Abstract. The purpose of this study was to explore the functional role of the cytoplasmic domain of the α5 subunit of the α5/β1 integrin, a fibronectin receptor. Mutant CHO cells that express very low levels of endogenous hamster α5 subunit (CHO clone B2) were transfected with an expression vector containing full-length or truncated human α5 cDNAs to form chimeric human α5/hamster β1 integrins. Three transfec-
tants were examined: B2a27 expresses a full-length human α5 subunit with 27 amino acids in the cyto-
plasmic domain; B2a10 expresses an α5 with a 17-
amino acid cytoplasmic truncation; B2a1 expresses an
α5 with a 26-amino acid truncation. Levels of α5/β1
surface expression in B2a27 and B2a10 cells were
similar to that in wild type CHO cells. The expression
of α5/β1 in B2a1 cells was less, amounting to
15-20% of WT levels, despite message levels that
were three to five times greater than those of B2a27.
The transfectants were used to examine the role of the
α5 cytoplasmic domain in cell adhesion, cell motility,
cytoskeletal organization, and integrin-mediated tyro-
sine phosphorylation.

The adhesion characteristics of B2a27 and B2a10
cells on fibronectin substrata were similar to each
other and to wild type CHO cells. B2a1 cells dis-
played slight reductions in the strength and rate of
adhesion to fibronectin. Cell motility in the presence
of fibronectin was similar for B2a27, B2a10, and wild
type CHO cells on fibronectin substrata. The B2a1 cells spread to a
lesser degree, and some organization of actin was ob-
served; the untransfected B2 cells remained round on
fibronectin substrata and showed no actin reorganiza-
tion. Since the reduced motility and cell spreading ob-
served in the B2a1 cells might be due either to re-
duced surface expression of α5/β1 or to the truncation
in the α5 cytoplasmic domain, we used flow cytomet-
ric cell sorting to select populations of B2a1 and
B2a27 cells expressing similar levels of cell surface
α5. The deficits in spreading and motility were present
in B2a1 cells expressing high levels of α5. Thus
the region of the α5 cytoplasmic domain adjacent to the
membrane seems to play an important role in
cytoskeletal organization and cell motility. We also ex-
amined whether α subunit truncation would affect integri-
mediated tyrosine phosphorylation. When
B2a27 cells interacted with fibronectin substrata, in-
creased tyrosine phosphorylation was observed in pro-
teins of ~125 kD. A similar pattern of phosphoryla-
tion was observed in wild type CHO, B2a10, and B2a1
cells, but not in B2 cells. Thus, the α5 cytoplasmic
domain does not seem to be essential for integri-
mediated tyrosine phosphorylation of intracellular pro-
teins.

Cell interactions with the extracellular matrix are a
vital aspect of embryogenesis, angiogenesis, the in-
flammatory response, tissue differentiation, and
hemostasis. These cellular processes are mediated in part by
the integrin family of cell surface glycoproteins (Buck and
Horwitz, 1987; Hynes, 1987; Juliano, 1987). Integrins are
formed by the noncovalent association of an α subunit with
a β subunit; to date, at least 14 α and 8 β subunits have been
described giving rise to at least 20 distinct integrin receptors
(Dedhar, 1990; Rouslahti, 1991; Hynes, 1992). The largest
subgrouping of integrin receptors are those that share the β1
subunit, including receptors for fibronectin, vitronectin,
 laminin, and collagen (Guan and Hynes, 1990; Sonnenberg
et al., 1988; Santoro et al., 1988; Takada, 1987; Elices et
al., 1991). Both the β1 subunit and its α partners have large
extracellular domains, transmembrane-spanning regions,
and short cytoplasmic domains. The extracellular domains
of integrin α and β subunits cooperate to bind matrix pro-
teins, while the cytoplasmic domains articulate with a num-
ber of cytoskeletal elements, including talin, vinculin, and
α actinin (Burridge et al., 1988; Otey et al., 1990).

Much of the research on integrins to date has focused on
the extracellular interactions of integrins with their ligands,
while the role of cytoplasmic domains of integrins has only
been explored to a limited degree. In an early study, Horwitz
et al. (1986) demonstrated the association of the cytoplasmic
domain of the β1 subunit with talin, while more recently an interaction between the β1 subunit and α actinin has been found (Otey et al., 1990). Using murine cells expressing avian β1 constructs, Hayashi et al. (1990) and Marcantonio et al. (1990), independently demonstrated that truncating the cytoplasmic domain by five or more amino acids resulted in partial or complete loss of β1 integrin localization in focal contacts, while Solowska et al. (1989) showed that truncation of the β1 cytoplasmic domain does not prevent the in vitro binding between fibronectin and chimeric fibronectin receptor. Hibbs et al. (1991) expressed various constructs of the LFA-1 receptor (a member of the β2 subclass of integrin receptors) in COS cells, and demonstrated that truncation of the carboxy terminus of the β subunit diminished phosphor ester stimulation of LFA-1 binding to ICAM. The importance of the cytoplasmic domain of integrin β subunits has also been demonstrated in vivo; antibodies against the cytoplasmic domain of the β1 subunit injected intracellularly into developing amphibian embryos prevented extracellular fibronectin fibril formation and delayed embryo development (Darribère et al., 1990).

It is apparent from the studies cited above that the cytoplasmic domain of the β subunit contributes significantly to integrin function. Less information, however, is available on the participation of the cytoplasmic domain of the α subunit. Hibbs et al. (1991) demonstrated that partially truncating the cytoplasmic domain of the α subunit of LFA-1 had no effect on receptor function. Truncating the entire cytoplasmic domain of the α subunit, however, did inhibit expression of the receptor on the cell surface. O'Toole et al. (1991) produced various constructs of the platelet glycoprotein αIIb/33, an integrin receptor that requires activation before binding; these were expressed in CHO cells. Interestingly, truncating the entire cytoplasmic domain of the α subunit of the platelet glycoprotein αIIb/33 yielded a functional receptor that bound fibrinogen without activation. Recently, Chan et al. (1992) have explored the role of the α subunit cytoplasmic domain by preparing a series of chimeras having the α2 external and transmembrane domains coupled to the cytoplasmic domains of α2, α4, or α5. They found that the presence of the α4 cytoplasmic domain contributed to cell motility while the α2 or α5 cytoplasmic domains permitted collagen gel contraction.

It seems clear that cytoplasmic domains play an important role in integrin interactions with cytoskeletal proteins and in cellular activation of integrins. Recently, however, another potential role has been identified. Studies of human carcinoma cells (Kornberg et al., 1991), rodent fibroblasts (Guan and Hynes, 1991), and platelets (Golden et al., 1991) all suggest that integrins can mediate a signal transduction process resulting in phosphorylation of tyrosine residues on intracellular proteins. Integrin-mediated tyrosine phosphorylation seems to involve a novel 125-kD cytoplasmic tyrosine kinase (Kanner et al., 1990; Shailer et al., 1992; Hanks et al., 1992). Early evidence suggests that integrin β subunit cytoplasmic domains may be required in this process (Guan and Hynes, 1991). Whether integrin-mediated tyrosine phosphorylation relates to other aspects of adhesion-related signal transduction such as de novo gene expression (Haskell et al., 1988; Werb et al., 1989) remains to be determined.

To address the role of the α5 cytoplasmic domain in fibronectin receptor (FnR) function, we have used an existing human α5 cDNA expression vector (Giancotti and Ruoslahti, 1990), to generate two constructs that give rise to α5 subunits with truncated cytoplasmic domains. One construct yields a protein with 17 amino acids deleted from the α5 cytoplasmic domain; the other essentially eliminates the entire cytoplasmic domain. These cDNAs were transfected into CHO cell α5/β1 FnR variants developed previously in our laboratory that fail to express significant levels of endogenous hamster α5 subunit (Schreiner et al., 1989). These FnR-deficient variants provide an elegant system to explore the contributions of normal or mutated truncated α5 subunits to α5/β1 FnR function.

Materials and Methods

Cell Culture

Wild type CHO cells, CHO 2% FnR variants (B2), and CHO 20% FnR variants (1-23), as well as the transfectants, were grown and maintained as previously described (Schreiner et al., 1989; Bauer et al., 1992). Cells were either grown as monolayers in α-MEM (GIBCO BRL, Gaithersburg, MD) supplemented with 10% FBS (Irvine Scientific, Santa Ana, CA) and 1% anti-biotic-antimycotic mixture (GIBCO BRL) or in suspension culture where the FBS was reduced to 5%. Cells grown in monolayer were routinely passaged with trypsin-EDTA (GIBCO BRL) and cell number was determined with an ElectroZone cellscopse (Particle Data, Inc., Elmhurst, IL).

Extracellular Matrix Proteins

Fibronectin was prepared from human plasma as described (Schwarz et al., 1984). Vitronectin was also prepared from human plasma using a modification (Danilov and Juliano, 1989) of a previously published procedure (Ruoslahti et al., 1987).

Vector Construction

A eukaryotic expression vector containing a full-length human cDNA insert (pECE-α5) was a gift from Dr. E. Ruoslahti (La Jolla Cancer Research Foundation, La Jolla, CA) (Giancotti and Ruoslahti, 1990). The pECE-α5/10 construct was generated by first digesting the original vector with restriction enzymes Ndel and Xbal, filling in the ends with the Klenow fragment of DNA polymerase 1, and religating the shortened vector. The pECE-α5/1 construct was generated by excising the section of DNA in the α5 insert within the HindIII and Xbal restriction sites. The pECE expression vector, however, also contained a HindIII site. Therefore, pECE-α5 was first partially digested with HindIII, filled in with the Klenow fragment of DNA polymerase 1, and blunt-end ligated (clones containing only one HindIII site within the α5 sequence were identified by restriction screening). The modified vector was then further digested with HindIII and Xbal. The ends of the shortened vector were filled in with the Klenow fragment of DNA polymerase I and religated. Both constructs were ~0.3-kb shorter than the intact construct (3.6 kb). Each construct was verified by didexoy sequencing using a 15-base primer 35 bases upstream of the truncated region. The pECE-α5/10 and pECE-α5/1 constructs gave rise to truncated α5 subunits expressing either a 10- or 1-amino acid cytoplasmic domain, respectively.

Transfection of FnR Deficient Variants

A FnR variant clone (B2) was chosen for expressing the α5 constructs; this clone expresses <2% of wild type levels of endogenous hamster α5/β1 (Schreiner et al., 1989). Cells were transfected with pECE-α5, pECE-α5/10, or pECE-α5/1. Cells were cotransfected with a neo-resistance plasmid (pSGNeo) for selection purposes. B2 cells were grown in monolayer, harvested with trypsin-EDTA and washed twice in ice cold electroporation media (PBS). The cells were then resuspended at a density of 1 × 107 cells/ml. Cells were then added to 0.4-cm electroporation Gene Pulser cu-

1. Abbreviations used in this paper: FnR, fibronectin receptor; RCF, relative centrifugal forces.

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vettes (Bio-Rad, Richmond, CA) and 20 µg of pECE-α5 construct vectors and 2 µg of pSVNeo were added to the cuvettes and incubated with the cells on ice for 10 min before electroporation. All plasmids were linearized by digestion with PvuI restriction enzyme before addition to the cells to facilitate insertion of the plasmids into host chromosomal DNA. Cells were pulsed using voltages between 200 and 800 V with a constant capacitance of 25 µF. Cells were selected with G418 (1,000 µg/ml) and isolated using cloning rings. After cloning, transfectants were maintained in normally supplemented α-MEM containing 200 µg/ml G418. Cells were screened for expression of the chimeric human α5/hamster β1 FnR by ELISA and by flow cytometry using the BIE5 anti-human α5 antibody (generous gift of Dr. C. Dansky, University of California, San Francisco, San Francisco, CA) (Werb et al., 1989).

**Integrin Analysis by Flow Cytometry**

The expression of α5/β1 FnR, α5 subunit, VnR, and the integrin β1 subunit was evaluated by indirect immunofluorescence using flow cytometric techniques similar to those previously (Schreiner et al., 1989). PBI (a mouse monoclonal anti-FnR antibody specific for the intact CHO α5/β1 FnR), 7E2 (a mouse mAb specific for the CHO β1 integrin subunit), BIE5 (a rat monoclonal anti-human α5 antibody), and rabbit polyclonal anti-VnR antibody (Suzuki et al., 1986) were used as primary antibodies, and FITC-conjugated IgG (anti-mouse, -rat, or -rabbit) was used as a secondary reagent. The PBI and 7E2 monoclonals have been fully described elsewhere (Brown and Juliano, 1985, 1988). Background staining was assessed by omitting the primary antibody. The FnR-deficient cells were also screened by an ELISA technique as described (Schreiner et al., 1989) to confirm the phenotype. The FnR-deficient variants were periodically screened thereafter by ELISA using PBI and BIE5 to ensure stability of the variant and mutant phenotypes.

**Adhesion Assays**

Adhesion assays using radiolabeled cells were conducted in tissue culture plates (48 well; Costar Corp., Cambridge, MA) coated with various amounts of fibronectin and subsequently blocked with bovine albumin. The assay procedure has been described in detail previously (Bauer et al., 1992; Schreiner et al., 1989). Adhesion assays were also conducted by reverse centrifugation to gauge the strength of adhesion of the modified receptors. These experiments were performed essentially as described by Lutz et al. (1989). Briefly, 96-well microtiter plates (3912 Microtiter Test II; Falcon Plastics, Cocheysville, MD) were coated as described above with 1 µg/ml Fn. Wells coated only with 3% BSA were used to determine background adhesion. Metabolically labeled cells (4 × 10^5) were added to the plates and the cells were then centrifuged onto the immobilized Fn substratum using a Beckman GP centrifuge at 17 g for 10 min at 4°C. The plates then were incubated at 37°C for 30 min while the cells were allowed to attach. The plates were then sealed with Costar plate sealers, inverted, and centrifuged at varying speeds corresponding to relative centrifugal forces (RCF) of 10, 50, 100, 500, and 1,000 g for 10 min. After centrifugation, the inverted plates were quick-frozen at ~70°C. The bottoms of the plate wells were clipped and the radioactivity of the remaining cells was quantitated. Background adhesion of cells to BSA was subtracted from specific adhesion of cells to Fn. Assays were performed in quadruplicate.

**Motility Assays**

Cell motility experiments were performed essentially as described by Bauer et al. (1992) using Transwell (Costar Corp.) motility chambers. Various concentrations of fibronectin were coated on to the lower side of the Transwell filter insert before the start of the assay. In one case a “wounding” type motility assay was used. Cell layers in 24-well tissue culture plates were scraped with a sharp plastic blade, thus demarcating a line where cells were removed. The migration of cells across the line and into the denuded area was observed as a function of incubation time at 37°C using an inverted phase microscope with a gridline eyepiece.

**Immunofluorescent Staining**

Cultured cells were harvested by adding 0.05% trypsin and 1 mM EDTA for 5 min at room temperature. The trypsin was neutralized by washing the harvested cells three times in serum-free α-MEM containing 1% BSA and 1% penicillin/streptomycin. The cells were resuspended in this same medium and counted. Cells (1 × 10^6) were seeded onto 22 mm^2 microscope coverslips that were pre-coated with 100 µg/ml Fn. Cells were allowed to attach for 4 h at 37°C, and then washed in universal buffer (UB; 150 mM NaCl, 50 mM Tris-HCl, pH 7.6, 0.1% NaN3) and fixed in 3.7% formaldehyde. The cells were further washed in UB and permeabilized with 0.4% Triton X-100. The cells were stained with rhodamine-labeled phalloidin (specific for F-actin; Barak et al., 1980) (Molecular Probes, Inc., Junction City, OR) for 45 min at 37°C. Cells were again washed with UB, mounted onto slides with Gelvatol® and visualized using a Zeiss Axiopt microscope (Carl Zeiss, Oberkochen, Germany).

**Immunoprecipitation**

Equal numbers (1 × 10^7) of cells were surface labeled in 1 ml PBS by addition of lactoperoxidase (100 µg), 1 mM NaC1, and 10-µl aliquots of 0.03% H2O2 added each 3 min for 20 min at room temperature. Cells were washed 4× in PBS and lysed in 1 ml of 2% NP-40, 150 mM NaCl, 50 mM Tris, 1 mM MgCl2, 0.01% aprotinin, 0.01% soybean trypsin inhibitor (pH 8.0) for 30 min on ice. Insoluble material was removed by centrifugation and lysates were stored at ~80°C. 50 µl of each lysate was pre-cleared with 30 µl of a 50% suspension of protein A-conjugated Sepharose beads and 10 µl goat anti-mouse or goat anti-rat IgG for 2 h on ice. Aliquots (10 µl) of either PBI (monoclonal mouse anti-hamster FnR antibody) or of BIE5 (monoclonal antibody against human α5 subunit antibody) were added to the pre-cleared lysates and allowed to incubate overnight on ice. The immune complexes were precipitated by addition of either 10 µl goat anti-mouse or 10 µl goat anti-rat IgG and 30 µl of 50% protein A-Sepharose beads to 300 µl lysate. The precipitates were washed 3× in a buffer containing 500 mM NaCl, 50 mM Tris, pH 8.0, 10 mM EDTA, 1 mg/ml BSA, 0.025% NaN3, and 0.5% NP-40. The precipitates were then washed 2× in a buffer containing 150 mM NaCl, 50 mM Tris, pH 8.0, 10 mM EDTA, 1 mg/ml BSA, 0.025% NaN3, and 0.5% NP-40. The precipitates were eluted by boiling 3 min in SDS sample buffer and analyzed by PAGE under nonreducing conditions. Immunoprecipitations were visualized by autoradiography using X-Omat AR film (Eastman Kodak Co., Rochester, NY). Comparisons of band intensities were made by densitometry; molecular weights of α5 subunits were determined by comparing the relative migrations of the α5 bands on the gel to the relative migrations of the molecular weight markers. Immunoprecipitations were also performed using a rabbit polyclonal anti-human α5 cytoplasmic domain antibody, graciously provided by Dr. R. O. Hynes, Massachusetts Institute of Technology, Cambridge, MA (Hynes et al., 1989). Antibody 161 cross-reacts with mouse, rat, chicken, and hamster α5 cytoplasmic domains.

**Analysis of Protein Tyrosine Phosphorylation**

Changes in the levels of phosphoryseryl residues subsequent to interactions of α5/β1 with its ligand were assessed by using Western blots probed with anti-phosphotyrosine antibody (Kornberg et al., 1991). CHO cells were dispersed using trypsin-EDTA and washed extensively with 1% BSA in α-MEM; cells were maintained in suspension and allowed to recover from trypsinization for 2 h before plating. Cells were then plated on 60-mm tissue culture plates previously coated with 10 µg/ml fibronectin; the cells were incubated for 90 min at 37°C. Control cells were maintained in suspension throughout this period. After the incubation, the cells were washed in PBS and lysed in ice-cold RIPA buffer (50 mM Tris-C1, pH 7.4, 150 mM NaCl, 1% deoxycholate, 1% Triton X-100, 0.1% SDS, 2 mM EDTA, 0.1% aprotinin, 1 mM PMSF, 100 µg/ml NaN3). The extracts were incubated with 20-µl packed protein A-Sepharose and then centrifuged at 10,000 g for 10 min. Total protein in the supernatant was measured using the BCA assay (Pierce Chemical Co., Rockford, IL). Equal amounts of protein were electrophoresed under reducing conditions on 8% denaturing polyacrylamide gels (Laemmli, 1970). The resolved proteins were transferred to nitrocellulose as described (McCune and Earp, 1989) and the blots were pre-blocked by overnight incubation in 3% BSA. Phosphotyrosyl-containing proteins were detected using the anti-phosphotyrosine antibody P766 (Signa Immunoclonicals, St. Louis, MO). Blots were incubated for 3–4 h in the presence of a 1:2,000 dilution of P766 and then washed; the blots were then incubated for 1 h in the presence of 2 µg/ml rabbit anti–mouse IgG and then re-washed. Finally, the blots were incubated with 2 µCi/10 ml 125I-protein A (New England Nuclear, Boston, MA) and washed extensively. Radioactive bands were detected by autoradiography; film exposures using Kodak...
Evaluation of Cells Expressing Similar Levels of Normal or Truncated \( \alpha_5 \)

\( \alpha \)-cells or \( \alpha_2/a_27 \) cells were labeled with B1E5 monoclonal followed by fluorescein anti-\( \alpha \)-rat polyclonal, as described above. The \( \alpha_2/a_27 \) population was analyzed for \( \alpha_5 \) expression by flow cytometry, and gates were chosen to include the main peak of fluorescence intensity. The labeled \( \alpha_2/a_27 \) cells were sorted using the gates set from the \( \alpha_2/a_27 \) population. A small population of high \( \alpha_5 \) expressors was selected from the \( \alpha_2/a_27 \) population (this population is termed \( \alpha_2/a_27H \)); the residual \( \alpha_2/a_27 \) cells comprised a low expressing population (termed \( \alpha_2/al \)). Cell populations were collected in \( \alpha \)-MEM plus 1% BSA and were maintained at 4°C. Light scatter characteristics were used to discriminate dead cells and to remove them from the sort. After the sorting runs, which lasted \( \sim 3 \) h, the level of \( \alpha_5 \) expression in the positively sorted \( \alpha_2/a_27 \) population (\( \alpha_2/a_27H \)) was analyzed and compared to the \( \alpha_2/a_27 \) population. The labeled, sorted, \( \alpha_2/a_27 \) and \( \alpha_2/al \) populations were briefly treated with trypsin-EDTA, rinsed in serum-containing medium, and then placed in suspension culture for 1 h at 37°C to regenerate cell surface integrins (Szekan and Juliano, 1990). Thereafter, the cells were suspended in \( \alpha \)-MEM plus 1% bovine albumin and plated on to 24-well tissue culture plates that had previously been coated with 10 \( \mu \)g/ml of fibronectin. Cell morphology was observed and photographed using a phase contrast microscope after 2 or 24 h of incubation at 37°C. Alternatively, cell motility was evaluated using a cell layer "wounding" assay, as described above.

Results

Preparation of Integrin \( \alpha_5 \) Subunits with Truncated Cytoplasmic Domains

Vectors capable of expressing truncated \( \alpha_5 \) subunits were constructed by deleting segments of DNA that corresponded to specific regions of the cytoplasmic domain from an \( \alpha_5 \) containing expression vector (pECE-\( \alpha_5 \)). The new cDNA constructs were sequenced through the altered regions by Sanger's dideoxy chain termination technique (data not shown). Translation of the truncated \( \alpha_5 \) constructs was terminated by stop codons present within the vector. The intact \( \alpha_5 \) contains a 27 amino acid cytoplasmic domain. Two constructs were generated; pECE-\( \alpha_5/10 \), which encodes an \( \alpha_5 \) subunit with the cytoplasmic domain truncated by 17 amino acids; pECE-\( \alpha_5/11 \), which encodes an \( \alpha_5 \) subunit that has 26 of the 27 amino acids of the cytoplasmic domain deleted (Fig. 1). Both pECE-\( \alpha_5 \) and the mutated \( \alpha_5 \) vector constructs were transfected into a CHO FnR variant (the B2 clonal cell line; originally described by Schreiner et al., 1989) that expresses very low levels of the endogenous hamster \( \alpha_5 \) subunit. The transfection yielded chimeric fibronectin receptors; i.e., human \( \alpha_5 \) associating with hamster \( \beta_1 \).

Expression of the Human \( \alpha_5/\)Hamster \( \beta_1 \) Constructs

Several cell lines that expressed either pECE-\( \alpha_5 \) or one of the truncated \( \alpha_5 \) constructs were screened by ELISA using the B1E5 anti-\( \alpha_5 \) subunit antibody (data not shown). The cell lines that expressed the greatest amounts of \( \alpha_5 \) subunit were chosen for study. The selected cell lines were \( B_2/a_27 \), \( B_2/a_10 \), and \( B_2/a_1 \), corresponding to cells expressing pECE-\( \alpha_5 \), pECE-\( \alpha_5/10 \), or pECE-\( \alpha_5/11 \), respectively. ELISA analysis showed that both \( B_2/a_27 \) and \( B_2/a_10 \) cells expressed similar levels of \( \alpha_5 \) subunit on the surface, while less \( \alpha_5 \) subunit was expressed by the \( B_2/a_1 \) cells (data not shown). Expression of chimeric \( \alpha_5/\beta_1 \) FnR on the surface of the transfecants was also evaluated by flow cytometry (Fig. 2). Analysis of cells stained with PB1, an antibody which recognizes the intact \( \alpha_5/\beta_1 \) FnR (Brown and Juliano, 1985), showed that wild type, \( B_2/a_27 \), and \( B_2/a_10 \) expressed similar levels of FnR. \( B_2/a_1 \) expressed substantially less \( \alpha_5/\beta_1 \) FnR than either the other transfectants or wild type cells. B2 cells exhibited levels of hamster \( \alpha_5/\beta_1 \) FnR expression only marginally above background. When cells were analyzed using B1E5, an antibody to the \( \alpha_5 \) subunit, wild type, \( B_2/a_27 \), and \( B_2/a_10 \) were shown to express similar levels of the subunit. In agreement with the PB1 antibody data, \( B_2/a_1 \) expressed substantially less \( \alpha_5 \) subunit, \( \sim 15-20\% \) of wild type cells. B2 cells were devoid of measurable \( \alpha_5 \) subunit expression. Expression of \( \beta_1 \) subunit was tested using an anti-hamster \( \beta_1 \) mAb (7E2). As reported previously, \( \beta_1 \) subunit expression in B2 cells was substantially reduced due to lack of \( \alpha_5 \) (Schreiner et al., 1989); \( \beta_1 \) expression in \( B_2/a_27 \) and \( B_2/a_10 \) was restored to wild type levels, while \( \beta_1 \) subunit expression was only marginally increased in \( B_2/a_1 \) cells. Finally, \( \alpha_5/\beta_3 \) vitronectin receptor expression was tested using a polyclonal anti-receptor antibody; all cell lines were shown to exhibit similar levels of vitronectin receptor expression. Thus levels

**Figure 1.** \( \alpha_5 \) amino acid sequences and diagrams of truncations. Only the cytoplasmic domain amino acid sequences are depicted and these are designated by the name of the construct. Diagrams represent the entire structures of the \( \alpha_5 \) subunits and are designated by the name of the cell lines expressing the constructs.
Figure 2. FACS Histograms of FnR, β1, α5, and VnR expression on CHO wild type, B2, B2/a27, B2/al and B2/al0. Equal numbers of each cell type were washed with cold α-MEM with 1% BSA and stained with primary antibodies PB1 (anti-hamster α5/β1), 7E2 (anti-hamster β1), B1E5 (anti-human α5), or an anti-VnR antibody (right side histogram in each panel). Cells were then stained with appropriate secondary antibodies, either fluorescein-conjugated sheep anti-mouse IgG (PB1, 7E2), fluorescein-conjugated goat anti-rat IgG (B1E5), or fluorescein-conjugated goat anti-rabbit IgG (anti-VnR). Cells not treated with primary antibody and stained with fluorescein-conjugated secondary antibody were used as negative controls (left histogram in each panel). The ordinate is the number of cells per channel of fluorescence intensity with the full height of the ordinate being 512. The abscissa is the log of the fluorescence intensity in arbitrary units. A three-log scale of fluorescence intensity is indicated under the lowest panel on the left and is applicable to all the panels.

Immunoprecipitation of Expressed α5 Constructs and Evaluation of mRNA Levels

Immunoprecipitations of 125I-labeled cell-surface proteins were performed on detergent lysates prepared from equal numbers of wild type CHO cells, B2 cells, and the α5 transfectants. PB1 was used to precipitate α5/β1 FnR from wild type and B2 cells, while chimeric FnR from transfected cells was immunoprecipitated with B1E5. Different antibodies were used to ensure the highest efficiency immunoprecipitation. The precipitated FnR was analyzed on a non-reducing 4.5–8.5% gradient polyacrylamide gel to discriminate size differences between the various α5 constructs (Fig. 3 A). Immunoprecipitation of native α5/β1 FnR or chimeric FnR with either PB1 or B1E5, respectively, yielded two bands of equal intensity; the 140-kD band is the α5 subunit while the 120-kD band is the β1 subunit. Apparent molecular weights of the precipitated α and β subunits were calculated from their relative migration factors (n = 2). α5 subunits from either PB1 (wild type, B2) or B1E5 (α5 transfectants) and resolved under nonreducing conditions on a 4.5–8.5% gradient polyacrylamide gel. Equal cpm were applied to each gel lane: lane 1, wild type; lane 2, B2; lane 3, B2/a27; lane 4, B2/al; and lane 5, B2/al0. (B) FnR was immunoprecipitated from detergent lysates with anti-α5 cytoplasmic domain polyclonal antibody (161). Proteins were resolved on a 7.5% polyacrylamide gel. Lane 1, wild type; lane 2, B2; lanes 3 and 6, B2/a27 (only half as much protein was loaded in lane 6); lane 4, B2/al; lane 5, B2/al0.
Figure 4. Adhesion of α5 transfectants to Fn-coated substrata. (A) Efficiency of adhesion to Fn-coated substrata. 48-well tissue culture plates were coated with various concentrations of Fn for 2 h at 37°C in PBS, and then blocked with 3% BSA as described in Materials and Methods. Cells that were metabolically labeled overnight with 35S-Trans label were washed and resuspended at 5 × 10^5 cells/ml in adhesion buffer. Cells were added to the plates and allowed to adhere to the substrata at 37°C for 60 min. The adherent cells were recovered and analyzed for radioactivity as described in Materials and Methods. Data is presented as a percentage of the total cells added to each well. (○--○), wild type; (●--●), B2; (▲--▲), B2/a27; (●--●), B2/al; (○--○), B2/al0.

(B) Kinetics of adhesion. 48-well tissue culture plates were coated with non-limiting concentrations of fibronectin (1 μg/ml) and then blocked with 3% BSA. Metabolically labeled cells were prepared as described in Fig. 5 A, added to the Fn-coated plates and allowed to attach at 37°C for varying times up to 60 min. The adherent cells were recovered and analyzed for radioactivity as described in Materials and Methods. Data is presented as a percentage of the total cells added to each well. (○--○), wild type; (●--●), B2; (▲--▲), B2/a27; (●--●), B2/al; (○--○), B2/al0.

Native and chimeric α5/β1 FnR were also immunoprecipitated with a rabbit polyclonal antibody (No. 161) directed against the cytoplasmic domain of the human α5 subunit and were resolved on a 7.5% polyacrylamide gel (Fig. 3 B). Antibody 161 shows cross-reactivity with α5 subunits originating from other species due to interspecies sequence homologies within the α5 cytoplasmic domain. Antibody 161 precipitated native α5/β1 FnR from wild type cells (Fig. 3 B, lane 1). Chimeric α5/β1 was also precipitated from B2/a27 (Fig. 3 B, lanes 3 and 6). In contrast, the truncated α5 subunits arising from B2/al cells (Fig. 3 B, lane 5) and wild type cells (Fig. 3 A, lane 1) or B2/a27 cells (Fig. 3 A, lane 3) exhibited similar mobilities on gradient gels, with apparent subunit molecular weights of 147 and 149 kD, respectively. In comparison, the truncated α5 subunits from B2/al cells (Fig. 3 A, lane 4) or B2/al0 cells (Fig. 3 A, lane 5) migrated on the gradient gel slightly faster than intact human α5 subunit, exhibiting apparent molecular weight values of 143 and 147 kD, respectively. The β1 subunit for all cell types tested, excluding B2, exhibited an apparent molecular weight of 117 kD; α5/β1 was absent in immunoprecipitates from B2 cells (Fig. 3 A, lane 2).
B2/a10 cells (Fig. 3 B, lane 6) were not precipitated by the
161 antibody, confirming that the α5 subunit is truncated in
these cells. No α5/β1 was detected in B2 cells with antibody
161.

Equal amounts of total cytoplasmic RNAs derived from
wild type, B2, B2/a27, B2/a10, and B2/al cells were evalu-
ated by Northern blot hybridization (Chomczynski and
Sacci, 1987; Sambrook et al., 1989) for the presence of hu-
man α5 subunit mRNA. Northern analysis showed a single
band at ~4.2 kb for cells transfected with pECE-α5, pECE-
α5/1, and pECE-α5/10 (data not shown), in agreement with
the sizes predicted from the cDNA constructs taking into ac-
count polyadenylation of the transcribed mRNA sequence.
Slot blot hybridizations were performed to compare relative
amounts of α5 mRNA present in all three transfectants. Den-
sitometric analysis of the slot blot hybridization indicated
that five times more α5 message was present in B2/a10, and
two times more message was present in B2/a1 when com-
pared to α5 message within B2/a27; thus, data from North-
ern and slot blot analysis, compared with surface expression
of the mutated α5 subunits, suggests that partial or complete
deletion of the cytoplasmic domain impedes effective trans-
lation or surface expression.

Adhesion of Cells to Fibronectin

Although the truncated α5 subunits were expressed and
could be precipitated using the BI65 antibody, it remained
to be seen whether the modified receptors were functional.
To explore this, cells were tested for their ability to adhere
to purified Fn immobilized on tissue culture plastic. These
experiments show that B2/a27 and B2/a10 adhered to Fn to
a similar extent as wild type CHO cells (Fig. 4 A). Despite
lower α5/β1 FnR expression, B2/a1 cells also adhered to Fn
in a manner similar to wild type cells. Initial binding was evi-
dent at coating concentrations of 0.1 μg/ml Fn, while maxi-
mum adhesion of cells to Fn was demonstrated at coating
concentrations of 1 μg/ml. The cells not expressing α5/β1
(B2 cells) showed very limited adhesion to Fn even at coating
concentrations of 10 μg/ml. To determine whether the trun-
cations within the α5 subunit could affect the rapidity of
binding to immobilized Fn, time courses of adhesion assays
were performed. Kinetic experiments were done using non-
limiting concentrations of Fn (1 μg/ml). Both B2/a27 and
B2/a10 cells displayed kinetics of adhesion to Fn similar to
wild type cells (Fig. 4 B), adhering maximally in 15 min.
B2/a1 cells showed a slower rate of adhesion to Fn than wild
type cells during the initial 10 min, but approached wild type
levels of adhesion at 15 min. This slower rate of adhesion
may be a reflection of the decreased surface expression of the
chimeric α5/β1 FnR. This latter result is in agreement with
results reported previously in our laboratory which showed
that CHO FnR variants that exhibit reduced (20% of wild
type) α5/β1 FnR expression, adhered to immobilized Fn
substrata at a slightly slower rate than wild type cells
(Schreiner et al., 1989).

Centrifugal force was used to gauge the strength of adhe-
sion of cells to non-limiting concentrations of immobilized
Fn (1 μg/ml) (Fig. 5). Wild type cells and B2/a27 cells were
completely adherent at relative centrifugal forces (RCFs) up
to 100 g. Beyond this, adhesive resistance to centrifugal
force decreased for both cell lines. At the maximal centrifu-
gal force used (RCF = 1,000 g), 80 and 75% of wild type
cells and B2/a27 cells, respectively, remain attached to the
Fn-coated substrata. B2/a1 and B2/a10 cells showed slight
decreases in adhesion at 100 g. The decrease in adhesion was
more pronounced at 500 g, falling to 50 and 75% for B2/a1
and B2/a10, respectively. At 1,000 g both B2/a1 and B2/a10
displayed ~50% adhesion. To distinguish between the
effects of reduced α5/β1 FnR expression, and effects caused
by the cytoplasmic domain truncation, a variant that ex-
presses 20% of wild type hamster α5/β1 FnR (clone 1-23;
previously described by Schreiner et al., 1989), was used as
a control. At all centrifugal forces tested, the adhesion of
B2/a1 to Fn paralleled that of the 20% FnR variant. The 2%
FnR variant B2 showed only background adhesion to Fn

Figure 5. Strength of adhesion of α5 transfectants to Fn. 96-
well microtiter plates were
coated with non-limiting con-
tentrations of Fn (1 μg/ml)
and then blocked with 3%
BSA. Metabolically labeled
(as described in Materials and
Methods) cells were added to
the plates and then were
spun
onto the substrata at 17 g for
10 min at 4°C. The plates
were then incubated at 37°C
for 30 min. After incubation,
plates were sealed, inverted,
and centrifuged at 4°C for 10
min at varying speeds to
dislodge non- or weakly adherent
cells. Adhesion was quan-
tified by counting the radioac-
tivity of the attached cells.
Wild type, m; B2, ○; B2/a27,
●; B2/a1, ▲; B2/a10, ▼;
1-23, ◇.

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Motility of Cells on Fn

It has been shown that the α5/β1 FnR is involved in the motility of cells on Fn substrata (Bauer et al., 1992). Using a modified Boyden chamber assay, we examined the contributions of the α5 subunit cytoplasmic domain to cellular haptotaxis on Fn. B2/a27 cells migrate on Fn to an extent similar to wild type cells. B2/a10 cells also exhibited wild type levels of motility on Fn, while migration of B2/a1 cells on Fn was substantially reduced (15–35% of wild type) (Fig. 6). The B2 cells failed to show any appreciable motility on Fn. Decreased motility of B2/a1 cells on Fn could be due to either decreased α5/β1 FnR expression or to less efficient receptor function due to the deletion of the α5 cytoplasmic domain.

To help evaluate these two possibilities, the motility of the B2/a1 on Fn was compared to that of FnR-deficient clone 1–23 (Fig. 6, inset). The B2/a1 and 1–23 both exhibited reduced degrees of motility as compared to wild type cells. This comparison suggested that the decreased motility of B2/a1 cells on Fn might be partly due to reduced α5/β1 FnR expression. However, additional experiments (see below) indicate that α5 truncation has a direct effect on motility. Our standard motility assay incubation is eight hours. A kinetic study was performed to determine whether the deletions in the cytoplasmic domain of the α5 might cause changes in the motility of cells on Fn discernable beyond this 8-h incubation time. On non-limiting concentrations of Fn (10 μg/ml) migration of B2/a27 and B2/a10 cells paralleled migration of wild type cells at all time points tested (Fig. 7). B2/a1, however, showed slower rates of motility on Fn initially, and failed to achieve wild type levels of motility even after 24 h of incubation.

Cytoskeletal Organization

Subsequent to integrin binding to ECM proteins, integrins cluster to form adhesion plaques (focal contacts), a process which involves cytoskeletal rearrangement and actin stress fiber development (Burridge et al., 1988). It has already been shown that truncation of the β1 subunit within the cytoplasmic domain significantly impairs the ability of integrins to participate in adhesion plaque formation and cytoskeletal organization (Hayashi et al., 1990; Marcantonio et al., 1990). We investigated whether cytoplasmic truncation of the α5 subunit also affects cytoskeletal organization. Morphology and stress fiber development were evaluated in cells plated on substrata coated with 100 μg/ml Fn. Wild type, B2/a27, and B2/a10 cells spread equally well on Fn exhibiting strong stress fiber formation (Fig. 8, A, C, and E, respectively). CHO cells do not produce well-developed focal contacts; however, in the wild type, B2/a27 and B2/a10 cells, α5/β1 was shown to localize at the edges of the stress fiber (data not shown). B2/a1 cells (Fig. 8, D) spread on Fn but differed morphologically from wild type cells, maintaining a less-elongated appearance. This was similar to the appearance previously described for 1–23 cells that express comparable levels of α5/β1 (Schreiner et al., 1989). A poorly spread morphology was also evident in several other independent clones arising from transfection with the fully truncated α5 construct (data not shown). As in the other cells (with the exception of B2), stress fibers were produced in
Figure 7. Kinetics of CHO motility on Fn. Transwell motility inserts were coated with 30 μg/ml Fn. 1 × 10^5 cells were added to the upper chamber and incubated for various times at 37°C. Motility was quantitated as before. (○—○), wild type; (●—●), B2; (Δ—Δ), B2/a27; (▲—▲), B2/al; (□—□) B2/al0.

Figure 8. Actin cytoskeletal stress fiber development in α5 transfectants spread on Fn. Trypsinized cells were plated onto fibronectin-coated (50 μg/ml) coverslips and allowed to spread for 4 h. Cells were then fixed in 3.7% formaldehyde, permeabilized in 0.4% Triton X-100, and stained with rhodamine-labeled phalloidin. Cells were observed using a 100× objective. A, wild type; B, B2; C, B2/a27; D, B2/al; and E, B2/al0.
B2/al cells, but cytoskeletal organization was less well defined. α5/β1 was detectable in adhesion plaques in B2al cells despite lower receptor expression (data not shown). The morphology of the B2/al cells resembled that previously described for clone 1–23, a line that expresses levels of α5/β1 similar to the B2/al cells (Schreiner et al., 1989). The B2 cells (Fig. 8, B) showed no spreading on Fn, maintaining a spherical appearance; stress fiber development in the B2 cells was conspicuously absent.

**Protein Tyrosine Phosphorylation**

Changes in the pattern of protein tyrosine phosphorylation have been observed subsequent to the clustering of integrins by antibodies (Kornberg et al., 1991), or as a result of adhesion to fibronectin (Guan and Hynes, 1991). In Fig. 9 protein tyrosine phosphorylation patterns subsequent to plating on Fn-coated substrata are illustrated for the FnR-deficient mutant B2, and for α5-transfected CHO cells. A substantial increase in tyrosine phosphorylation was seen in a band of ~125 kD in both B2/a27 cells and B2/al cells adherent to Fn substrata as compared with non-adherent cells. In other experiments, similar increases in tyrosine phosphorylation were observed in B2a/10 cells and WT CHO cells subsequent to adhesion to fibronectin (data not shown). Exposure to Fn substrata had no effect on the tyrosine phosphorylation pattern of B2 cells; as indicated above, these cells are almost completely nonadherent on Fn substrata. In CHO cells interactions with fibronectin seem to be almost exclusively dependent on the α5/β1 integrin, since these interactions can be completely abolished by mAbs directed against this integrin (Brown and Juliano, 1985; Bauer et al., 1992). Thus, the results shown in Fig. 9 suggest that the α-cytoplasmic do-

**Figure 9.** Protein tyrosine phosphorylation subsequent to adhesion on Fn substrata. CHO mutants or transfectants were incubated for 90 min at 37°C on 60-mm dishes coated with 0, 0.5, or 10 μg/ml of fibronectin. The cells were then washed, lysed in 300 μl of RIPA buffer, and then processed for gel electrophoresis and transblotting; gels were run with equal protein loads in each lane. Phosphotyrosyl proteins were detected by Western blotting with an anti-P-tyrosine antibody as described in Materials and Methods. A 24-h autoradiographic exposure is shown. The position of the 125-kD tyrosine phosphorylated protein(s) is indicated by an arrow. Positions of molecular weight markers are indicated at the left of the figure. (Lanes 1–3) B2 cells on 0, 0.5, or 10 μg/ml of fibronectin; (lanes 4–6) B2/a27 cells on 0, 0.5, or 10 μg/ml of fibronectin; (lanes 7–9) B2a27 cells on 0, 0.5, or 10 μg/ml of fibronectin.

**Evaluation of Normal and Truncated α5 Function at Similar Levels of Expression**

Although a comparison of clones 1–23 and B2al (Figs. 6 and 8) suggested that the deficits in cell motility and cell spreading exhibited by B2al might be due simply to low levels of α5 expression rather than to truncation of the cytoplasmic domain, these results were not unambiguous. Thus we examined cell morphology and motility in cells from clones B2a27 and B2al under conditions where the full-length (a27) and truncated (al) α5 subunits were expressed at similar levels. This was done by using a flow cytometer to sort out a population of B2al cells that expressed high levels of truncated α5, similar to the levels of full-length α5 expressed in the B2a27 population (in fact, the average α5 expression in the sorted B2al cells was slightly higher than the B2a27 cells). The high-expressor B2al cells ( termed B2a1H) were compared to B2 and B2a27 in terms of ability to spread on Fn substrata, and in terms of cell motility in a "wounding" assay (we were not able to obtain sufficient numbers of B2a1H cells to study cell motility using the Boyden chamber type motility assay). As seen in Fig. 10, during a 2-h incubation at 37°C, B2a27 cells attached and became fully spread on a Fn substratum; in contrast, the B2a1H cells attached, but only a few cells were able to spread. The B2 cells did not attach to the Fn substratum and remained round. The negatively selected, low-expressing population (B2a1L) as well as the unselected B2al population attached to the Fn substratum (although some "floaters" were seen in the B2a1L population); these two populations largely remained round after 2h. After a more extended incubation (24 h) a large fraction of the B2a1H cells and some of the B2al cells had partially spread; however, obvious differences with the B2a27 population persisted (data not shown). Table I compiles the number of fully spread, partially spread, or round cells from each population, and confirms the impressions of Fig. 10. Table II illustrates that B2a1H cells migrate less well than B2a27 cells using the wound-type motility assay.

**Discussion**

Integrins play a vital role in the adhesion and motility of cells on ECM proteins. In doing this, integrins must interact with

**Table I. Cell Spreading**

| Cell type | Fully spread | Partly spread | Round |
|-----------|--------------|---------------|-------|
| B2a27     | 76 ± 7       | 14 ± 8        | 9 ± 7 |
| B2a1H     | 3 ± 3        | 37 ± 2        | 59 ± 6|
| B2a1      | 0            | 23 ± 10       | 77 ± 11|
| B2a1L     | 0            | 8 ± 3         | 92 ± 4|
| B2        | 0            | 3 ± 3         | 96 ± 6|

Cell populations (1 x 10⁵) in α-MEM + 1% albumin were plated into 24-well tissue culture plates pre-coated with 10 μg/ml fibronectin and incubated for 2 h at 37°C in a CO₂ incubator. The cells were observed with a 40× phase contrast objective and the number of fully, partially spread, or round cells per objective field was counted. For each sample fields in three different wells were observed. All cells tested were exposed to primary and secondary antibodies and processed in the same manner. The B2a1H and B2a1L are the sorted populations. Results are given as means and standard errors.
the cytoskeletal apparatus in some fashion (Burridge et al., 1988). Recent evidence also suggests that integrins both influence cellular signal transduction mechanisms (Kornberg et al., 1991), and are in turn acted upon by cellular regulatory processes (Van Kooyk et al., 1989). Thus, integrins participate in both "outside in" and "inside out" interactions between the extracellular matrix and the cell interior (Hynes, 1992). It seems reasonable to assume that integrin cytoplasmic domains are essential to these transactions. In this study we have investigated the role of the or5 cytoplasmic domain in several aspects of integrin function including cell adhesion, motility, cytoskeletal organization, and protein tyrosine phosphorylation.

To this end, we engineered two or5 constructs (pECE-or5/1 and pECE-or5/10) that are truncated within the cytoplasmic domain, from an existing full-length human or5 cDNA expression vector (pECE-or5). A CHO cell clone (B2) that produces very low levels of endogenous hamster or5 subunit was chosen as a host system to test the function of these constructs. Transfection of B2 cells with either pECE-or5 or pECE-or5/10 resulted in wild type levels of surface or5/β1 FnR expression. After transfection, pECE-or5/1 was also expressed at the cell surface, albeit at much reduced levels, approximating 15–20% of wild type or5/β1FnR expression. CHO cells transfected with pECE-or5 have previously been shown to give rise to functional chimeric human or5/hamster β1 (Bauer et al., 1992; Giancotti and Ruoslahti, 1990). Our results showed that the truncated or5 constructs can also associate with β1 and that these complexes were expressed on the surface of the B2 cells. Chimeric or5/β1 FnR was immunoprecipitated with the B1E5 anti–human or5 antibody from lysates derived from the three transfectants. Precipitated or5 subunits arising from either B2/al or B2/al0 showed altered mobility on gradient polyacrylamide gels, migrating slightly faster than the full-length human or5 subunit. Hamster or5 subunit migrated slightly faster than the intact human or5 presumably due to differences in subunit glycosylation. Additionally, anti-or5 cytoplasmic domain antibody (161) did not precipitate FnR derived from either B2/al or B2/al0, thus confirming the truncated nature of the cytoplasmic

Figure 10. Morphology of cells expressing similar levels of full-length or truncated or5. Using flow cytometry a population of B2al cells was obtained (B2alH) that expresses a level of truncated or5 similar to the expression of full-length or5 in B2a27 cells (see Materials and Methods). A negatively selected low-expressor population was also obtained (B2alL). Cell populations (B2, B2al, B2alL, B2alH, B2a27) in α-MEM + 1% albumin were placed in 24-well tissue culture plates pre-coated with 10 μg/ml Fn, incubated for 2 h at 37°C, and photographed using a phase contrast photo microscope with a 40× objective. A, B2a27; B, B2al; C, B2alH; D, B2; E, Flow cytometric analysis of or5 expression in B2a27 and B2alH cells.
domains, as well as the absence of significant residual hamster α5.

As mentioned above, complete removal of the cytoplasmic domain of the α5 subunit results in lower FnR surface expression. Reduced expression was not due to lower mRNA levels. Thus, B2aα1 cells, which express low levels of cell surface α5/β1, produced three times more α5 message than B2aα27 cells, which express wild type levels of α5/β1, while B2aα10 expressed five times more α5 message than B2aα27, but expressed comparable levels of cell surface α5/β1; both of these observations suggest that truncation of the cytoplasmic domain reduces surface expression. This result is in agreement with the work of Hibbs et al. (1991) who noted reduced surface expression of LFA-1 in COS cells after complete truncation of the cytoplasmic domain of the α subunit. Our pECE α5/1 construct is truncated in the midst of the highly conserved KXGFFR sequence that follows the transmembrane domain in all α chains (Dedhar, 1990); this sequence may be important for efficient cell surface expression of α/β heterodimers (Chan et al., 1992).

Expression of the truncated human α5 constructs in CHO cells gave rise to functional chimeric α5/β1 fibronectin receptor. This conclusion is based on the following observations: (a) transfected cells adhered to immobilized Fn substrate; (b) the cells exhibited motility on Fn-coated substrata that was consistent with receptor expression; (c) cells adhering to Fn underwent cytoskeletal reorganization and focal adhesion plaque development. B2aα27 and B2aα10 cells behaved similarly to wild type cells in terms of adhesion, motility, and cytoskeletal organization. In contrast, B2aα27 cells showed decreased motility on Fn; strength and kinetics of adhesion to Fn were also somewhat attenuated. To understand the behavior of B2aα1 cells, it was essential to dissociate functional effects imposed by the truncation from those due to decreased α5/β1 expression. Thus, we used fluorescence cell sorting to obtain a sub-population of B2a1 cells (B2aα1H) that expressed levels of truncated α5 similar to the level of expression of full-length α5 found in B2aα27. Despite similar levels of a subunit expression, there were distinct functional differences between the B2a1H cells and B2aα27 cells. The latter cells can rapidly spread on fibronectin substrata and develop a fully extended fibroblastic morphology; the B2a1H cells spread to a very limited degree in the short term. After overnight incubation there is considerable spreading of B2a1H, although not as extensive as for B2a27, however, results at these longer times may be partly due to modification of the fibronectin substratum by cell secretion of other adhesion factors. Since both B2aα27 cells and B2aα10 cells can rapidly spread and develop stress fibers, while B2a1H cannot, it seems that the ability to rapidly reorganize the cytoskeleton and to engage in cell spreading is markedly affected by deletion of the portion of α5 which is proximate to the membrane. This portion of the α5 subunit also seems to be involved in cell migration since the B2a1H cells are less motile than the B2aα27 cells. However, the level of expression of α5 is also an important determinant of cell function. This is emphasized by the different behavior of wild type CHO and clone 1–23, which expresses reduced levels of intact hamster α5. It is also apparent from the observation that, although the B2a1H cells spread rather poorly, they do spread more than B2aα1L or B2a1 cells that express lower levels of the truncated α5.

Other laboratories have delineated an important role for the β chain cytoplasmic domain in integrin function. For example, Hayashi et al. (1990) found that deleting five or more amino acids from the carboxy terminus of the β1 subunit significantly impaired integrin function, in terms of failure to promote adhesion or to permit integrin co-localization in focal adhesion plaques. Similar results were found by Hibbs et al. (1991) in their experiments truncating the β subunit of LFA-1. Our studies indicate that the situation may be more complex for α chains. Thus, essentially complete truncation of the α5 cytoplasmic domain seems to have little effect on functions such as cell adhesion or integrin-mediated tyrosine phosphorylation. Partial truncation, as in the B2α10 clonal line, also seems to have little effect on the cell’s ability to rapidly organize the cytoskeleton or to be motile. However, truncation of the portion of the α5 subunit immediately adjacent to the transmembrane domain, as in the B2α1 clonal line, does seem to produce marked effects on the cell. At minimum, truncation of this region seriously retards the ability of the cell to organize its cytoskeleton and to spread on the substratum; effects on motility are also seen.

Cells seem somewhat more tolerant of modifications of α chain cytoplasmic domains than of modification of corresponding regions in β chains. For example, when Hibbs et al. (1991) truncated the α chain cytoplasmic domain of LFA-1, little effect was noted. This seems consistent with the observations of LaFlamme et al. (1992) who prepared chimeras of the external domain of IL-2 receptor and integrin α or β cytoplasmic domains; these investigators noted that the β subunit chimeras were capable of focal contact localization, while α chimeras were not. Thus our observations, along with those of Hibbs et al. (1991) and LaFlamme et al. (1992), suggest that α chain cytoplasmic domains may not be essential for several important integrin-mediated cellular functions. Our own observations show that partial truncation of the α5 cytoplasmic domain, as in the B2α10 cells, can be tolerated with little recognizable impact on cell function. Truncated of virtually the entire cytoplasmic domain, as in the B2α1 cells, still permits efficient adhesion and presumptive signaling paths mediated by tyrosine phosphorylation; however, these cells are deficient in their ability to rapidly deploy the cytoskeleton.
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