Antibodies to the Conserved Cytoplasmic Domain of the Integrin $\beta_1$ Subunit React with Proteins in Vertebrates, Invertebrates, and Fungi

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Abstract. The integrin family of cell surface receptors can be divided into three groups on the basis of their homologous $\beta$ subunits: $\beta_1$, $\beta_2$, and $\beta_3$. We have raised an antibody against a synthetic peptide corresponding to the COOH-terminal domain of the chicken integrin $\beta_1$ subunit that reacts with $\beta$ subunits from a variety of vertebrates, invertebrates, and fungi, demonstrating strong evolutionary conservation of sequences in this domain. In Drosophila cells, the antibody recognizes integrin $\alpha\beta$ complexes that appear to be identical with position-specific antigens. Cross-reactive proteins are also detected in Caenorhabditis elegans and Candida albicans. The antiserum is specific for $\beta_1$ subunits and does not recognize other integrin $\beta$ subunits in humans. In immunofluorescence analyses of cultured cells, the antibody reacts only with permeabilized cells confirming that this highly conserved COOH-terminal segment is a cytoplasmic domain.

Integrins comprise a large family of cell surface receptors which function in cell–cell and cell–matrix adhesion in many cell types (Hynes, 1987; Ruoslahti and Pierschbacher, 1987). These receptors clearly participate in a wide variety of processes, including cellular differentiation and migration in development, as well as thrombosis, wound healing, and leukocyte helper and killer functions.

The structures of integrins have been deduced by cDNA sequence and immunological analysis (Tamkun et al., 1986; Argraves et al., 1987; Suzuki et al., 1987; Kishimoto et al., 1987; Law et al., 1987; Poncz et al., 1987; Fitzgerald et al., 1987; Hemler et al., 1987; DeSimone and Hynes, 1988). These receptors consist of transmembrane, noncovalently linked heterodimers with distinct $\alpha$ and $\beta$ subunits. There are at least three different integrin $\beta$ subunits and 10 different $\alpha$ subunits. For simplicity, we have divided the integrins into three classes on the basis of their component $\beta$ subunits (Hynes, 1987). The $\beta_1$ class includes the chicken integrin complex (Horwitz et al., 1985), the human fibronectin receptor (Pytel et al., 1985a), and the very late antigens (VLAs).1 The VLAs, which are present on a wide variety of human cells, were initially discovered on activated human T cells (Hemler et al., 1985) and consist of five distinct $\alpha$ subunits each associated with a common $\beta$ subunit (Hemler et al., 1987). The $\beta_2$ class appears to be leukocyte specific and includes the heterodimers LFA-1, Mac-1, and p50,95 (Anderson and Springer, 1987). Finally the $\beta_3$ class includes the human vitronectin receptor and platelet glycoprotein IIb/IIIa (Pytela et al., 1986; Ginsburg et al., 1987).

All three classes of $\beta$ subunits have a large extracellular domain with a total of 56 cysteine residues including four cysteine-rich repeats. This high cysteine content may account for the shift in electrophoretic mobility of the $\beta$ subunit to a higher apparent molecular mass on SDS-PAGE when reducing agent is present. The $\alpha$ subunits of integrins have a large extracellular domain with a series of metal-binding domains (Argraves et al., 1987; Suzuki et al., 1987; Poncz et al., 1987) and, in some cases, consist of two disulfide-linked polypeptides, termed a heavy and a light chain (Hynes, 1987; Ruoslahti and Pierschbacher, 1987). This structure accounts for the decrease in apparent molecular weight of many $\alpha$ subunits on SDS-PAGE when reducing agent is included. Different $\alpha/\beta$ combinations have different ligand specificities, interacting via their extracellular domains with a wide variety of ligands, including extracellular matrix molecules such as fibronectin (Pytela et al., 1985a; Gardner and Hynes, 1985; Akiyama et al., 1986), laminin (Horwitz et al., 1985), collagen types I and III–VI (Wayner and Carter, 1987), vitronectin (Pytela et al., 1985b), von Willebrand factor, and fibrinogen (Pytela et al., 1986), as well as the complement component C3b (Wright et al., 1987).

There is a single, strongly hydrophobic region in each of the $\alpha$ and $\beta$ subunits of integrin, preceding short, hydrophilic COOH-terminal (putative cytoplasmic) domains. These domains are thought to interact with the cytoskeleton, consis-
tent with the coalignment of matrix and cytoskeletal proteins with integrins (Damsky et al., 1985; Chen et al., 1985). Within the COOH-terminal domain of the β1 subunit, there is a putative site of tyrosine phosphorylation (Tamkun et al., 1986). Indeed, the β1 subunit of Rous sarcoma virus-transformed chicken cells has been shown to be phosphorylated on tyrosine (Hirst et al., 1986). The COOH-terminal segments of integrin β subunits are remarkably well conserved in sequence among vertebrates (DeSimone and Hynes, 1988).

To study the function of the integrin β, COOH-terminal domain, we have used a synthetic peptide comprising this putative cytoplasmic domain to generate a domain-specific antisera. We have used this antisera to verify the cytoplasmic localization of this domain and report that the antisera reacts with proteins from vertebrates, invertebrates, and fungi, suggesting the widespread occurrence of integrin receptors.

**Materials and Methods**

**Cells**

Chicken embryo fibroblasts (CEFs) were prepared from day-11 embryos (Spassa, Inc., Norwalk, CT) as described (Rein and Rubin, 1968). Cells were grown in DME plus 5% FCS (Gibco, Grand Island, NY) and used between passages 3 and 6. The NIH hamster cell line (Mautner and Hynes, 1977) was cultured in DME with 5% FCS. MG-63 human osteosarcoma cells (Billius et al., 1977) were grown in DME with 10% FCS. Human platelets were isolated from citrated platelet-rich plasma as described by Lawler et al. (1982). Human peripheral blood monocytes were isolated as described by Wright and Silverstein (1982) and were kindly provided by L. Van De Water (Beth Israel Hospital, Boston, MA). S-2 cells were grown in Schneider medium with 10% FCS and were kindly provided by M. L. Pardee (Massachusetts Institute of Technology [M.I.T.], Cambridge, MA). Caenorhabditis elegans adults and embryos were kindly provided by L. Miller and B. Meyer (M.I.T.). Membranes from Saccharomyces cerevisiae (strain CHS1) and Candida albicans (strain 73/055) were prepared as described (Orlean, 1987) and were generously provided by J. Au-Yong (M.I.T.). Extracts of C. elegans and day-11 chicken embryos were prepared as described by Knudsen et al. (1985).

**Immunoprecipitation**

Cells were labeled with 125I and lactoperoxidase (Sigma Chemical Co., St. Louis, MO) either in suspension or as a monolayer as described (Hynes, 1975). 106 cells and 1.0 mCi/ml were used per experiment. Cells were washed three times with PBS (phosphate-buffered saline with 1 mM CaCl2 and 1 mM MgCl2), resuspended in 1 ml of extraction buffer (50 mM Tris, pH 8.0, 150 mM NaCl, 0.5% NP-40), and incubated for 15 min on ice, then sedimented for 10 min at 10,000 g. The supernatant was preincubated with 50 μl of protein A-Sepharose for 5 min and the beads sedimented for 2 min at 10,000 g. The resulting supernatant was used for immunoprecipitation. On average 5 × 108 TCA-precipitable counts were used per sample.

In some experiments, the integrin complex was denatured: 1/20 vol of 20% SDS was added, followed by boiling for 3 min. After cooling, a fivefold excess of Triton X-100 was added, and this sample was then treated as the nondenatured ones.

2 μl of primary antiserum was added and incubated for 1 h at 4°C. 30 μl of protein A-Sepharose was added and the samples were rotated overnight at 4°C. After spinning the sample for 2 min at 10,000 g, the beads were washed four times with 1 ml extraction buffer, followed by elution with sample buffer.

**Production of Anti-peptide Antibodies**

100 ng of crude 39-mer (Fig. 1) was synthesized by Peninsula Laboratories, Inc. (Belmont, CA). The synthesis was monitored by HPLC and amino acid analysis of the products. The peptide was coupled to keyhole limpet hemocyanin (KLH) (Calbiochem-Behring Corp., La Jolla, CA) using m-maleimidobenzoylsulfo-succinimide ester (sulfo-MBS) (Pierce Chemical Co., Rockford, IL) essentially as described (Kitagawa and Aikawa, 1976). KLH was dialyzed against 10 mM KPO4, pH 7.0, and sedimented at 10,000 g for 10 min. 6.2 mg of KLH in 1 ml KPO4 was mixed with 0.24 ml of sulf-o-MBS (5 mg/ml stock) and stirred for 15 min at 4°C. The reaction mixture was desalted using a 10 × 0.5-cm G-25 column (Pharmacia Fine Chemicals, Piscataway, NJ) with 100 mM KPO4, pH 6.0. Protein-containing fractions were pooled and incubated with 35 mg of peptide. The reaction mixture was adjusted to pH 7.0 with NaOH and stirred overnight at 4°C, then quenched with 0.5 M ethanolamine, pH 8.0, for 1 h. This mixture was dialyzed against NH4HCO3 and lyophilized.

Trace amounts of the peptide 23I labeled with Bolton-Hunter reagent (2,000 Ci/mmol; New England Nuclear, Boston, MA and Dupont Co., Wilmington, DE; Bolton and Hunter, 1973) were added to monitor coupling efficiency, with a 90% recovery of input counts. For injections, 100 μg of peptide conjugate in PBS was mixed with 1 ml of complete Freund's adjuvant (Gibco) by sonication and was injected subcutaneously at multiple sites along the dorsal midline of rabbits. At 3-4 wk intervals, booster injections of 100 μg of protein in incomplete adjuvant were given. Significant antibody titers were observed after two boosts. This response contrasts with the poor one obtained when a l0-aminoc acid peptide corresponding to the tryptic peptide containing the dot tyrosine in Fig. 1 was used.

**Other Antibodies**

Rabbit anti-ⅡB was prepared as described (Gardner and Hynes, 1985). Rabbit antisera to the β subunit of LFA-1 and to native Mac-1 complex were kindly donated by T. K. Kishimoto and T. Springer (Dana-Farber Cancer Institute, Boston, MA). Monoclonal anti-VLA β ascites was a gift of M. Hemler (Dana-Farber Cancer Institute). CSAT antibody was a gift of C. Buck (Wisconsin Institute, Philadelphia, PA) and R. Horwitz (University of Pennsylvania, Philadelphia, PA) and monoclonal anti-PS-3 conjugated to agarose was a gift of M. Wilcox (Laboratory of Molecular Biology, Cambridge, England) and N. Brown (Harvard University, Cambridge, MA).

**Gel Electrophoresis and Immunoblotting Analysis**

SDS-PAGE was performed by the method of Laemmli (1970). Separation gels were 70% acrylamide with a 30% stacking gel. Samples were prepared in sample buffer (5% SDS, 100 mM Tris-HCl, pH 6.8, 10 mM EDTA, 10% glycerol, and bromphenol blue) and boiled for 3 min. Reduced sample buffer contained 50 mM dithiothreitol (DTT). The following were used as reduced molecular mass markers: myosin (M, 200,000), beta-galactosidase (M, 116,000), phosphorylase B (M, 97,000), and BSA (M, 66,000).

In immunoblotting experiments, polypeptides were transferred from the gels to nitrocellulose (Schleicher & Schuell, Inc., Keene, NH; BAB3) using a Hoeffer transfer apparatus for 1,500 mA/h in 20 mM Tris-HCl, pH 6.8 (Towbin et al., 1979). After transfer, strips were cut and washed three times with buffer A (25 mM Tris-HCl, pH 8.0, 150 mM NaC1, 0.1% Tween 20), then blocked with 5% BSA (fraction V) in buffer A for 30 min at 25°C. Strips were then incubated with primary antisera at a 1:100 dilution in buffer A for 1 h at room temperature, washed three times with buffer A, and stained using the Vecta-stain kit (Vector Laboratories, Inc., Burlingame, CA) as per instructions, using 4-chloro-I-naphthol as the enzyme substrate.

**Immunofluorescence**

NIH fibroblasts were stained using minor modifications of the method of Mautner and Hynes (1977) with 0.5% NP-40 used to permeabilize cells. The cells were plated on coverslips and grown in DME with 0.3% FCS to allow maximum spreading. CEFs were plated for 2 h on coverslips previously coated with human plasma fibronectin, which was purified as described (Engvall and Ruoslahti, 1977). These cells were used either live or after fixation. For fixation, cells were rinsed twice in PBS and fixed for 15 min in a freshly prepared 4% solution of paraformaldehyde (Fluka Chemical Co., Bern, Switzerland) in PBS, rinsed, and permeabilized with 0.5% NP-40 in PBS for 15 min at room temperature. Fixed and live cells were stained as described (Mautner and Hynes, 1977) except for washing and incubation with DME instead of PBS. After the final wash, live cells were fixed with 4% paraformaldehyde for 15 min. Coverslips were examined using a Zeiss Photo-Ill microscope and photographed using Kodak Tri-X film.

**Results**

**Production of Anti-peptide Antisera**

To study the COOH-terminal (putative cytoplasmic) domain

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of the integrin β1 subunit, we generated an antiserum against a peptide comprising the bulk of this domain which was coupled to KLH via an amino-terminal cysteine (Fig. 1). The synthetic peptide sequence is highly conserved among vertebrate integrins and contains the putative site of tyrosine phosphorylation.

When an extract of 125I surface-labeled CEFs was immunoprecipitated using this anti-peptide antiserum, multiple bands corresponding to the chicken integrin complex were recovered (Fig. 2, lane b). Unlabeled peptide completely blocked this precipitation, but had no effect on the recovery of the integrin complex when the mAb CSAT was used (Fig. 2, lane c and e). When the extract was denatured by heating in SDS, only the β subunit was immunoprecipitated using the anti-peptide antiserum (Fig. 2, lane d).

Since cDNA sequence analysis has shown that the COOH termini of the β1 subunits of chicken, Xenopus, and humans are virtually identical (DeSimone and Hynes, 1988), we would expect that an antibody to this domain would react with integrin β1 subunits of these and other vertebrate species. In Fig. 2, extracts of surface-labeled hamster cells (Nil8) and human osteosarcoma cells (MG-63) were immunoprecipitated. The anti-peptide antiserum specifically immunoprecipitated α/β heterodimers from both species (Fig. 2, lanes g and k). Again, only the β subunit was recovered by immunoprecipitation from denatured extracts (Fig. 2, lanes i and m) while no integrin bands were observed after immunoprecipitation using either preimmune serum or anti-peptide antiserum with competing peptide added (Fig. 2, lanes f, h, j, and l). These results confirm the specific reactivity of this anti-peptide antiserum with β subunits from several vertebrates and demonstrate coprecipitation of several different α subunits with these β subunits. Fig. 2 also shows that the human integrin heterodimers recovered with anti-peptide antiserum comigrate with those immunoprecipitated with monoclonal anti-VLA β (Fig. 2, lanes k and n), confirming the previous conclusion that the VLA β subunit is similar or identical to integrin β (Takada et al., 1987).

**β1 Specificity**

Since the COOH-terminal domains of the three classes of β subunits—β1, β2, and β3—are homologous, with many conservative substitutions (see Fig. 1) we investigated whether the antibodies directed against the β1 COOH terminus would also react with β2 or β3. For the analysis of β1 vs. β2, extracts of surface-labeled human monocytes (Fig. 3, lanes a-i) were immunoprecipitated either with anti-peptide antiserum or with antisera directed against either the β2 subunit or native Mac-1. The results show that the anti-peptide antiserum immunoprecipitated only β1 and associated α subunit (Fig. 3, lanes a and g), while β2 and associated α subunits (p150 and Mac-1) were precipitated with the appropriate antisera (Fig. 3, c, d, h, and i). The β subunit precipitated with the anti-peptide antiserum (β1) showed a characteristic shift in electrophoretic mobility upon reduction while the β2 subunit showed only a minor shift in mobility (cf. Fig. 3, lanes a, c, and d with lanes g-i).

For the analysis of β1 vs. β3, extracts of surface-labeled human platelets were immunoprecipitated with various antibodies (Fig. 3, lanes j-n). Anti-IIb immunoprecipitated IIb complexes with IIIa (β3) (Fig. 3, lane j), while anti-peptide antiserum and anti-VLA β immunoprecipitated β1 complexes with no β3 seen (Fig. 3, k and n). Immunoprecipitation of denatured extracts with the anti-peptide antiserum yielded only β1 (lane m). The specificity of this antiserum is again seen by the absence of integrin in immunoprecipitates recovered with preimmune or immune serum plus com-

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**Figure 1.** Conservation in COOH-terminal sequence of vertebrate integrin β subunits. COOH-terminal amino acid sequences derived from cDNA sequences of a number of integrin β subunits are shown in the single letter code. Upper-case letters indicate residues conserved in all or most β subunits, while lower-case letters indicate differences from the conserved β sequence. The asterisk marks the tyrosine residue proposed to be phosphorylated in chicken integrin β1. The sequence of the synthetic peptide is shown at the bottom. Sources of the sequence data are as follows: chicken β1 (Tamkun et al., 1986); *Xenopus* β1 and β3 (DeSimone and Hynes, 1988); human β1 (Argraves et al., 1987); human β1 (LFA-1 β; Kishimoto et al., 1987); and human β1 (IIIα; Fitzgerald et al., 1987).

**Figure 2.** Immunoprecipitation of integrins from chicken, hamster, and human cells. Extracts of 125I surface-labeled CEFs (lanes a-e), Nil8 hamster cells (lanes f-i), and MG63 human osteosarcoma cells (lanes j-n) were incubated with preimmune (P) serum (lanes a, f, and j); immune (I) anti-peptide antiserum (lanes b, g, and k); anti-peptide antiserum with 10 μg of (+) peptide added (lanes c, h, and l); anti-peptide antiserum after SDS (S) denaturation of the extracts (lanes d, i, and m); CSAT mAb (M) plus peptide (lane e), or anti-VLA beta antibody (lane n). The samples were immunoprecipitated using protein A-Sepharose and analyzed by SDS-PAGE (nonreduced) followed by autoradiography of the dried gels. β subunits are specifically precipitated from all three cell lines, whereas α subunits are precipitated only under nondenaturing conditions.
Figure 3. Immunoprecipitation of integrins from human monocytes and platelets. Extracts of $^{35}$S surface-labeled monocytes (lanes a-i) or platelets (lanes j-n) were incubated with (l) anti-peptide antiserum (lanes a, g, and k); anti-peptide antiserum with 10 µg of peptide (T+) added (lanes b, f, and l); anti-peptide antiserum after SDS denaturation (S) of the extracts (lane m); preimmune (P) serum (lane e); anti-LFA beta antiserum (lanes c and h); anti-native Mac-1 antiserum (lanes d and i); anti-IIIb antiserum (lane j); or anti-VLA antibody (lane n). The samples were immunoprecipitated using protein A-Sepharose and analyzed by SDS-PAGE under nonreduced (lanes a-e and j-n) or reduced (lanes f-i) conditions followed by autoradiography of the dried gels. $\alpha_2$ subunits are precipitated by the anti-peptide antiserum from both cell types, together with associated $\beta_2$ subunits. $\alpha_2$ and $\alpha_3$ subunits and their associated $\beta_2$ subunits are precipitated by the relevant antisera but not by the anti-peptide antiserum. The lower band of the pair marked $\alpha$IIb in lane j is probably a degradation product of glycoprotein IIIb. Molecular mass markers (200, 116, 97, 66 kD) are shown by bars on the left.

peting peptide (Fig. 3, lanes b, e, f, and l). Thus, there was no detectable cross-reactivity of the anti-$\beta_1$ peptide antiserum with $\beta_2$ or $\beta_3$, and this antiserum is specific for the $\beta_1$ class in humans and can be used to identify $\beta_1$ integrins in many tissues and species.

**Invertebrate Integrins**

Since we had observed cross-reaction of integrin $\beta_1$ cDNA probes with genomic DNAs of several invertebrate species (DeSimone and Hynes, 1988), we investigated whether the anti-$\beta_1$ antiserum would react with proteins of invertebrates. In the case of *Drosophila melanogaster* it had also been suggested that the position-specific antigens (PS1, PS2, and PS3) are related to vertebrate integrins (Leptin et al., 1987). Extracts of surface-labeled S-2 *Drosophila* cells were immunoprecipitated using the anti-integrin peptide antiserum and monoclonal anti-PS-3. Both antibodies immunoprecipitated $\alpha\beta$ heterodimers, which showed characteristic shifts in mobility upon reduction (Fig. 4, lanes b, d, f, and h). Integrins were absent after immunoprecipitation with immune serum plus peptide (Fig. 4, lanes c and e) and the anti-peptide antiserum precipitated only the smaller ($\beta_2$) subunits from SDS-denatured extracts (data not shown). The two $\beta_2$-related bands seen under reducing conditions (Fig. 4, lanes f and h) were a consistent finding in repeated experiments with both the anti-peptide antiserum and anti-PS-3 and presumably are due to proteolysis. The immunologic identity of the bands recovered with the anti-peptide antiserum and with the anti-PS-3 antibody was proven by preclearing experiments, in which either antibody could remove all of the antigen (data not shown).

The striking cross-reactivity between the anti-peptide antiserum and *Drosophila* integrin led us to investigate other species as well. Immunoblotting experiments demonstrated cross-reactivity in embryo extracts of the nematode *C. elegans*. Two bands were observed at apparent molecular masses of 120 and 70 kD, respectively, both of which could be completely competed with added peptide (Fig. 5, lanes a and b). These two bands comigrated with two immunoreactive bands observed in chicken embryo extract (Fig. 5, lane c). The lower molecular mass band (M, 70,000) seen in both cases is probably a degradation product since a similar band is seen in chicken embryo extracts with many anti-chicken $\beta_1$ antibodies and it shifts in electrophoretic mobility to a higher apparent molecular mass upon reduction. Furthermore, this band is only seen in blots of embryo extracts or stored extracts of tissue culture cells, but is not seen in freshly prepared cell extracts (Marcantonio and Hynes, unpublished observations).

Finally, we investigated the degree of cross-reactivity in yeasts. Immunoblotting experiments showed no specific bands in membrane extracts of *Saccharomyces cerevisiae* (data not shown). However, immunoblotting experiments
Drosophila S-2

Figure 4. Immunoprecipitation of extracts of Drosophila S-2 cells. Extracts of 35S surface-labeled S-2 cells (lanes b-h) were incubated with either immune (I) anti-peptide antiserum (lanes b and f), anti-peptide antiserum with 10 μg of peptide (I+) added (lanes c and e), or anti-PS-3 (PS) agarose beads (lanes d and h). After immunoprecipitation, samples were analyzed by SDS-PAGE under nonreduced (lanes a-d) or reduced (lanes e-h) conditions. An immunoprecipitate of the chicken integrin complex (M; lane a) using CSAT antibody is shown for comparison; arrowheads indicate bands 1, 2, and 3 of the complex. Molecular markers are indicated at left.

with another yeast, C. albicans, showed a band of M, 95,000 with anti-peptide antiserum, which was absent when preimmune or immune serum plus peptide were used (Fig. 5, lanes d-f).

Topology of the COOH Terminus

Since the COOH-terminal segment of integrin β1 is preceded in the primary sequence by a stretch of hydrophobic amino acids characteristic of a transmembrane domain, it has been proposed that the COOH terminus is the cytoplasmic domain (Tamkun et al., 1986). Antibodies to the amino-terminal third of the integrin β subunit stain live cells, indicating that this portion of the molecule is extracellular (Tamkun et al., 1986). In contrast, when live CEFs were stained with the anti-peptide antiserum, no staining was seen (Fig. 6, A and B). However, when the cells were fixed and permeabilized, staining characteristic of the integrin distribution in CEFs was seen (Fig. 6, C and D). These results confirm the proposed topology of the integrin β subunit.

In contrast with the CEFs, where the staining appeared as multiple short contact sites, fixed and permeabilized Nil8 hamster cells stained with the anti-peptide antiserum (Fig. 6, E and F) exhibited long delicate fibrils corresponding with the longer contact sites typical of these cells (see Discussion). As in the case of CEFs, live Nil8 cells showed no staining (data not shown). In both cell types, the integrin staining pattern coalesced with both the fibronectin and actin staining patterns (data not shown). In all cases, no specific staining was seen with preimmune serum or with immune serum plus competing peptide (data not shown).

Discussion

The antiserum we describe here has allowed demonstration of three main points concerning integrins: proof of the presumed topology of the β1 subunit, conservation of the COOH-terminal cytoplasmic domain, and wide phylogenetic distribution.

The wide cross-reactivity of the anti-peptide antiserum with proteins of similar sizes in vertebrates, invertebrates, and fungi shows that sequences in the cytoplasmic domain of integrin β subunits have been strongly conserved during long periods of evolution (up to 10⁹ yr). Intimations of this high degree of conservation had already been obtained from primary sequence data but the present results considerably extend the known phylogenetic distribution of integrins. We have shown that in human cells the antiserum is specific for the integrin β1 subunit and does not cross-react with the closely related β2 and β3 subunits (Fig. 3). Thus, the widely distributed β subunit which we have detected is presumably the β1 subunit. It is currently unclear whether β2 and β3 subunits, which appear to have diverged from β1 before the evolution of vertebrates (DeSimone and Hynes, 1988) are as widely distributed as the β1 subunit.

In immunoblotting experiments or in immunoprecipitates of denatured material, the antiserum recognizes only the β1 subunit (Figs. 2, 3, and 5). However, when extractions and immunoprecipitations are performed under nondenaturing conditions, larger α subunits are coprecipitated as would be expected given the known αβ structure of integrins. In many cell types, more than one α subunit coprecipitates with the β1 subunit indicating the presence in the cells of two or more αβ heterodimers as previously reported.

In the human cells analyzed here, we can relate the heterodimers precipitated with the anti-β1 antiserum to the VLA antigens found on these cells. In both MG-63 osteosarcoma
cells (Fig. 2) and in platelets (Fig. 3), the αβ complexes precipitated by the anti-β₁ antiserum appear identical to the VLAα₃ and α₅ on MG63 osteosarcoma cells, and predominantly VLAα₂ on platelets (Hemler et al., 1987; Takada et al., 1987; Giancotti et al., 1987).

In monocytes (Fig. 3) both subunits of the complex immunoprecipitated with the anti-β₁ antiserum shifted in electrophoretic mobility upon reduction, a property consistent with the presence of VLA-5 (α₅ β₁) on monocytoid cell lines (Hemler et al., 1987) and the binding studies indicating the presence of fibronectin receptors on monocytes (Hosein and Bianco, 1985). Interestingly, β₂ found on monocytes (Fig. 3) had only a small shift in electrophoretic mobility upon reduction, despite the fact that all 56 cysteine residues of integrin β subunits are conserved. The β₂-associated α subunits are probably the Mac-1 α subunit and p150 (Miller et al., 1986). It is worth noting that the results shown in Fig. 3 indicate that each β subunit associates with a specific set of α subunits and that interaction of α subunits with other β subunits is not observed.

The results with the Drosophila S-2 cells confirm the identity of the position-specific antigens as integrins (Fig. 4). These antigens are expressed in many insect cell lines and
in embryos, where they occur in characteristic patterns in developing tissues (Brower et al., 1984; Wilcox and Leptin, 1985). Their biochemical structure is typical of integrins, with a common β subunit (PS3) and variable α subunits (PS1 and PS2) associated in noncovalent complexes (Brower et al., 1984). Our anti-β1 peptide antiserum precipitates αβ complexes which appear identical with those precipitated by anti-PS3 mAb (Fig. 4). Previous data have shown homology in NH2-terminal sequence between PS-1 and vertebrate integrin α chains (Leptin et al., 1987). Recent cDNA sequence data for PS2 published while this paper was in review confirm that it is an integrin α subunit (Bogaert et al., 1987). The present data show that PS3 is probably a β subunit; it remains unclear whether other β subunits occur in Drosophila.

We also detect what appear to be integrin β subunits in the nematode C. elegans and the fungus C. albicans. In these two species, we have so far been unsuccessful in performing immunoprecipitations because of difficulties with extraction and degradation. Therefore, we cannot demonstrate αβ complexes here. However, the immunoblotting experiments (Fig. 5) indicate the presence of specifically cross-reactive bands of sizes similar to integrin β subunits. It will be interesting to see whether the cross-reacting protein found in C. albicans plays a role in the ability of these fungi to attach to and invade blood vessels and tissues. Integrins may play a role in the pathogenesis of infectious diseases; e.g., infection of cells in vitro by the parasite Trypanosoma cruzi can be blocked by peptides derived from the cell-binding domain of fibronectin (Ouaissi et al., 1986).

Finally, our immunofluorescence data (Fig. 6) establish that the COOH-terminal segment of the β1 subunit is indeed the cytoplasmic domain as has been widely assumed. We have previously reported that antiserum specific for segments in the NH2-terminal third of the β1 subunit stain live cells, placing this part of the molecule outside the membrane (Tamkun et al., 1986). Since only a single strongly hydrophobic stretch is found in the sequence immediately preceding the COOH-terminal cytoplasmic domain, it seems highly likely that this short segment comprises the entire cytoplasmic domain of this subunit.

As shown here and elsewhere, this domain is highly conserved suggesting that it plays an important role in the functions of integrin presumably including interactions with the cytoskeleton. It has been demonstrated that integrins coalign with both fibronectin and cytoskeletal proteins (Chen et al., 1985; Damsky et al., 1985) and that purified chicken integrins bind to talin in vitro (Horwitz et al., 1986). The distribution of staining we observe in CEFs is very similar to that reported by others and corresponds with the distribution of cytoskeletal proteins such as talin and vinculin and with attachment plaques. The staining pattern of Nil8 cells (Fig. 6, E and F) is somewhat different. The integrin is arrayed in very long fine striae similar to the elongated attachment sites observed in these cells (Hynes et al., 1982; Singer, 1982). The codistinctions between fibronectin and cytoskeletal proteins in these cells are also very extended (Hynes and Destree, 1978; Singer, 1982; Singer and Paradiso, 1981; Hynes et al., 1982). Thus, in both cell types the distribution of integrin β1 subunits corresponds well with the coalignments observed between fibronectin and the cytoskeleton.

The availability of this cytoplasmic domain peptide and of its antibody should facilitate future investigations of the role of the cytoplasmic domain of integrin β1 in the transmembrane link between the extracellular matrix and the cytoskeleton. In addition, the cross-reactivity of this antiserum with proteins in invertebrate species where genetic analysis is feasible should facilitate investigation of the role of integrins in development.

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