*
1% S	β_1-CHO	0.08	−
	$\beta_{1/5}$-CHO	0.11	−
1% S; TS2/16	β_1-CHO	0.19	−
	$\beta_{1/5}$-CHO	0.14	−
1% S; TS2/16; FN	β_1-CHO	0.65	+
	$\beta_{1/5}$-CHO	0.12	−
1% S; 4B4; FN	β_1-CHO	0.08	−
	$\beta_{1/5}$-CHO	0.12	−
10% S	β_1-CHO	0.72	+
	$\beta_{1/5}$-CHO	0.68	+

* Proliferation of β_1-CHO and $\beta_{1/5}$-CHO cells was determined as described in the legend for Fig. 6. +, > 10-fold greater β_1 proliferation compared to $\beta_{1/5}$ after subtraction of CHO-MTX background; −, less than twofold greater proliferation for β_1-CHO cells.
‡ Ratios of the levels of phosphorylated p90 relative to p95 were based on peak areas obtained from gel scans such as shown in Fig. 7.





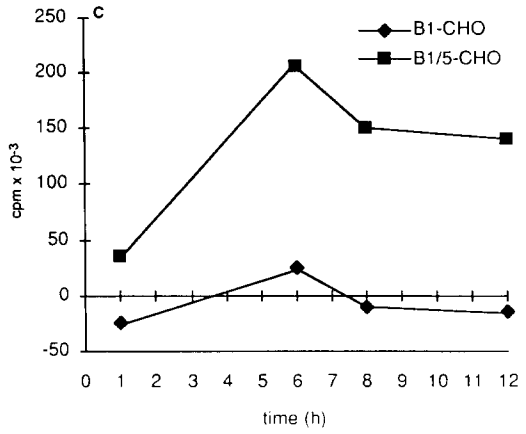


Figure 8. Haptotactic migration of CHO, β_1-CHO and $\beta_{1/5}$-CHO cells. As described in Materials and Methods, [^{35}S]methionine-labeled cells were placed into the upper compartment of a modified Boyden chamber in the presence of mAb PB1 (to block hamster $\alpha^5\beta_1$ function). (*A*) After 6 h, all radioactivity on the upper surface of the membrane was removed by extensive washing, and then radioactive cells that had traversed the membrane towards the bottom surface were quantitated. The bottom surface was coated with fibronectin, vitronectin, or pronectin (or nothing), and in two experiments, 1 μg/ml of TS2/16 was also included. (*B*) Migration towards fibronectin was carried out in the presence of 0.1% FCS, 10% FCS, 0.1% FCS plus 100 nM PMA, or 0.1% FCS plus 40 ng/ml PDGF (Promega Biotec) as indicated. In one experiment, the bottom surface was coated with collagen and migration was carried out in 10% FCS. (*C*) Migration towards fibronectin, in the presence of TS2/16, was determined at the indicated time points using conditions described in (*A*). Background migration displayed by CHO-MTX control cells was subtracted. These background values were 65,000, 58,000, 48,000, and 129,000 cpm at 1, 6, 8, and 12 h, respectively.

β_1-CHO cells showed only background cell motility towards fibronectin, vitronectin, pronectin, and towards surfaces not specifically coated with any matrix protein. Because the migration of β_1-CHO cells was not significantly elevated or depressed compared to untransfected or mock-transfected CHO cells (Fig. 8 *A*), human β_1 did not appear to exert either a positive or negative effect on CHO cell migration. In contrast, $\beta_{1/5}$-CHO cells displayed markedly increased migration toward a fibronectin source (Fig. 8 *A*), and even a higher level of migration in the presence of the anti-β_1 stimulating antibody TS2/16. The presence of TS2/16 alone had no effect on cell migration if ECM protein was omitted. In separate experiments, cells crossing the membrane towards the lower compartment were counted by microscopic examination, yielding data comparable to that obtained using ^{35}S-labeled cells (not shown). The difference in migration between β_1-CHO and $\beta_{1/5}$-CHO cells was maintained for ≥12 h (Fig. 8 *C*). These results point to a pronounced difference in the contribution of the β_5 and β_1 cytoplasmic domains towards cell migration.

Analysis of additional experimental conditions revealed that migration of $\beta_{1/5}$-CHO cells was markedly elevated in the presence of 10% serum, compared to 0.1% serum (Fig. 8 *B*). This serum effect could not be mimicked by adding PMA or PDGF. Also, the presence of 10% serum did not support nonspecific migration when a control ligand (collagen) was used.

Discussion

The central finding in this paper is that the β_1 and β_5 cytoplasmic domains were equally able to support cell adhesion, but showed marked differences in multiple postligand binding functions. Whereas the β_1 cytoplasmic domain was better suited for localizing into focal adhesion-like structures, and selectively contributed to cell proliferation (associated with p90 phosphorylation), the β_5 cytoplasmic domain facilitated migration of CHO cells on fibronectin.

Differences in Subcellular Localization

Previously, it was found that $\alpha^v\beta_5$ and $\alpha^v\beta_3$ had different subcellular distribution patterns in cells spread on vitronectin (82), but it was not shown that the β chain cytoplasmic domains were responsible. Here, we provide explicit evidence that replacement of only the cytoplasmic domain of

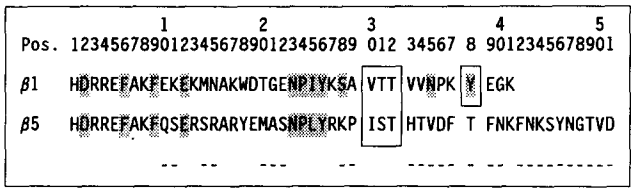


Figure 9. Comparison of the human β_5 (51, 61, 77) and β_1 (2) cytoplasmic domains. Residues important for focal adhesion formation are shaded (64), residues of likely importance for adhesion regulation are boxed (31), and nonconservative differences between β_1 and β_5 are indicated by the dashes below.

β_1 with that of β_5 was sufficient to alter dramatically the subcellular distribution of the integrin towards a more diffuse state.

Approximately 10 key residues within the β_1 cytoplasmic domain have been identified that determine whether β_1 will localize to focal adhesions or will be distributed more diffusely (64). Three of these residues in β_1 (Fig. 9, positions 28, 35, and 38) are missing in β_5, thus perhaps explaining why β_5 fails to localize to focal adhesions. Related to this, the same three β_1 residues (positions 28, 35, and 38) occur within a potential α-actinin binding region (57) that may be important for focal adhesion formation. Attention is now also focused on the 10 additional residues that make β_5 longer than β_1 (Fig. 9), which could potentially exert a negative effect.

Similarities in Cell Adhesion

The β_1 and $\beta_{1/5}$ subunits made virtually identical contributions towards CHO cell adhesion regardless of fibronectin levels or divalent cation levels, and both were equally responsive to stimulation by an anti-β_1 activating antibody (TS2/16). Because of these nearly identical cell adhesion results, we consider it highly likely that the differences we observed in other functions were not simply caused by a gross alteration of the overall integrin structure. Even more importantly, the inside-out signaling mechanisms regulating the constitutive adhesive activity of β_1 integrin in CHO cells appear to be independent of the mechanisms regulating subcellular localization, cell migration, or cell proliferation. This leads to an important qualitative distinction between the type of integrin clustering that may facilitate cell adhesion in a short-term assay of 0.5–1 h (30) and the integrin clustering that is visible in focal adhesions after 6–12 h.

Key residues within the β_2 (31) and β_3 (12) cytoplasmic domains have been identified that are involved in the regulation of integrin-ligand adhesive interactions. For the most part, those residues are conserved in both β_1 and β_5 (Fig. 9, positions 30–32, 38, 39) if you allow the phenylalanine at position 39 in β_5 to substitute for the Tyr at position 38 in β_1. Thus, a common mechanism for controlling cell adhesion/ligand binding appears to be shared by multiple integrin β subunit tails, even though they may differ markedly in other functional respects.

Differences in Cell Proliferation

A large body of literature (reviewed in references 33 and 46) has addressed the effects of fibronectin and other ECM proteins on cell proliferation. These ECM proteins act at least partially through integrins, because many integrin ligands or anti-integrin antibodies had costimulatory effects on cell proliferation (15, 50, 55, 66, 73, 85). Although a few studies have suggested that mitogenesis can be achieved directly through integrins (53, 78, 80), most studies have failed to sort out direct proliferative signals from other events such as integrin-mediated cell adhesion, shape change, and potentiation of other growth triggering agents. Our results now provide a clear demonstration that the β_1 and β_5 cytoplasmic domains differ markedly in their ability to influence cell proliferation, even though they support cell adhesion to a similar extent. We hypothesize that the ability of β_1 to be recruited more readily into macromolecular subcellular structures could facilitate signaling required for cell proliferation. Conversely, the more diffuse localization of the β_5 cytoplasmic domain might prevent molecular associations needed for proliferation. In this regard, subcellular structures such as focal adhesions are increasingly seen as sites of signaling, where relevant cytoskeletal proteins and other proteins may become exposed to kinases and/or phosphatases (24, 34, 38, 48).

We consider it unlikely that the observed proliferation differences are caused by an alteration in the pattern of associated α chains between β_1 and $\beta_{1/5}$ because (*a*) the major α subunit associated with β_1 on CHO cells (i.e., α^5) was expressed at identical levels in β_1- and $\beta_{1/5}$-CHO cells; (*b*) both β_1 and $\beta_{1/5}$ yielded identical immunoprecipitation patterns; and (*c*) there was no evidence for other β_1-associated α chains, based on negative collagen and laminin adhesion assays and immunoprecipitation results. Furthermore, all available evidence indicates that integrin $\alpha\beta$ pairing specificity is determined by extracellular (and perhaps also transmembrane), but not cytoplasmic domains (5, 6, 14, 20, 56, 74).

Because both TS2/16 and intact fibronectin together were required to trigger proliferation of β_1-CHO cells, we hypothesize that TS2/16 strengthens the affinity of $\alpha^5\beta_1$ for fibronectin (as previously described [3, 8, 19, 81]) thus allowing the integrin, along with proteins interacting with other domains in the intact fibronectin, to be gathered into the appropriate membrane-spanning signaling complex. Alternatively, the anti-β_1 antibody itself could be sending a costimulatory signal that acts synergistically with intact fibronectin, but not fibronectin fragments or peptides.

In the many previous studies showing cell proliferation as a result of costimulatory signals acting through β_1 integrins (10, 15, 50, 55, 73, 85), it was often not clear whether the contribution of the β_1 integrin went beyond simply providing cell adhesion. Our results now indicate clearly that adhesive capability alone is not sufficient (such as in $\beta_{1/5}$-CHO cells), and that the β_1 cytoplasmic domain is serving additional signaling and/or localization functions required for cell proliferation.

The nearly all-or-none appearance of phosphorylated p90 in response to fibronectin plus TS2/16 showed a strong correlation with enhanced cell proliferation. At the present time, we cannot determine whether this event is an essential part of the downstream signaling pathway leading to proliferation or part of a divergent pathway. We suspect that p90 is phosphorylated on serine or threonine because it was readily detected at a high level in a whole cell extract, and it was not

seen with antiphosphotyrosine antibody. Among known proteins of ~90 kD, such as HSP-90 (84), PKC-ϵ (67), and pp90^{rsk} (11), that are phosphorylated on serine or threonine, pp90^{rsk} has the most obvious association with cell proliferation (11). However, we found no difference in the level of phosphorylated pp90^{rsk} immunoprecipitated from β_1-CHO and $\beta_{1/5}$-CHO cells, regardless of the stimulation of those cells with fibronectin and TS2/16 (not shown). Thus at present, the identity of our p90 protein is unknown. Nonetheless, its phosphorylation is closely correlated with cell proliferation, and it provides an independent assay indicating that the β_1 tail has a unique signaling function not seen with the β_5 tail.

Differences in Cell Migration

We hypothesize that differences between the β_1 and β_5 cytoplasmic domains with respect to subcellular localization are likely to be closely associated with differences in cell migration. For example, the β_5 cytoplasmic domain, by promoting more diffuse subcellular localization, could thus facilitate cell migration. In this regard, others have also noted an inverse correlation between focal adhesion formation and cell motility (1, 17, 63). Although $\alpha^5\beta_1$ can have a negative effect on random cell migration when overexpressed in CHO cells (22), our β_1-CHO cells did not migrate any less than untransfected or mock-transfected CHO cells in a haptotactic migration assay. Thus, rather than the β_1 tail having a negative effect, the β_5 cytoplasmic domain seems to be playing a positive role.

In the complex multistep process of cell migration, we propose that β_1 and $\beta_{1/5}$ may not differ in the initial adhesive steps that are required, but rather, they may show critical differences in a later step involving integrin-cytoskeletal interactions at the leading edge of the cell (68). Alternatively, considering that the $\beta_{1/5}$-CHO cells were more migratory than β_1-CHO cells only when serum was present, the β_5 and β_1 cytoplasmic domains may differ in their abilities to translate signals resulting from serum stimulation into a migratory phenotype. Another possibility is that processes such as phosphatase-dependent deadhesion, also known to influence cell motility (29), could differentially act on β_1 and β_5 cytoplasmic domains.

While the precise biochemical mechanism by which the β_5 cytoplasmic tail facilitates migration remains to be determined, we do not wish to imply that the β_5 tail is never able to form focal adhesion-like structures during cell migration, especially since the transient occurrence of such structures may be an essential step in cell migration (62). Rather, we simply propose that the β_5 tail has properties that make it averse to localization into focal adhesions in highly spread cells, but at the same time, contribute to enhanced cell migration.

The independent regulation of cell adhesion and migration has also been observed in studies involving integrin α chain cytoplasmic domains. Whereas the α^2, α^4, and α^5 cytoplasmic domains contributed similarly to cell adhesion (9, 39, 41), the α^4 cytoplasmic domain was more effective at supporting migration (9).

In another study, β_3 but not β_5 supported cell migration, even though the latter was more diffusely distributed (44), thus contrasting with the results shown here. Possibly β_5 did not support migration in the previous study because serum was not present, a different cell type was used (a pancreatic carcinoma) or a different α chain (α^v) was involved (44).

In conclusion, our results support a general scheme in which different integrins use commonly shared mechanisms to control cell adhesion (inside-out signaling), but translate adhesive information into sharply contrasting subsequent events (outside-in signaling), depending on the unique feature of their different cytoplasmic domains. Thus, we predict that other β subunits with even more divergent cytodomains (β_6, β_8, and β_4) will differ from β_1 and β_5 in several of the same parameters examined here. In addition, our results begin to suggest how subcellular localization of integrins could exert control over diverse functions such as cell proliferation and cell migration. The challenge in the future will be to identify specific biochemical associations for β_1 and β_5 cytoplasmic domains that are relevant to their specific functions.

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