Epithelial Integrin $\alpha_6\beta_4$: Complete Primary Structure of α_6 and Variant Forms of β_4

Richard N. Tamura, Carla Rozzo, Lisa Starr, James Chambers, Louis F. Reichardt,* Helen M. Cooper, and Vito Quaranta

Department of Immunology, IMM-8, Research Institute of Scripps Clinic, La Jolla, California 92037; and * Department of Physiology, Division of Neuroscience and Howard Hughes Medical Institute, University of California, San Francisco, California 94143-0724

Abstract. The integrin $\alpha_6\beta_4$ is a heterodimer predominantly expressed by epithelia. While no definite receptor function has yet been assigned to it, this integrin may mediate adhesive and/or migratory functions of epithelial cells. We have determined the complete primary structure of both the α_6 and β_4 subunits from cDNA clones isolated from pancreatic carcinoma cell line libraries. The deduced amino acid sequence of α_6 is homologous to other integrin α chains (18–26% identity). Antibodies to an α_6 carboxy terminus peptide immunoprecipitated $\alpha_6\beta_4$ complexes from carcinoma cells and $\alpha_6\beta_1$ complexes from platelets, providing further evidence for the association of α_6 with more than one β subunit. The deduced amino acid sequence of β_4 predicts an extracellular portion homologous to other integrin β chains, and a unique cytoplasmic domain comprised of > 1,000 residues. This agrees with the structures of the β_4 cDNAs from nor-

mal epithelial cells (Suzuki, S., and Y. Naitoh. 1990. *EMBO [Eur. Mol. Biol. Organ.] J.* 9:757–763; Hogervost, F., I. Kuikman, A. E. G. Kr. von dem Borne, and A. Sonnenberg. 1990. *EMBO [Eur. Mol. Biol. Organ.] J.* 9:765–770). Compared to these structures, however, the β_4 cDNAs that we have cloned from carcinoma cells contain extra sequences. One of these is located in the 5′-untranslated region, and may encode regulatory sequences. Another specifies a segment of 70 amino acids in the cytoplasmic tail. Amplification by reverse transcription-polymerase chain reaction of mRNA indicated that multiple forms of β_4 may exist, possibly due to cell-type specific alternative splicing. The unique structure of β_4 suggests its involvement in novel cytoskeletal interactions. Consistent with this possibility, $\alpha_6\beta_4$ is mostly concentrated on the basal surface of epithelial cells, but does not colocalize with components of adhesion plaques.

THE interaction of cells with the extracellular matrix is important for the formation, maintenance, and repair of tissues as well as for other biological processes such as the metastasis of cancer cells. This interaction is mediated, in part, by a family of cell surface receptors called integrins (Hynes, 1987; Ruoslahti and Pierschbacher, 1987; Buck and Horwitz, 1987). These receptors form a link between the extracellular matrix and the cytoskeleton and may transmit signals from the extracellular to the intracellular environment that affect cell behavior.

Integrins are heterodimers comprised of α and β subunits, that are noncovalently associated transmembrane glycoproteins. At least 11 α chains (Ruoslahti and Giancotti, 1989) and 6 β chains (Sheppard et al., 1990) have been recognized in man. Each α subunit tends to associate with only one type of β subunit, but there are several exceptions to this rule (Hemler et al., 1989; Cheresh et al., 1989; Holzmann et al., 1989; Freed et al., 1989).

We and others have previously reported the identification of an integrin expressed primarily on epithelial-type cells, termed $\alpha_E\beta_4$ or $\alpha_6\beta_4$ (Kajiji et al., 1987, 1989; Hemler et al., 1989). The alpha subunit, α_6, also appears to associate with the β_1 integrin subunit and, on platelets, $\alpha_6\beta_1$ has been shown to function as a laminin receptor (Sonnenberg et al., 1988).

Here we report the complete cDNA sequence and the deduced amino acid sequence of both the α_6 and the β_4 subunits. Both α_6 and β_4 are homologous with the other integrin α and β chains, respectively. However, they contain unique structural features which may suggest novel functional properties.

Materials and Methods

Cells

The human pancreatic carcinoma cell line FG was cultured as described previously (Kajiji et al., 1989) in RPMI 1640 supplemented with 10% fetal calf serum, 2 mM glutamine, and penicillin-streptomycin (50 IU/ml–50 μg/ml). The human colon carcinoma cell line LoVo (Drewinko et al., 1976)

This is publication No. 6533-IMM from the Research Institute of Scripps Clinic.

was cultured in DME supplemented as above. Human platelets were the generous gift of Dr. Mark Ginsberg (Research Institute of Scripps Clinic, La Jolla, CA).

Cell Labeling

FG cells (10^7) were metabolically labeled with [^{35}S]methionine as described previously (Kajiji et al., 1989). Platelets were surface labeled with [^{125}I]sodium iodide and lactoperoxidase essentially as described by Roth (1975). Preparation of nonionic detergent cell extracts, immunoprecipitation, and analysis by SDS-PAGE have been described previously (Kajiji et al., 1989).

Antibodies

The mouse monoclonal antibody S3-41 and the rabbit polyclonal antibody 5710 recognize $\alpha_6\beta_4$ (Kajiji et al., 1987, 1989). The rat monoclonal antibody GoH3 (Sonnenberg et al., 1987), which is specific for α_6, was the generous gift of Dr. Arnoud Sonnenberg (University of Amsterdam, The Netherlands). The "anti-α_6 cyto" rabbit antiserum was raised to a synthetic peptide (IHAQPSDKERLTSDA) corresponding to the carboxy terminus of α_6 based on the deduced amino acid sequence reported here. The monoclonal antibody, AA3, was produced using standard hybridoma procedures (Kohler and Milstein, 1975) from mice immunized with placental $\alpha_6\beta_4$ purified on S3-41 affinity columns (Kajiji et al., 1989). AA3 has been shown to be specific for the β_4 subunit by immunoprecipitation and Western blotting. Full characterization of this antibody will appear elsewhere (Cooper, H. M., and V. Quaranta, manuscript in preparation).

cDNA Library Screening

Three different cDNA libraries were constructed (Invitrogen, San Diego, CA) from mRNA isolated from FG cells: an oligo-dT primed expression library in λgt11 and two plasmid (pTZ18R Bst XI; Invitrogen) libraries, one oligo-dT primed, and the other random primed.

The following oligonucleotides were synthesized with a Gene Assembler (Pharmacia, Uppsala, Sweden) according to the amino-terminal sequence of the mature α_6 protein (Kajiji et al., 1989): (a) 40-mers with 64-fold redundancy covering the first 13 amino acids (FNLDTREDNVIRK)

5'-TTCAACTTAGACACGCGAGAGGACAACGTAATCCGAAAGT-3'.
          C   G   T   T   G   G

(b) 14-mers covering the complete redundancy of the first 5 amino acids

(1) 5'-TTTAATCTAGATAC-3'
          C   C   T   C
(2) 5'-TTTAATCTGGATAC-3'
          C   C   C   C
(3) 5'-TTTAATTTGGACAC-3'.
          C   C   A   T

Screening of the random-primed cDNA library was performed with the 40-mers, labeled with [γ^{32}P]ATP and T4 polynucleotide kinase (labeling kit from Pharmacia), using low stringency conditions (hybridization at 37°C overnight and washes with 2× SSC at room temperature and at 46°C, 30-min each). 44 positives were then hybridized with the complete set of 14-mers followed by washes in 3.0 M tetramethylammonium chloride (TMAC) at progressively higher temperatures as described by Wood et al., (1985). Three clones, α6.1, α6.31, and α6.44, remained positive up to the melting temperature of 14-mers (46°C).

The insert from the α6.1 cDNA clone was isolated and used to screen the oligo-dT primed cDNA library. Preparation of probes, filters, hybridization, and washes were performed according to Maniatis et al. (1982).

The λgt11 library ($\sim$1 × 10^6 plaques) was screened (Young and Davis, 1983) with the rabbit antiserum 5710, which was raised against purified human $\alpha_6\beta_4$ and previously shown by Western blots to react predominantly with β_4 (Kajiji et al., 1989). 72 positive clones from the antibody screening were plaque purified and the cDNA inserts were amplified directly from bacteriophage plaques using the polymerase chain reaction (PCR).[1] Briefly, a plug of agar containing the plaque was transferred to 1 ml of SM (10 mM Tris-HCl pH 8.0, 50 mM NaCl, 5 mM MgCl$_2$) and incubated for either 1 h at room temperature or overnight at 4°C. 10 μl of supernatant

---

1. *Abbreviations used in this paper*: ORF, open reading frame; PCR, polymerase chain reaction.

was used in a 50 μl PCR containing 67 mM Tris-HCl pH 8.8, 1.5 mM MgCl$_2$, 10 mM β-mercaptoethanol, 1.25 U of TAQ I polymerase, 0.25 mM each of dATP, dTTP, dCTP, and dGTP, and 0.1 μg each of 24-mer oligonucleotides corresponding to sequences of λgt11 flanking the Eco RI cloning site. The PCR program consisted of 3 steps: (a) one cycle at 94°C for 4 min; (b) 40 cycles of 1 min at 94°C, 2 min at 55°C, and 3 min at 72°C with a 5-per cycle extension on the 72°C segment; (c) 10 min at 72°C and a final shift to 4°C. These amplified fragments were isolated using Gene Clean (Bio 101, La Jolla, CA), digested with either Eco RI or Not I, repurified with Gene Clean, and subcloned into pKS+ (Stratagene, La Jolla, CA).

The 72 positive inserts were arranged into 11 groups based on cross-hybridization. Fusion proteins produced by clones representative of a group were used to select epitope specific antibodies (Weinberger et al., 1985) from the rabbit antiserum, 5710. These antibodies were tested for their ability to immunoprecipitate β_4 from a SDS/heat-denatured FG lysate. The clone lam 18.2.1 (part of a group of 13 crosshybridizers) was identified as a β_4 clone by this epitope selection method (Fig. 5 A). The insert from this clone was used to screen both of the plasmid cDNA libraries to isolate overlapping clones. Additional screenings were done using radiolabeled inserts from β_4 positive cDNA clones until the complete β_4 cDNA was isolated.

DNA Sequencing

The α_6 cDNA clones were sequenced from restriction fragments (Pvu II or Sma I for α6.1; Ava I + Bam HI or Eco RI + Hind III for α6.1-7) subcloned into pKS+. The β_4 cDNA clones were sequenced from nested deletion subclones created using a kit from Pharmacia. Both strands were sequenced by dideoxy chain termination (Sanger et al., 1977) either on an ABI 370A DNA Sequencer (Applied Biosystems, Inc. Foster City, CA) or manually (Sequenase kit; USB, Cleveland, Ohio) using either the T3 and T7 polymerase vector primer sequences or specific oligonucleotide primers synthesized to appropriate regions of the α_6 or β_4 sequence. Sequences were analyzed on a VAX-VMS version 5.2 computer, with the programs of the University of Wisconsin Genetics Computer Group (Devereux et al., 1984).

cDNA Synthesis and PCR

Poly-A+ RNA was isolated from human placenta and human carcinoma cells (Fast Track Kit; Invitrogen). 2-5 μg was used to synthesize cDNAs with AMV reverse transcriptase (20 U; Molecular Genetics Resources, Tampa, FL) and 1 μg of random hexamer primers (Pharmacia). The cDNAs were extracted with phenol/chloroform, precipitated with ethanol, and resuspended in 100 μl of water. 1 μl was amplified using the PCR conditions described above except for the primer concentrations (1 μM each) and, for reactions using oligos 3 + 4 and oligos 5 + 6, the annealing temperature (52°C). The following oligonucleotides were used (see Fig. 6 for numbering; 5' nucleotide is in bold face type): (a) (82-98), (b) (310-329), (c) (4441-4482), (d) (4679-4697), (e) (4679-4697), and (f) (4805-4820). 10 μl of the PCR mixture were separated on a 3.5% acrylamide gel along with 1 kb molecular size markers (Bethesda Research Laboratories, Gaithersburg, MD) and the DNA was visualized by staining with ethidium bromide.

To confirm the identity of PCR bands, these were subcloned and sequenced. Briefly one-half of PCR reaction was purified on glass beads (Geneclean, Bio 101) and ligated in the Sma I site of the vector pKS+ (Stratagene). Plasmids prepared from bacteria transformed with the ligation mixture were restriction digested and their inserts, if present, were sequenced in both directions as described above.

Immunofluorescence

Cultured carcinoma cells were trypsinized, plated on coverslips coated with Matrigel (Collaborative Research, Lexington, MA) and cultured for 2 d. They were then fixed and stained with appropriate antibodies as described (Marchisio et al., 1984). Briefly, cells were fixed in 3% paraformaldehyde for 10 min at room temperature, permeabilized with the nonionic detergent Triton X100 (0.5%) for 5 min on ice, and then incubated with primary antibodies: mouse monoclonal AA3 specific for human β_4, control monoclonal Q5/13 specific for HLA-DR, a monoclonal antibody specific for vinculin (Sigma Chemical Co., St. Louis, MO), affinity-purified rabbit antibodies to talin (a gift from F. Giancotti, La Jolla Cancer Research Foundation, La Jolla, CA), protein A-purified anti-α_6 cytoplasmic tail antibodies, control, irrelevant, rabbit immunoglobulin. Actin was stained with fluorescein isothiocyanate-conjugated phalloidin (Sigma Chemical Co.).

Secondary antibodies were affinity purified goat anti-rabbit or anti-mouse immunoglobulins, conjugated to either fluorescein or rhodamine (Sigma Chemical Co.). Cells were viewed with a Zeiss Axiophot microscope equipped with an epifluorescence source, and photographed on Kodak T-max 3200.

Results

Isolation of cDNA Clones Encoding α_6

We have previously isolated the integrin $\alpha_6\beta_4$ from human carcinoma cells and human placenta and determined the amino-terminal sequence of both subunits (Kajiji et al., 1989). Based on the sequence of the amino terminus for α_6, degenerate oligonucleotides were synthesized and used to screen 3×10^5 colonies from a random primed human pancreatic carcinoma (FG) cDNA library as described in Materials and Methods. Three clones with inserts of ~ 1.2 kb were isolated and sequenced. All three clones ($\alpha 6.1$, $\alpha 6.31$, and $\alpha 6.44$) overlapped and contained open reading frames (ORFs) whose deduced amino acid sequence matched exactly the protein sequence of the amino terminus of α_6 (Kajiji et al., 1989; Hemler et al., 1989).

To isolate the rest of the α_6 gene, $\sim 7.2 \times 10^5$ colonies of the oligo-dT primed cDNA plasmid library was screened with the radiolabeled insert from the $\alpha 6.1$ clone. Of 16 positive clones, one ($\alpha 6.1$-7) had an insert of 4.8 kb. Overlapping restriction endonuclease fragments of the α_6 cDNA inserts were subcloned into pKS and sequenced in both orientations. Fig. 1 A shows the overall relationship of the α_6 cDNA clones to one another and the restriction sites relevant for subcloning and sequencing. The resulting sequence consists of 146 bp of the 5'-untranslated region, an ORF of 3,219

bp encoding 1,078 amino acids, and 2,264 bp of the 3'-untranslated region (Fig. 2).

Analysis of the Primary Structure of α_6

Preceding the amino terminus of the mature protein is a possible signal sequence of 23 amino acids. The mature protein is comprised of 1,055 amino acids with a M_r $\sim 117,000$. The addition of carbohydrate with an average 2,500 M_r to the protein core at the 10 potential N-glycosylation sites (N-X-S/T) would result in an estimated size of 142,000 M_r. This relative molecular mass corresponds well with the 150,000 M_r estimated from the migration of α_6 on SDS-polyacrylamide gels under nonreducing conditions.

A hydropathy profile (Kyte and Doolittle, 1982) of the deduced protein sequence identified a putative transmembrane region from amino acid residues 1,012–1,037. After this transmembrane region are 36 amino acids (residues 1,038–1,073) which comprise the presumed cytoplasmic domain. Analysis of the amino-terminal portion of the molecule revealed seven homologous repeat (domain I, residues 42–79; II, 113–145; III, 185–217; IV, 256–292; V, 314–352; VI, 375–411; VII, 430–470). The last three domains each contain a sequence motif resembling the cation binding site consensus sequence of D-X-D/N-X-D/N-G-X-X-D found in a number of calcium-binding proteins (Van Eldik et al., 1982). A fourth site, residues 230–238, with weak homology to this calcium binding site motif resides between repeated domains III and IV. These potential cation binding sites are in a region (residues 189–488) of the molecule that is devoid of cysteine residues.

The migration of α_6 in SDS-polyacrylamide gels under reducing conditions indicated that α_6 consisted of two poly-
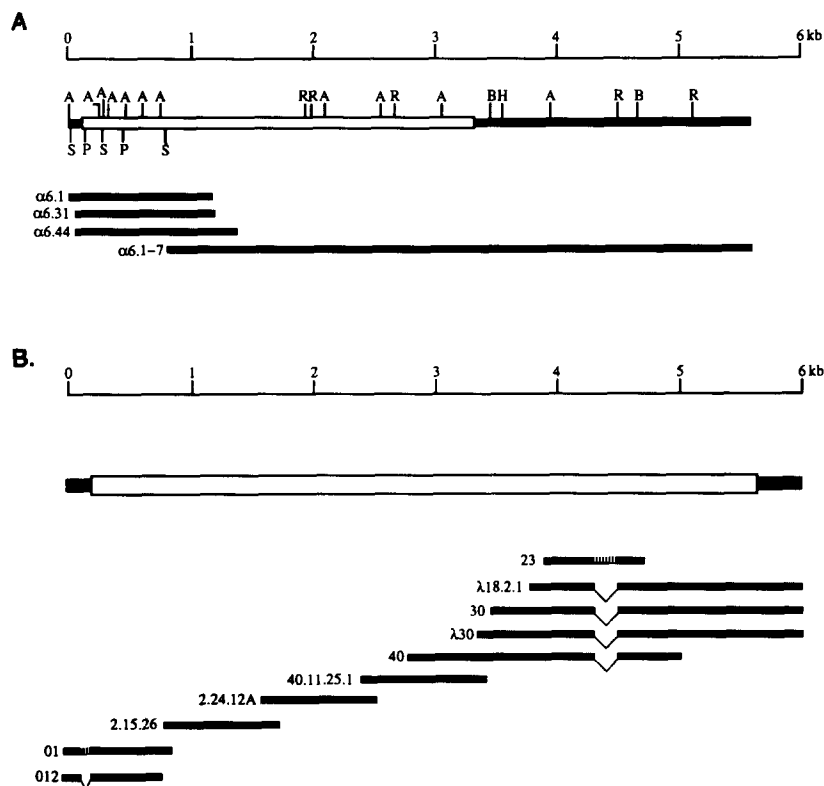
Figure 1. Restriction map of the α_6 (A) and β_4 (B) cDNA clones. The ORFs for α_6 and β_4 are shown as open bars. The lines indicate the size and position of the plasmid and phage cDNA clones isolated from the three cDNA libraries. Restriction sites relevant to subcloning and sequencing are Ava I (A), Bam HI (B), Pvu II (P), Eco RI (R), and Sma I (S). Representative clones out of a total of 54 are shown for β_4.

```
   1 GCGCGACCGTCCCGGGGGTGGGGCCGGGCGCAGCGGCGAGAGGAGGCGAAGGTGGCTGCGGTAGCAGCAGCGCGGCAGCCTCGGACCCAGCCCGGAGCGC
 101 AGGGCGGCCGCTGCAGGTCCCCGCTCCCCTCCCCGTGCGTCCGCCCATGGCCGCGCGCCGGGCAGCTGTGCTTGCTCTACCTGTCGGCGGGGCTCCTGTCC
                                            M  A  A  A  G  Q  L ©  L  L  Y  L  S  A  G  L  L  S        18
 201 CGGCTCGGCGCAGCCTTCAACTTGGACACTCGGGAGGACAACGTGATCCGGAAATATGGAGACCCCGGGAGCCTCTTCGGCTTCTCGCTGGCCATGCACT
       R  L  G  A  A  F  N  L  D  T  R  E  D  N  V  I  R  K  Y  G  D  P  G  S  L  F  G  F  S  L  A  M  H  W    52
 301 GGCAACTGCAGCCCGAGGACAAGCGGCTGTTGCTCGTGGGGGCCCCGCGCGGAGAAGCGCTTCCACTGCAGAGAGCCTTCAGAACGGGAGGGCTGTACAG
       Q  L  Q  P  E  D  K  R  L  L  L  V  G  A  P  R  G  E  A  L  P  L  Q  R  A  F  R  T  G  G  L  Y  S      85
 401 CTGCGACATCACCGCCCGGGGGCCCATGCACGCGGATCGAGTTTGATAACGATGCTGACCCCACGTCAGAAAGCAAGGAAGATCAGTGGATGGGGGTCACC
     ©  D  I  T  A  R  G  P ©  T  R  I  E  F  D  N  D  A  D  P  T  S  E  S  K  E  D  Q  W  M  G  V  T       118
 501 GTCCAGAGCCAAGGTCCAGGGGGCAAGGTCGTGACATGTGCTCACCGATATGAAAAAAGGCAGCATGTTAATACGAAGCAGGAATCCCGAGACATCTTTG
       V  Q  S  Q  G  P  G  G  K  V  V  T ©  A  H  R  Y  E  K  R  Q  H  V  N  T  K  Q  E  S  R  D  I  F  G    152
 601 GGCGGTGTTATGTCCTGAGTCAGAATCTCAGGATTGAAGCAGATATGGATGGGGGAGATTGGAGCTTTTGTGATGGGCGATTGAGAGGCCATGAGAAATT
       R ©  Y  V  L  S  Q  N  L  R  I  E  D  D  M  D  G  G  D  W  S  F ©  D  G  R  L  R  G  H  E  K  F       185
 701 TGGCTCTTGCCAGCAAGGTGTAGCAGCTACTTTTACTAAAGACTTTCATTACATTGTATTTGGAGCCCCGGGTACTTATAACTGGAAAGGGATTGTTCGT
       G  S ©  Q  Q  G  V  A  A  T  F  T  K  D  F  H  Y  I  V  F  G  A  P  G  T  Y  N  W  K  G  I  V  R      218
 801 GTAGAGCAAAAGAATAACACTTTTTTTGACATGAACATCTTTGAAGATGGGCCTTATGAAGTTGGTGGAGAGACTGAGCGATGATGAAAGTCTCGTTCCTG
       V  E  Q  K  N  N  T  F  F  D  M │N  I  F  E  D  G  P  Y  E│ V  G  G  E  T  E  H  D  E  S  L  V  P  V    252
 901 TTCCTGCTAACAGTTACTTAGGTTTTTCTTTGGACTCAGGGAAAGGTATTGTTTCTAAAGATGAGATCACTTTTGTATCTGGTGCTCCCAGAGCCAATCA
       P  A  N  S  Y  L  G  F  S  L  D  S  G  K  G  I  V  S  K  D  E  I  T  F  V  S  G  A  P  R  A  N  H      285
1001 CAGTGGAGCCGTGGTTTTGCTGAAGAGAGACATGAAGTCTGCACATCTCCTCCCTGAGCACATATTCGATGGAGAAGGTCTGGCCTCTTCATTGGCTAT
       S  G  A  V  V  L  L  K  R  D  M  K  S  A  H  L  L  P  E  H  I  F  D  G  E  G  L  A  S  S  F  G  Y      318
1101 GATGTGGCGGTGATGGACCTCAACAAGGATGGGTGGCAAGATATAGTTATTGGAGCCCCACAGTATTTTGATAGAGATGGAGAAGTTGGAGGTGCAGTGT
       D  V  A  V  M │D  L  N  K  D  G  W  Q  D│ I  V  I  G  A  P  Q  Y  F  D  R  D  G  E  V  G  G  A  V  Y    352
1201 ATGTCTACATGAACCAGCAAGGCAGATGGAATAATGTGAAGCCAATTCGTCTTAATGGAACCAAAGATTCTATGTTTGGCATTGCAGTAAAAAAATATTGG
       V  Y  M  N  Q  Q  G  R  W  N  N  V  K  P  I  R  L  N  G  T  K  D  S  M  F  G  I  A  V  K  N  I  G      385
1301 AGATATTAATCAAGATGGCTACCCAGATATTGCAGTTGGAGCTCCGTATGATGACTTGGGAAAGGTTTTTATCTATCATGGATCTGCAAATGGAATAAAT
     │D  I  N  Q  D  G  Y  P  D│ I  A  V  G  A  P  Y  D  D  L  G  K  V  F  I  Y  H  G  S  A  N  G  I  N      418
1401 ACCAAACCAACACAGGTTCTCAAGGGTATATCACCTTATTTTGGATATTCAATTGCTGGAAACATGGACCTTGATCGAAATTCCTACCCTGATGTTGCTG
       T  K  P  T  Q  V  L  K  G  I  S  P  Y  F  G  Y  S  I  A  G  N  M │D  L  D  R  N  S  Y  P  D│ V  A  V    452
1501 TTGGTTCCCTCTCAGATTCAGTAACTATTTTCAGATCCCGGCCTGTGATTAATATTCAGAAAACCATCACAGTAACTCCTAACAGAATTGACCTCCGCCA
       G  S  L  S  D  S  V  T  I  F  R  S  R  P  V  I  N  I  Q  K  T  I  T  V  T  P  N  R  I  D  L  R  Q      485
1601 GAAAACAGCGTGTGGGGCGCCTAGTGGGATATGCCTCCAGGTTAAATCCTGTTTTGAATATACTGCTAACCCCGCTGGTTATAATCCTTCAATATCAATT
       K  T  A ©  G  A  P  S  G  I ©  L  Q  V  K  S ©  F  E  Y  T  A  N  P  A  G  Y  M  P  S  I  S  I       518
1701 GTGGGCACACTTGAAGCTGAAAAAGAAAGAAGAAAATCTGGGCTATCCTCAAGAGTTCAGTTTCGAAACCAAGGTTCTGAGCGCAAATATACTCAAGAAC
       V  G  T  L  E  A  E  K  E  R  R  K  S  G  L  S  S  R  V  Q  F  R  N  Q  G  S  E  P  K  Y  T  Q  E  L    552
1801 TAACTCTGAAGAGGCAGAAACAGAAAGTGTGCATGGAGGAAACCCTGTGGCTACAGGATAATATCAGGGATAAGCTGCGTCCCATTCCCATAACTGCCTC
       T  L  K  R  Q  K  Q  K  V ©  M  E  E  T  L  W  L  Q  D  N  I  R  D  K  L  R  P  I  P  I  T  A  S      585
1901 AGTGGAGATCCAAGAGCCAAGCTCTCGTAGGCGAGTGAATTCACTTCCAGAAGTTCTTCAATTCTGAATTCAGATGAACCCAAGACAGCTCATATTGAT
       V  E  I  Q  E  P  S  S  R  R  R  V  N  S  L  P  E  V  L  P  I  L  N  S  D  E  P  K  T  A  H  I  D      618
2001 GTTCACTTCTTAAAAGAGGGATGTGGAGACGACAATGTATGTAACAGCAACCTTAAACTGAATATAAATTTTGCACCCGAGAAGGAAATCAAGACAAAT
       V  H  F  L  K  E  G ©  G  D  D  N  V ©  N  S  N  L  K  L  E  Y  K  F ©  T  R  E  G  N  Q  D  K  F     652
2101 TTTCTTATTTACCAATTCAAAAAGGTGTACCAGAACTAGTTCTAAAAGATCAGAAGGATATTGCTTTAGAAATAACAGTGACAAACAGCCCTTCCAACCC
       S  Y  L  P  I  Q  K  G  V  P  E  L  V  L  K  D  Q  K  D  I  A  L  E  I  T  V  T  N  S  P  S  N  P     685
2201 AAGGAATCCCACAAAAGATGGCGATGACGCCCATGAGGCTAAACTGATTGCAACGTTTCCAGACACTTTAACCTATTCTGCATATAGAGAACTGAGGGCT
       R  N  P  T  K  D  G  D  D  A  H  E  A  K  L  I  A  T  F  P  D  T  L  T  Y  S  A  Y  R  E  L  R  A      718
2301 TTCCCTGAGAAACAGTTGAGTTGTGTTGCCAACCAGAATGGCTCGCAAGCTGACTGTGAGCTCGGAAATCCTTTTAAAAGAAATTCAAATGTCACTTTTT
       F  P  E  K  Q  L  S ©  V  A  N  Q  N  G  S  Q  A  D ©  E  L  G  N  P  F  K  R  N  S  N  V  T  F  Y    752
2401 ATTTGGTTTTAAGTACAACTGAAGTCACCTTTGACACCCCATATCTGGATATTAATCTGAAGTTAGAAACAACAAGCAATCAAGATAATTTGGCTCCAAT
       L  V  L  S  T  T  E  V  T  F  D  T  P  Y  L  D  I  N  L  K  L  E  T  T  S  N  Q  D  N  L  A  P  I      785
2501 TACAGCTAAAGCAAAAGTGGTTATTGAACTGCTTTTATCGGTCTCGGGAGTTGCTAAACCTTCCCAGGTGTATTTTGGAGGTACAGTTGTTGGCGGAGCAA
       T  A  K  A  K  V  V  I  E  L  L  L  S  V  S  G  V  A  K  P  S  Q  V  Y  F  G  G  T  V  V  G  E  Q      818
2601 GCTATGAAATCTGAAGATGAAGTGGGAAGTTTAATAGAGTATGAATTCAGGGTAATAAACTTAGGTAAACCTCTTACAAACCTCGGCACAGCAACCTTGA
       A  M  K  S  E  D  E  V  G  S  L  I  E  Y  E  F  R  V  I  N  L  G  K  P  L  T  N  L  G  T  A  T  L  N    852
2701 ACATTCAGTGGCCAAAAGAAATTAGCAATGGGAAATGGTTGCTTTATTTGGTGAAAGTAGAATCCAAAGGATTGGAAAAGGTAACTTGTGAGCCACAAA
       I  Q  W  P  K  E  I  S  N  G  K  W  L  L  Y  L  V  K  V  E  S  K  G  L  E  K  V  T ©  E  P  Q  K     885
2801 GGAGATAAACTCCCTGAACCTAACGGAGTCTCACAACTCAAGAAAGAAACGGGAAATTACTGAAAAACAGATAGATGATAACAGAAAATTTTCTTTATTT
       E  I  N  S  L  M  L  T  E  S  H  N  S │R  K  K  R│ E  I  T  E  K  Q  I  D  D  N │R  K│ F  S  L  F    918
2901 GCTGAAAGAAAATACCAGACTCTTAACTGTAGCGTGAACGTGAACTGTGTGAACATCAGATGCCCGCTGCGGGGGCTGGACAGCAAGGCGTCTCTTATTT
       A  E │R  K│ Y  Q  T  L  M ©  S  V  N  V  N ©  V  N  I  R ©  P  L  R  G  L  D  S  K  A  S  L  I      952
3001 TGCGCTCGAGGTTATGGAACAGCACATTTCTAGAGGAATATTCCAAACTGAACTACTTGGACATTCTCATGCGAGCCTTCATTGATGTGACTGCTGCTGC
       R  S  R  L  W  M  S  T  F  L  E  E  Y  S  K  L  N  Y  L  D  I  L  M  R  A  F  I  D  V  T  A  A  A      985
3101 CGAAAATATCAGGCTGCCAAATGCAGGCACTCAGGTTCGAGTGACTGTGTTTCCTCAAAGACTGTAGCTCAGTATTCGGAGTACCTTGGTGGATCATC
       E  N  I  R  L  P  N  A  G  T  Q  V  R  V  T  V  F  P  S  K  T  V  A  Q  Y  S  G  V  P  W  W  I  L     1018
3201 CTAGTGGCTATTCTCGCTGGGATCTTGATGCTTGCTTTATTAGTGTTTATACTATGGAAGTGTGGTTTCTTCAAGAGAAATAAGAAAGATCATTATGATG
       L  V  A  I  L  A  G  L  L  M  L  A  L  L  V  F  L  L  W  K  C ©│G  F  F  K  R│ N  K  K  D  H  Y  D  A  1052
3301 CCACATATCACAAGGCTGAGATCCATGCTCAGCCATCTGATAAAGAGAGGCTTACTTCTGATGCATAGTATTGATCTACTTCTGTAATTGTGTGGATTCT
       T  Y  H  K  A  E  I  H  A  Q  P  S  D  K  E  R  L  T  S  D  A                                         1073
3401 TTAAACGCTCTAGGTACGATGACAGTGTTCCCCGATACCATGCTGTAAGGATCCGGAAAGAAGAGCGAGAGATCAAAGATGAAAAGTATATTGATAACCT
3501 TGAAAAAAAACAGTGGATCACAAAGTGGAACAGAAATGAAAGCTACTCATAGCGGGGGCCTAAAAAAAAAAAAAGCTTCACAGTACCCAAACTGCTTTTTC
3601 CAACTCAGAAATTCAATTTGGATTTAAAAGCCTGCTCAATCCCTGAGGACTGATTTCAGAGTGACTACACACAGTACGAACCTACAGTTTTAACTGTGGA
3701 TATTGTTACGTAGCCTAAGGCTCCTGTTTTGCACAGCCAAATTTAAAACTGTTGGAATGGATTTTTCTTTAACTGCCGTAATTTAACTTTCTGGGTTGCC
3801 TTTGTTTTTGGCGTGGCTGACTTACATCATGTGTTGGGGAAGGGCCTGCCCAGTTGCACTCAGGTGACATCCTCCAGATAGTGTAGCTGAGGAGGCACCT
3901 ACACTCACCTGCACTAACAGAGTGGCCGTCCTAACCTCGGGCCTGCTGCGGCAGACGTCCACATCACAAGACTATGCCATTGGG
4001 GTAGTTGTGTGTTTCAACGGAAAGTGCTGTCTTAAACTAAATGTGCAATAGAAGGTGATGTTGCCATCCTACCGTCTTTTCCTGTTTCCTAGCTGTGTGAAT
4101 ACCTGCTCACGTCAAATGCATACAAGTTTCATTCTCCCTTTCACTAAAAACACACAGGTGCAACAGACTTGAATGCTAGTTATACTTATTTGTATATGGT
4201 ATTTATTTTTTCTTTTCTTTACAAACCATTTTGTTTATTGACTAACAGGCCAAAGAGTCCTCCAGTTTACCCTTCAGGTTGGTTTAATCAATCAGAATTAGA
4301 ATTAGAGCATGGGAGGGTCATCACTATGACCTAAATTATTTTACTGCAAAAAGAAAATCTTTATAAATGTACCAGAGAGAGTTGTTTTAATAACTTATCTA
4401 TAAACTATAACCTCTCCTTCATGACAGCCTCCACCCCACAACCCAAAAGGTTTAAGAAATAGAATTATAACTGTAAAGATGTTTATTTCAGGCATTGGAT
4501 ATTTTTTACTTTAGAAGCCTGCATAATGTTTCTGGATTTACATACTGTAACATTCAGGAATTCTTGGAGAAGATGGGTTTATTCACTGAACTCTAGTGCG
4601 GTTTACTCACTGCTGCAAATACTGTATATTCAGGACTTGAAAGAAATGGTGAATGCCTATGGAACTGGATCCAAACTGATCCAGTATAAGACTACTG
4701 AATCTGCTACCAAAACAGTTAATCAGTGAGTCGAGTGTTCTATTTTTTGTTTTGTTTCCTCCCCTATCTGTATTCCCAAAAATTACTTTGGGGCTAATTT
4801 AACAAGAACTTTAAATTGTGTTTTAATTGTAAAAATGGCAGGGGGTGGAATTATTACTCTATACATTCAACAGAGACTGAATAGATATGAAAGCTGATTT
4901 TTTTTAATTACCATGCTTCACAATGTTAAGTTATATGGGGAGCAACAGCAAACAGGTGCTAATTTGTTTTGGATATAGTATAAGCAGTGTCTGTGTTTTG
5001 AAAGAATAGAACACAGTTTGGTAGTGCCACTGTTGTTTTGGGGGGGCTTTTTTCTTTTTCCGGAAAATCCTTAAACCTTAAGATACTAAGGACGTTGTT
5101 TTGGTTGTACTTGGAATTCTTAGTCACAAAATATATTTTGTTTACAAAAATTTCTGTAAAACAGGTTATAACAGTGTTTAAAGTCTCAGTTTCTTGCTTG
5201 GGGAACTTGTGTCCCTAATGTGTTAGAGTTGCTAGATTGCTAAGGAGCTGATACTTGACAGTTTTTTAGACCTGTGTTACTAAAAAAAAAGATGAATGTCGG
5301 AAAAGGGTGTTGGGAGGGTGGTCAACAAAGAAACAAAGATGTTATGGTGTTTAAAAATGTCATCTCAAGTCAAGTCACTGGTCTG
5401 TTTGCATTTGATACATTTTTGTACTAACTAGCATTGTAAAATTATTTCATGATTAGAAATTACCTGTGGATATTTGTATAAAAAGTGTG│AAATAAA│TTTTT
5501 TATAAAAGTGTTCATTGTTTCGTAACACAGCATTGTATATGTGAAGCAAACTCTAAAATTATAAATGACAACCTGAATTTATCTATTTCATCAAAAAAAAA
5601 AAAAAAAAAAACTTTATGGGCACAACTGG
```

peptides joined by a disulfide bridge (Sonnenberg et al., 1987; Kajiji et al., 1989) similar to the integrin α subunits α₅, αᵥ, αₗₗᵦ, and αₚₛ₂. In the region of α₆ corresponding to this potential cleavage site (residues 899–923), there are three sets of dibasic residues. The use of two of these sites could account for the appearance of the smaller polypeptide as a doublet on SDS-polyacrylamide gels (Sonnenberg et al., 1987). This doublet may also arise from differences in glycosylation at the two sites present in the light chain. The sequence RKKRE, which most closely resembles the cleavage site of other integrin α chains, is at position 899–903. Cleavage at this site would result in the formation of a heavy chain of 118,000 M_r and a light chain of 24,000 M_r, which are close to the 125,000 M_r and 30,000 M_r estimated by SDS-PAGE.

Comparison of α₆ with the Other Integrin α Subunits

Alignment with the other integrin α subunits shows that α₆ shares several structural features with these proteins (Fig. 3). Of the 20 cysteines in the mature α₆ protein, 11 are in equivalent positions to those found in the other 10 α chains, 5 are shared with 9 of the other 10 alphas, and 2 are shared with the other 5 α chains which do not contain the I domain. One cysteine (residue 1039) is shared with the PS2 α chain, and another cysteine (residue 643) is unique to α₆. Cysteine 643 is in a region of the molecule (residues 641–689) in which two gaps of about 15 and 9 amino acids were placed in the other α chains in order to optimize the alignment. Three of the four putative cation binding sites of α₆ align with similar sites in the other α subunits. A fourth weakly homologous cation binding site (residues 230–238) precedes, slightly, the cation binding sites present in the other α chains which lack the I domain. The cytoplasmic domain of α₆ contains the sequence GFFKR which is absolutely conserved in all but the *Drosophila* PS2 α subunit, where the K is replaced by an N.

Some of the integrin α chains contain additional polypeptide sequence. α₂ and the α chains associated with β₂ (αₗ, αₘ, αₚₗₛₒ) contain an insert domain (I domain) near the amino terminus of these molecules and the *Drosophila* PS2 α subunit contains an insert just preceding the transmembrane portion of this molecule. α₆ does not contain either of these inserts.

Based on the alignment shown in Fig. 3, α₆ shares 18–26% identity with the other integrin alpha subunits. The highest homology is with αᵥ, α₅, and αₚₛ₂ (25–26% identity) and the lowest homology is with those α chains which contain the I-domain (18% identity).

Further Evidence for the Identity of the α₆ cDNA Clones and Association of α₆ with Both β₄ and β₁

A rabbit antiserum (anti-α₆ cyto) was prepared to a syn-

thetic peptide corresponding to the last 15 amino acids of the deduced α₆ amino acid sequence. This antiserum precipitated the α₆β₄ complex from a radiolabeled FG lysate (Fig. 4 A), indicating that the cDNA clones isolated in this study encode the α₆ protein. We then tested this antiserum with platelets that express α₆β₁ (Sonnenberg et al., 1987) but not α₆β₄. Fig. 4 B shows that this antiserum precipitated the α₆β₁ complex from a radiolabeled platelet lysate. These results provide further evidence that α₆ forms stable heterodimers with both β₁ and β₄ (Hemler et al., 1989). However, they do not exclude the possibility of slight structural variations in α₆ which may be responsible for preferential association with either of the β chains.

Isolation of cDNA Clones Encoding β₄

The cDNA for β₄ was isolated by antibody screening of a phage expression library followed by colony hybridization of two plasmid libraries (see Materials and Methods) (Fig. 5 A). Overlapping clones covering ∿5.9 kb were sequenced (Fig. 1 B) and shown to encode β₄ protein because: (a) they contain an ORF of 1,822 amino acids starting with a 27-residue putative signal peptide followed by a 13 amino acid sequence identical to the β₄ protein amino terminus we previously determined (Kajiji et al., 1989); (b) their nucleotide sequence is essentially identical to that of recently reported β₄ cDNA clones from normal epithelial cells (Suzuki and Naitoh, 1990; Hogervorst et al., 1990), with the exception of two locations described in detail below. To summarize the salient features, the mature β₄ protein contains a 710 amino acid extracellular domain, showing obvious sequence homology to integrin β chains (including cysteine conservation at 47/56 positions), followed by a predicted 23 amino acid transmembrane region, and finally 1,089 amino acids that presumably represent an unusually large cytoplasmic domain. The total mass of the mature protein is 200,000 or 212,500 if all five extracytoplasmic putative N-glycosylation sites are used.

To test whether or not the large carboxyl-terminal sequence was part of the β₄ protein in complex with α₆, a native FG lysate was immunoprecipitated with the antibodies eluted from the cytoplasmic domain fusion protein (clone lam-18.2.1). These antibodies precipitated a complex of proteins with SDS-PAGE mobilities identical to those of authentic α₆β₄ heterodimers (Fig. 5 B).

Evidence for Possible Multiple Forms of β₄ mRNAs

Some of the cDNA clones were found to contain insertions in two locations: one insertion was identified in the 5′-untranslated region, and the other in the coding region corresponding to the cytoplasmic tail.

Thirteen independent clones, covering the 5′ end of the β₄ cDNA, were sequenced. Six of them had an insertion of 49

Figure 2. Nucleotide sequence and deduced amino acid sequence of the human α₆ subunit. The sequence corresponding with the amino-terminal sequence determined on α₆ protein (Kajiji et al., 1989) is underlined. The putative transmembrane region is shown by a dashed underline. The arrowhead marks the position of cleavage between the signal sequence and the mature protein. Cysteines are circled. Potential sites of N-linked glycosylation are shown in bold face. Closed boxes outline the putative cation binding domains. Dashed boxes outline dibasic residues that may represent sites of cleavage in the formation of a heavy and a light chain. The cytoplasmic sequence GFFKR, which is conserved in virtually all of the integrins α chains, is denoted by a stippled dashed box. A potential polyadenylation signal is boxed. These sequence data are available from EMBL/GenBank/DDBJ under accession number X53586.
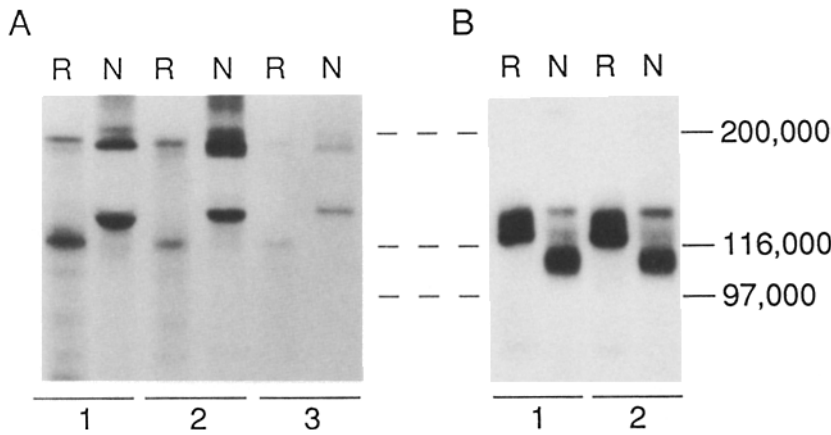
Figure 4. Immunoprecipitation of $\alpha_6\beta_4$ and $\alpha_6\beta_1$ with anti-α_6 cyto, an antibody raised to a synthetic peptide corresponding to the carboxy terminus of α_6. (A) Autoradiogram of immunoprecipitations of a detergent lysate of [^{35}S]methionine-labeled FG cells analyzed by SDS-PAGE under reducing (R) or nonreducing (N) conditions. Antibodies used were: anti-α_6 cyto (lanes 1), antiserum 5710 (lanes 2), and the monoclonal antibody S3-41 (lanes 3), both to $\alpha_6\beta_4$. (B) Autoradiogram of a SDS-PAGE analysis of immunoprecipitations of ^{125}I surface-labeled platelets with the monoclonal antibody to α_6, GoH3 (lanes 1), and the antiserum anti-α_6 cyto (lanes 2).

bp in the 5' untranslated sequence after position 129, 9 bp upstream of the ATG initiation codon (Fig. 6).

The second insertion sequence was found in the cytoplasmic tail where, in one out of the five clones analyzed, there was an insertion of 70 amino acids precisely in frame between threonine 1369 and glutamic acid 1440, located after bp 4294 (Fig. 6).

These extra sequences raised the possibility that multiple forms of β_4 cDNA exist in carcinoma cells. In addition, a β_4 cDNA from normal epithelial cells recently reported (Hogervorst et al., 1990) contains a 159-bp sequence in the cytoplasmic region not present in any of our clones and located after base pair 4,744 (Fig. 6, arrowhead).

To determine whether these sequences were actually present in cellular mRNAs, we amplified mRNAs from various cells using PCR. Oligonucleotide primers flanking the sites of insertion were synthesized (in one case, see Fig. 7 A, an internal primer was used) as described in Materials and Methods. PCRs using the primers flanking the 5' region insert generated products, consistent with the two types of cDNA we found, i.e., with or without the 49-bp insert (248 and 200 bp, respectively; Fig. 7 B, lanes 1 and 2), in two carcinoma cell lines tested (FG and LoVo). Of these, only the 200-bp band (i.e., without the insert) was obtained from placental mRNA (Fig. 7 B, lane 3). Unexpectedly, one band of 260 bp was seen in placental amplification products, suggesting the possibility of additional insertions in this region. The low intensity of the bands obtained from placental cDNA for the amplification of this region may be explained by low representation of 5' mRNA sequences due to degradation during mRNA extraction from tissues.

To confirm their identity, these PCR bands were subcloned and sequenced. From FG and LoVo PCRs, clones corre-

sponding to the 200- and 248-bp bands were found and shown to contain the correct nucleotide sequences. However, clones for the 260-bp band, as well as clones from placenta PCRs were not obtained. The identity of these bands deserves further study.

Using oligonucleotide primers specific to the region containing the insert at 4,294 bp (Fig. 7 B, lanes 4-6), a band of 255-bp was amplified from both LoVo and FG cDNAs but not from placenta cDNA, suggesting that mRNA containing this insert is expressed in carcinoma but not in normal cells.

PCR amplifications with primers flanking the insert at base pair 4,744 (Fig. 7 B, lanes 7-9) suggested that mRNAs, both with and without this insert, are present in placental tissue and that the form containing the insert is prevalent. Instead, LoVo and FG carcinoma cell mRNA produce only PCR bands corresponding to the insert-minus form. The identity of the 144-, 255-, and 354-bp bands was confirmed by the sequences of subclones from the respective PCRs.

These results suggest that multiple alternatively spliced forms of β_4 may exist in normal as well as transformed epithelial cells.

Cellular Distribution of $\alpha_6\beta_4$

The long cytoplasmic tail of β_4 is suggestive of unique interactions with cytoskeletal components. To gain some information on this possibility, the cellular distribution of $\alpha_6\beta_4$ with respect to adhesion plaques was examined by immunofluorescence studies. FG carcinoma cells were plated on Matrigel-coated glass, fixed, permeabilized, and stained by specific antibodies after 2 d of culture. The majority of the cells in these cultures had a typical epithelial appearance, forming clusters of 3-20 cells with epithelioid geometry, as

---

Figure 3. Alignment of α_6 deduced amino acid sequence with the other integrin α subunits. The other α chains are from human sources except where noted: 5 = fibronectin receptor (Argraves et al., 1987; Fitzgerald et al., 1987), V = vitronectin receptor (Suzuki et al., 1987), IIB = gpIIB (Poncz et al., 1987), 4 = VLA-4 (Takada et al., 1989), 2 = VLA-2 (Takada and Hemler, 1987), P150 (Corbi et al., 1987), M = MacI (Arnaout et al., 1988; Corbi et al., 1988), L = LFAI (Larson et al., 1989); M(M) = murine MacI (Pytela, 1988), and the position specific (PS2) α chain from Drosophila (Bogaert et al., 1987). Initial alignment was generously provided by Dr. Robert Pytela and modified to include the α_6 sequence with the aid of sequence comparison programs (Devereux et al., 1984). Closed circles and open circles represent positions were a residue is conserved among all 11 α chains or in 10 of 11 chains, respectively. Inverted triangles denote residues found in all six (closed) or five or six (open) of the α subunits which do not contain the I-domain. Potential cation binding sites are boxed with a closed line and a highly conserved cytoplasmic sequence (GFFKR) is boxed with a dashed line.
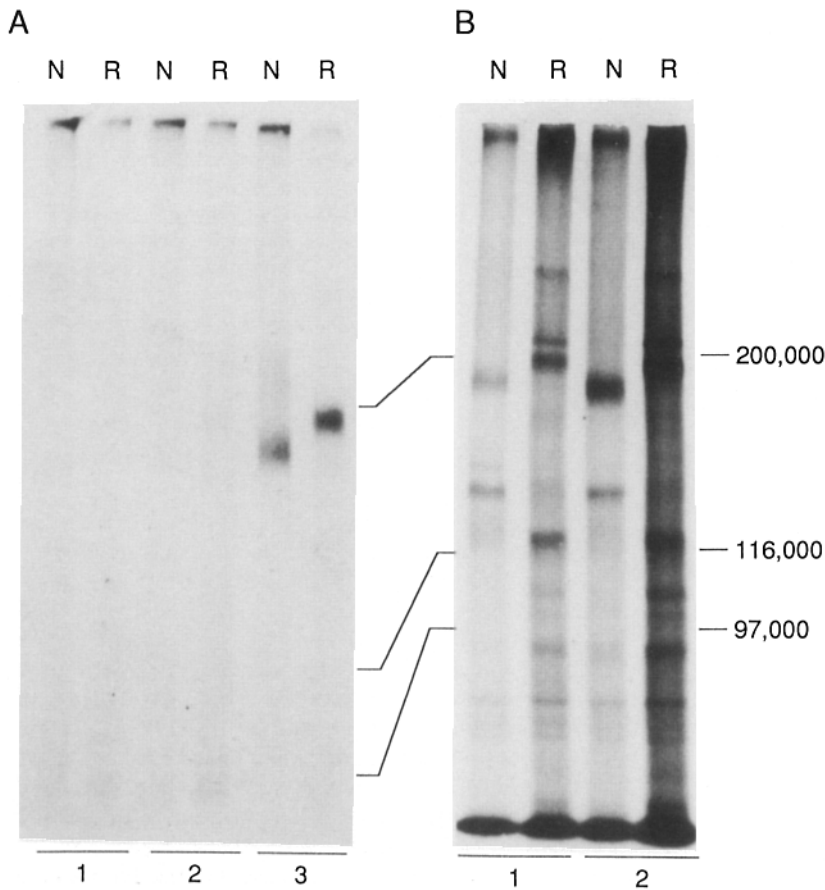
A   **B**

N R N R N R   N R N R

— 200,000

— 116,000

— 97,000

1   2   3   1   2

Figure 5. Immunoprecipitation of β_4 with antibodies selected with the lam 18.2.1 fusion protein. (*A*) Antibodies eluted from plaques lam 23.1.1 (lanes *1*), lam 8B (lanes *2*), and lam 18.2.1 (lanes *3*) were used for immunoprecipitations of a denatured [^{35}S]methionine FG lysate and analyzed by SDS-PAGE under reducing (*R*) or nonreducing (*N*) conditions. (*B*) β_4 specific monoclonal antibody, AA3 (lanes *1*) and antibodies eluted from plaque lam 18.2.1 (lanes *2*) were used for immunoprecipitations of a nondenatured [^{35}S]methionine FG lysate and analyzed by SDS-PAGE.

evidenced by phase-contrast microscopy (Fig. 8, *1 A*, *2 A*, and *3 A*). That these cells form adhesion plaques was shown by staining with antibodies to vinculin and talin, which can be visualized as spots on the external edges of spread cells in the periphery of the clusters (Fig. 8, *1 B* and *3 B*). Actin, specifically stained by fluorescent phalloidin, was also observed on the outer edges of spread cells in a pattern of spikes, and on the inside of these cells in small patches (Fig. 8, *2 B*). However, no clearly recognizable actin stress fibers were seen.

In cells stained simultaneously for both talin and β_4, the talin staining on the outer edges was often juxtaposed to that of β_4 (Fig. 8, *3 B* and *3 C*, *arrows*) but was not overlapping. In parts of the basal surface interior to the edge of the cells, talin was not detectable, while β_4 was visible and appeared to be arranged in small patches and streaks (Fig. 8, *3 C*, *arrows*). Cells double-stained with both anti-vinculin (mouse monoclonal) and anti-α_6 cytoplasmic tail (rabbit antibodies) gave very similar patterns to the cells double stained for talin and β_4 (not shown). These results suggest that the cellular distribution of $\alpha_6\beta_4$ is distinct from that of adhesion plaques.

Discussion

We have isolated cDNA clones encoding both α and β subunits of the epithelial integrin, $\alpha_6\beta_4$. The α subunit was sequenced from two overlapping clones encoding a protein of 1,073 amino acids. Identification of these clones as α_6

came from two pieces of evidence. First, the ORF contained a sequence that matched exactly the sequence of the amino terminus of the mature α_6 protein (Kajiji et al., 1989). Second, an antiserum to a synthetic peptide corresponding to the putative α_6 carboxy terminus in the ORF recognized the $\alpha_6\beta_4$ complex in immunoprecipitations of radiolabeled carcinoma cell lysates.

Structurally, α_6 is very similar to the other integrin α subunits that have been cloned to date. It shares 23–26% identity with those subunits which do not contain the I domain insert (α_5, α_V, α_{IIb}, α_4, and α_{PS2}) and ~18% identity with those α chains that do contain it (α_M, α_L, α_{P150}, and α_2) (see alignment shown in Fig. 3).

All of the integrin α proteins contain three potential cation binding sites. A fourth potential site exists in four of the α chains which do not contain the I domain. In contrast, α_6 lacks this fourth cation binding site which makes it, along with α_4, an exception to the the classification of integrin α subunits proposed by Takada and Hemler (1989). There is a site (residues 230–238) with weak homology to the cation binding site consensus sequence. However, this site does not align with a potential site in any of the other α chains. It starts with an asparagine instead of an aspartic acid residue, and it contains a phenylalanine where an aspartic acid or asparagine residue is expected. These differences are, for the moment, of unclear significance, and await elucidation from x-ray crystallographic data.

Similar or identical forms of α_6 have been reported to associate with either of two integrin β subunits, β_1 or β_4

1   GCGCTGCCCGCCTCGTCCCCACCCCCCAACCCCCCGCGCCGCGCCCTCGGACAGTCCCTGCTCGCCCGCGCGCTGCAGCCCCATCTCCTAGCGGCAGCCCAGGCGCGGAGGGAGCGAGTCC

121 GCCCCGAGC[TAGGTCCAGGACGGGCGCACAGCAGCAGCCGAGGCTGGCCGGGAGAGC]GAGGAAGAGGATGGCAGGGCCACGCCCCAGCCCATGGGCCAGGCTGCTCCTGGCAGCCTTGAT
                                                                  M A G P R P S P W A R L L L A A L I 18

241 CAGCGTCAGCCTCTCTGGGACCTTGGCAAACCGCTGCAAGAAGGCCCCAGTGAAGAGCTGCACGGAGTGTGTCCGTGTGGATAAGGACTGCGCCTACTGCACAGACGAGATGTTCAGGGA
       S V S L S G T L A N R C K K A P V K S C T E C V R V D K D C A Y C T D E M F R D 58

361 CCGGCGCTGCAACACCCAGGCGGAGCTGCTGGCCGCGGGCTGCCAGCGGGAGAGCATCGTGGTCATGGAGAGCAGCTTCCAAATCACAGAGGAGACCCAGATTGACACCACCCTG.....
       R R C N T Q A E L L A A G C Q R E S I V V M E S S F Q I T E E T Q I D T T L...... 96

2288 .......CACAAGAAGAAGGACTGCCCTCCGGGCTCCTTCTGGTGGCTCATCCCCCTGCTCCTCCTCCTCCTGCCGCTCCTGGCCCTGCTACTGCTGCTATGCTGGAAGTACTGT.....
       ......H K K K D C P P G S F W W L I P L L L L L L L P L L A L L L L L C W K Y C...... 736

4085 ....GCGCGCAACGGGGCCGGCTGGGGGCCTGAGCGGGAGGCCATCATCAACCTGGCCACCCAGCCCAAGAGGCCCATGTCCATCCCCATCATCCCTGACATCCCTATCGTGGACGCCCA
       ...A R N G A G W G P E R E A I I N L A T Q P K R P M S I P I I [P D I P I V D A Q 1338

4201 GAGCGGGGAGGACTACGACAGCTTCCTTATGTACAGCGATGACGTTCTACGCTCTCCATCGGGCAGCCAGAGGCCCAGCGTCTCCGATGACAC]GGCTGCGGCTGGAAGTTCGAGCCCCT
       S G E D Y D S F L M Y S D D V L R S P S G S Q R P S V S D D T [G C G W K F E P L 1378

4321 GCTGGGGGAGGAGCTGGACCTGCGGCGCGTCACGTGGCGGCTGCCCCCGGAGCTCATCCCGCGCCTGTCGGCCAGCAGCGGGCGCTCCTCCGACGCCGAGGCCCCACGGCCCCCCGGAC
       L G E E L D L R R V T W R L P P E L I P R L S A S S G R S S D A E A P T A P R T] 1418

4441 GACGGCGGCGCGGGCGGGAAGGGCGGCAGCCGTGCCCCGCAGTGCGACACCCGGGCCCCCCGG[A]GAGCACCTGGTGAATGGCCGGATGGACTTTGCCTTCCCGGGCAGCACCAACTCCCT
       T A A R A G R A A A V P R S A T P G P P G [E H L V N G R M D F A F P G S T N S L 1458

4561 GCACAGGATGACCACGACCAGTGCTGCTGCCTATGGCACCCACCTGAGCCCACACGTGCTAAGCACATCCTCCACCCTCACACGGGACTACAACTCACTGACCCGCTC
       H R M T T T S A A A Y G T H L S P H V P H R V L S T S S T L T R D Y N S L T R S 1498

4681 AGAACACTCACACTCGACCACACTGCCCAGGGACTACTCCACCCTCACCTCCGTCTCCTCCCACG[A]CTCTCGCCTGACTGCTGGTGTGCCCGACACGCCCACCCGCCTGGTGTTCTCTGC
       E H S H S T T L P R D Y S T L T S V S S H D S R L T A G V P D T P T R L V F S A 1538

4801 CCTGGGGCCCACATCTCTCAGAGTGAGCTGGCAGGAGCCGCGGTGCGAGCGGCCGCTGCAGGGCTACAGTGTGGAGTACCAGCTGCTGAACGGCGGTGAGCTGCATCGGCTCAAC.....
       L G P T S L R V S W Q E P R C E R P L Q G Y S V E Y Q L L N G G E L H R L N 1576

5525 ....ACCCTGGGGGCCCAGCACCTGGAGGCAGGCGGCTCCCTCACCCGGCATGTGACCCAGGAGTTTGTGAGCCGGACACTGACCACCAGCGGGAACCCTTAGCACCCACATGGACCAACA
       .. T L G A Q H L E A G G S L T R H V T Q E F V S R T L T T S G T L S T H M D Q Q 1818

5641 GTTCTTCCAAACTTGACCGCACCCTGCCCCACCCCCGCCATGTCCCACTAGGCGTCCTCCCGACTCCTCTCCCGGAGCCTCCTCAGCTACTCCATCCTTGCACCCCTGGGGGCCCAGCCC
       F F Q T *   1822

5761 ACCCGCATGCACAGAGCAGGGGCTAGGTGTCTCCTGGGAGGCATGAAGGGGGCAAGGTCCGTCCTCTGTGGGCCCAAACCTATTTGTAACCAAAGAGCTGGGAGCAGCACAAGGACCCAG

5881 CCTTTGTTCTGCACTTAATAAATGGTTTTGCTACTGCTAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA

Figure 6. Nucleotide sequence and deduced amino acid sequence of the human β_4 subunit, highlighting the regions where insertions occur, presumably based on alternative mRNA splicing. The beginning and end of the sequence as well as the position of the predicted transmembrane segment (*dark bar underline*) are shown for orientation. The region containing the amino-terminal sequence determined on β_4 protein (Kajiji et al., 1989) is underlined with a lightly shaded bar. Alternate splice regions are boxed. The arrowhead at base pair 4,744 shows the site of insertion of 159-bp stretch reported by Hogervorst et al. (1990). The complete sequence data (5, 918 bp) are available from EMBL/GenBank/DDBJ under accession number X53587.

(Hemler et al., 1989). We present further evidence of this alternative association. Antiserum raised to a synthetic peptide corresponding to the carboxy terminus of α_6 reacts with both $\alpha_6\beta_4$ from pancreatic carcinoma cells and $\alpha_6\beta_1$ from human platelets. This reinforces the evidence, which includes identical amino-terminal sequences (Kajiji et al., 1989; Hemler et al., 1989) and the sharing of monoclonal antibody epitopes and peptide maps (Hemler et al., 1989), for a close structural similarity between the α_6 molecules associated with either β_4 or β_1. However, these results do not exclude the possibility that small structural differences, perhaps due to alternative exon splicing or posttranslational modifications, may be responsible for the differential association with either β_4 or β_1. Alternatively, if such differences do not exist, the relative affinities of β_4 and β_1 for α_6 may regulate their association.

In cells that express both β_1 and β_4, α_6 appears to form heterodimers preferentially with β_4 as observed by ourselves (DeLuca et al., 1990) and others (Hemler et al., 1989; Kennel et al., 1989). Regions unique to α_6 may be relevant for this preferential association. In this regard, two short regions of the α_6 molecule (554–561 and 641–690) are not present in the other α subunits (Fig. 3). Further experiments are needed to examine the possibility that they may be involved in the formation of heterodimers with β_4, possibly by creating a higher affinity for β_4 than β_1.

The primary structure of the integrin subunit β_4 showed some unusual features. Homology to other integrin β chains ends at the border of the extracytoplasmic region and the predicted transmembrane region. After this point, the β_4 sequence is completely divergent, with the exception of two conserved residues (WK) positioned at the end of the transmembrane region, which may, therefore, be essential for structure or function. While β_1, β_2, and β_3 have cytoplasmic tails of the same length (47 amino acids), with many identities or conserved substitutions, these sequences are entirely absent from β_4. Instead, β_4 contains a surprisingly large cytoplasmic domain comprised of >1,000 residues. The prediction of a long cytoplasmic tail for β_4 confirms recent reports from two other laboratories which isolated similar cDNAs from normal epithelial cells (Suzuki and Naitoh, 1990; Hogervorst et al., 1990).

In addition, we provide direct evidence that the putative cytoplasmic domain deduced from these cDNA clones is, in fact, part of the β_4 protein (Fig. 5). Antibodies eluted from a fusion protein containing the carboxy terminus of the ORF of these cDNAs immunoprecipitated the $\alpha_6\beta_4$ complex from nondenatured lysates, and a β_4-like protein from cell lysates in which the interaction of α_6 with β_4 was disrupted by denaturation. Since the 205,000-M_r form of β_4 can be accounted for entirely by the size of the polypeptide backbone and the addition of N-linked carbohydrates, our previ-

A.

5' untranslated

200 bp   129

248 bp

Cytoplasmic domain

0 bp   4294

255 bp

144 bp   4744

354 bp

B

129   4294   4744

1 2 3   4 5 6   7 8 9
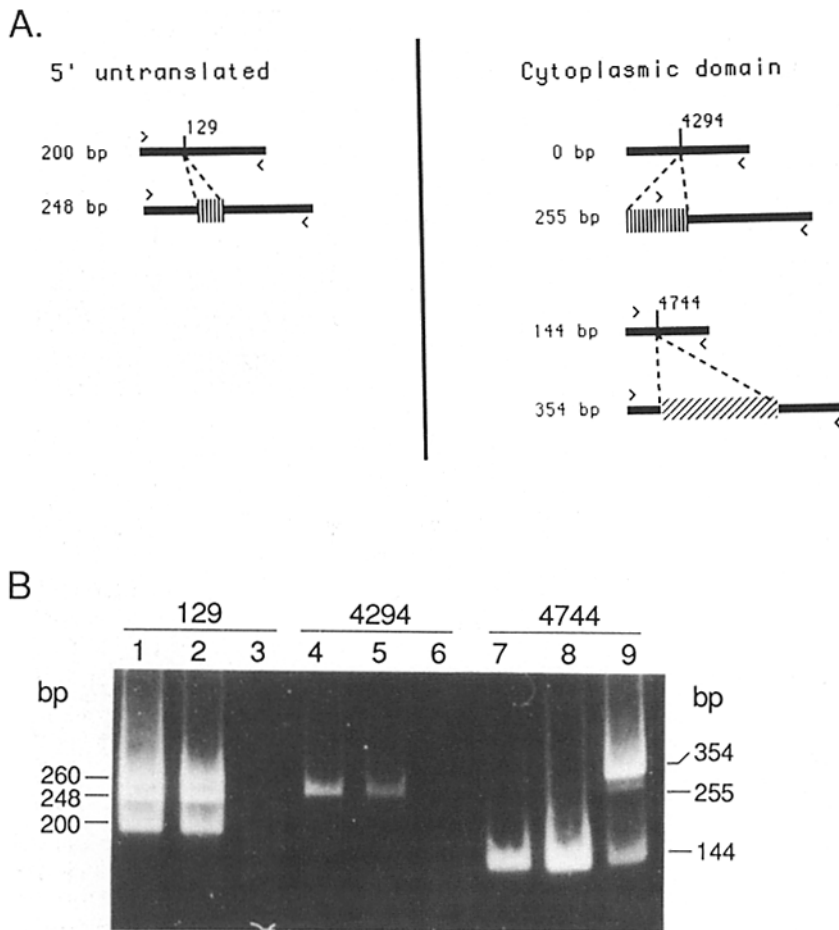
bp

260—
248—
200—

bp

354
255
144

Figure 7. PCR products from carcinoma and placental cDNA primed with oligonucleotides for potential alternative splice sites. (*A*) Diagrams illustrating the location and expected size of PCR products. Solid lines represent the regions of the cDNAs being amplified. The boxes with vertical lines represent the potential inserts observed in this study, and the box with diagonal lines represents the potential insert in the β_4 sequence reported by Hogervorst et al. (1990). Numbers to the left of each line are the size of the expected PCR product. Numbers above each line are the nucleotide positions just preceding the insert based on the numbering scheme shown in Fig. 6. Position and orientation of primers is indicated by the arrowheads. Oligonucleotides are described in Materials and Methods: 1 + 2 were used for insert 129, 3 + 4 were used for insert 4,294, and 5 + 6 were used for insert 4,744. (*B*) PCR products from FG cDNA (lanes *1, 4,* and *7*), LoVo cDNA (lanes *2, 5,* and *8*), and placental cDNA (lanes *3, 6,* and *9*) were analyzed on a polyacrylamide gel as described in Materials and Methods.

ous suggestion that the three different forms of β_4 (M_r = 205,000, 180,000, and 150,000, Kajiji et al., 1989) may be due to large amounts of sialic acid appears to be incorrect. Proteolytic cleavage may be a more accurate explanation for the heterogeneity in the molecular mass of β_4 as suggested by others (Hemler et al., 1989).

The β_4 sequences we obtained from carcinoma cells contain a few differences compared to the β_4 cDNAs isolated from normal epithelial cells (Hogervorst et al., 1990; Suzuki and Naitoh, 1990). A 49-bp insertion located 9-bp upstream of the ATG translational start site was found in 6 of 13 cDNAs we sequenced. It is possible that this region may be of regulatory importance (e.g., for translation efficiency). Furthermore, we found clones encoding two versions of the cytoplasmic tail of β_4. One of these is identical to that reported by Suzuki and Naitoh (1990). The other contains an insertion of 70 amino acids after threonine 1,369. Both of our sequences are different from that reported by Hogervorst et al. (1990), which contains an insertion of 53 amino acids after histidine 1,519. Thus, it appears that the β_4 cytoplasmic domain can exist in at least three versions.

A likely explanation for some or all of these insertions is alternative exon splicing of primary mRNA transcripts. Such mechanisms have been described in many other mRNAs (Smith et al., 1989), including the integrin subunits β_3 (Van Kuppevelt et al., 1989) and PS2 α (Brown et al., 1989). Consistent with the possibility of alternative splicing, we detected PCR amplification products of the expected size using primers located either within or adjacent to the insertions. That mature cytoplasmic mRNA, rather than immature nuclear transcripts, acted as templates in these PCR reactions is suggested by the fact that independent poly-(A) + RNA preparations yielded identical results, and that the relative ratio of the various forms remained constant within, and was characteristic of, a given cell type. For instance, the 4,744 insert was detectable in placenta but not in carcinoma cells, while the 4,294 insert had the opposite pattern (Fig. 7 *B*). Further experiments are, however, necessary to conclusively identify the origin of the multiple forms of the β_4 mRNA and to gain insight into their functional significance. In particular, it is necessary to verify the existence of distinct protein forms corresponding to the three versions of the β_4 cytoplasmic domain.

The unique cytoplasmic domain of β_4 suggests intracellular interactions distinct from those of other integrins. When engaged with extracellular matrix ligands, several types of integrins participate in the formation of structures called adhesion plaques (or focal adhesions) characterized by the colocalization of integrin receptors with the adhesion plaque components vinculin and talin, and with the ends of actin stress fibers (Buck and Horwitz, 1987). By immunostaining, we have shown here that the $\alpha_6\beta_4$ integrin is localized at the basal surface of adhering carcinoma cells (i.e., in areas of the plasma membrane contacting substrate). However, the distribution of $\alpha_6\beta_4$ is distinct from that of talin, suggesting that $\alpha_6\beta_4$ may not participate in adhesion plaque formation
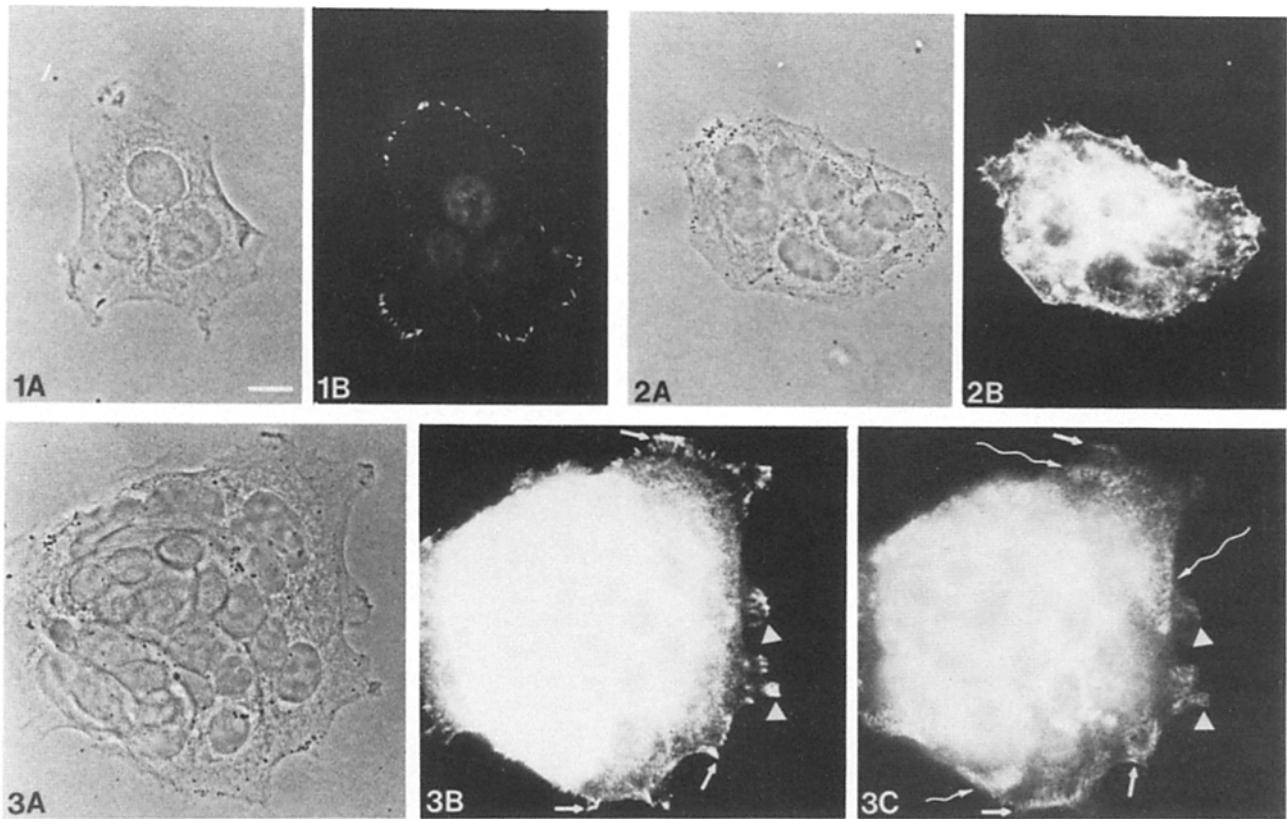
Figure 8. Immunofluorescence staining of FG carcinoma cells. The cells were cultured on Matrigel-coated glass coverslips for 2 d, and then fixed and detergent permeabilized. (*1 A*) Phase contrast; (*1 B*) anti-vinculin antibodies. (*2 A*) Phase contrast; (*2 B*) phalloidin (actin-binding). (*3 A*) Phase contrast; (*3 B*) anti-talin antibodies; (*3 C*) simultaneous staining with anti-β_4 antibodies. The plane of focus was kept at the basal surface of the cells. In *3 B* and *3 C* areas of interest are indicated as follows: (*a*) wavy arrows, areas internal to the edges stained by anti-β_4 antibodies but not anti-talin antibodies; (*b*) straight arrows, areas on the edges of peripheral, spread cells where talin and β_4 staining are juxtaposed but not overlapping; (*c*) arrowheads, areas where talin and β_4 staining were observed by careful focussing to be distinct. See text for further explanations. Bar, 10 μm.

in adhering carcinoma cells. It is possible that an inappropriate substrate was used in our experiments, as the ligand of $\alpha_6\beta_4$ is not biochemically identified. However, we have made similar observations on cultured keratinocytes that can be detached by anti-β_4 antiserum (DeLuca et al., 1990). Together with the unusual β_4 cytoplasmic tail, these data suggest that the biology of $\alpha_6\beta_4$ may differ substantially from other well-characterized integrins.

In conclusion, while the role of $\alpha_6\beta_4$ in epithelial cell adhesion remains to be established, the availability of α_6 and β_4 cDNAs should now facilitate this task.

Note Added in Proof. The sequence of α_3 from hamster was recently published (Tsuji, T., F. Yamamoto, Y. Miura, K. Takio, K. Tatani, S. Pawar, T. Osawa, and S. Hakomori. 1990. *J. Biol. Chem.* 265:7016–7021). Alignment with the α_6 amino acid sequence resulted in 37% identity, significantly higher than all other integrin α chains.

References

Argraves, W. S., S. Suzuki, H. Arai, K. Thompson, M. D. Pierschbacher, and E. Ruoslahti. 1987. Amino acid sequence of the human fibronectin receptor. *J. Cell Biol.* 105:1183–1190.

Arnaout, M. A., S. K. Gupta, M. W. Pierce, and D. G. Tenen. 1988. Amino acid sequence of the alpha subunit of human leukocyte adhesion receptor Mo1 (complement receptor type 3). *J. Cell Biol.* 106:2153–2158.

Bogaert, T., N. Brown, and M. Wilcox. 1987. The Drosophila PS2 antigen is an invertebrate integrin that, like the fibronectin receptor, becomes localized to muscle attachments. *Cell.* 51:929–940.

Brown, N. H., D. L. King, M. Wilcox, and F. C. Kafatos. 1989. Developmentally regulated alternative splicing of Drosophila integrin PS2 α transcripts. *Cell.* 59:185–195.

Buck, C. A., and A. F. Horwitz. 1987. Cell surface receptors for extracellular matrix molecules. *Annu. Rev. Cell Biol.* 3:179–205.

Cheresh, D. A., J. W. Smith, H. M. Cooper, and V. Quaranta. 1989. A novel vitronectin receptor integrin ($\alpha_v\beta_x$) is responsible for distinct adhesive properties of carcinoma cells. *Cell.* 57:59–69.

Corbi, A. L., L. J. Miller, K. O'Connor, R. S. Larson, and T. A. Springer. 1987. cDNA cloning and complete primary structure of the alpha subunit of a leukocyte adhesion glycoprotein p150,95. *EMBO (Eur. Mol. Biol. Organ.) J.* 6:4023–4028.

Corbi, A. L., T. K. Kishimoto, L. J. Miller, and T. A. Springer. 1988. The human leukocyte adhesion glycoprotein Mac-1 (complement receptor type 3, CD11b) α subunit. Cloning, primary structure, and relation to the integrins, von Willebrand factor and factor B. *J. Biol. Chem.* 263:12403–12411.

DeLuca, M., R. N. Tamura, S. Kajiji, S. Bondanza, R. Cancedda, P. Rossino, P. C. Marchisio, and V. Quaranta. 1990. Polarized integrin mediates keratinocyte adhesion to basal lamina. *Proc. Natl. Acad. Sci. USA.* In press.

Devereux, J., P. Haeberli, and O. Smithies. 1984. A comprehensive set of sequence analysis programs for the VAX. *Nucleic Acids Res.* 12:387–395.

Drewinko, B., M. M. Romsdahl, L. Y. Yang, M. J. Ahearn, and J. M. Trujillo. 1976. Establishment of a human carcinoembryonic antigen-producing colon adenocarcinoma cell line. *Cancer Res.* 36:467–475.

Fitzgerald, L. A., M. Poncz, B. Steiner, S. C. Rall, Jr., J. S. Bennett, and D. R. Phillips. 1987. Comparison of cDNA-derived protein sequences of the human fibronectin and vitronectin receptor α-subunits and platelet glycoprotein IIb. *Biochemistry.* 26:8158–8165.

Freed, E., J. Gailit, P. van der Geer, E. Ruoslahti, and T. Hunter. 1989. A novel integrin β subunit is associated with the vitronectin receptor a subunit (α_v) in a human osteosarcoma cell line and is a substrate for protein kinase C. *EMBO (Eur. Mol. Biol. Organ.) J.* 8:2955–2965.

Hemler, M. E., C. Crouse, and A. Sonnenberg. 1989. Association of the VLA α_6 subunit with a novel protein. A possible alternative to the common VLA β_1 subunit on certain cell lines. *J. Biol. Chem.* 264:6529–6535.

Hogervorst, F., I. Kuikman, A. E. G. Kr. von dem Borne, and A. Sonnenberg. 1990. Cloning and sequence analysis of β_4 cDNA: an integrin subunit that contains a unique 118 kd cytoplasmic domain. *EMBO (Eur. Mol. Biol. Organ.) J.* 9:765–770.

Holzmann, B., B. W. McIntyre, and I. L. Weissman. 1989. Identification of a murine Peyer's patch-specific lymphocyte homing receptor as an integrin molecule with an α chain homologous to human VLA-4α. *Cell.* 56:37–46.

Hynes, R. O. 1987. Integrins: a family of cell surface receptors. *Cell.* 48:549–554.

Kajiji, S. M., B. Davceva, and V. Quaranta. 1987. Six monoclonal antibodies to human pancreatic cancer antigens. 1987. *Cancer Res.* 47:1367–1376.

Kajiji, S., R. N. Tamura, and V. Quaranta. 1989. A novel integrin (α_Eβ_4) from human epithelial cells suggests a fourth family of integrin adhesion receptors. *EMBO (Eur. Mol. Biol. Organ.) J.* 8:673–680.

Kennel, S. J., L. J. Foote, R. Falcioni, A. Sonnenberg, C. D. Stringer, C. Crouse, and M. E. Hemler. 1989. Analysis of the tumor-associated antigen TSP-180. Identity with α_6-β_4 in the integrin superfamily. *J. Biol. Chem.* 264:15515–15521.

Kohler, G., and C. Milstein. 1975. Continuous cultures of fused cells secreting antibody of pre-defined specificity. *Nature (Lond.).* 256:495–497.

Kyte, J., and R. F. Doolittle. 1982. A simple method for displaying the hydropathic character of a protein. *J. Mol. Biol.* 157:105–132.

Larson, R. S., A. L. Corbi, L. Berman, and T. Springer. 1989. Primary structure of the leukocyte function-associated molecule-1 α subunit: an integrin with an embedded domain defining a protein superfamily. *J. Cell Biol.* 108:703–712.

Maniatis, T., E. F. Fritsch, and J. Sambrook. 1982. Molecular Cloning: A Laboratory manual. Cold Spring Harbor Laboratory. Cold Spring Harbor, New York.

Marchisio, P. C., D. Cirillo, L. Naldini, M. V. Primavera, A. Teti, and A. Zambonin-Zallone. 1984. Cell-substratum interaction of cultured avian osteoclasts is mediated by specific adhesion structures. *J. Cell. Biol.* 99:1696–1705.

Poncz, M., R. Eisman, R. Heidenreich, S. M. Silver, G. Vilaire, S. Surrey, E. Schwartz, and J. S. Bennett. 1987. Structure of the platelet membrane glycoprotein IIb. Homology to the α subunits of the vitronectin and fibronectin membrane receptors. *J. Biol. Chem.* 262:8476–8482.

Pytela, R. 1988. Amino acid sequence of the murine Mac-1 a chain reveals homology with the integrin family and an additional domain related to von Willebrand factor. *EMBO (Eur. Mol. Biol. Organ.) J.* 7:1371–1378.

Roth, J. 1975. Methods for assessing immunologic and biologic properties of iodinated peptide hormones. *Methods Enzymol.* 37(Pt. B):223–233.

Ruoslahti, E., and F. G. Giancotti. 1989. Integrins and tumor cell dissemination. *Cancer Cells.* 1:119–126.

Ruoslahti, E., and M. D. Pierschbacher. 1987. New perspectives in cell adhesion: RGD and integrins. *Science (Wash. DC).* 238:491–497.

Sanger, F., S. Nicklen, and A. R. Coulson. 1977. DNA sequencing with chain-terminating inhibitors. *Proc. Natl. Acad. Sci. USA.* 74:5463–5467.

Sheppard, D., C. Rozzo, L. Starr, V. Quaranta, D. Erle, and R. Pytela. 1990. Complete amino acid sequence of a novel integrin β subunit (β_6) identified in epithelial cells using the polymerase chain reaction. *J. Biol. Chem.* 265: 11502–11507.

Smith, C. W. J., J. G. Patton, and B. Nadal-Ginard. 1989. Alternative splicing in the control of gene expression. *Annu. Rev. Genet.* 23:527–577.

Sonnenberg, A., H. Janssen, F. Hogervorst, J. Calafat, and J. Hilgers. 1987. A complex of platelet glycoproteins Ic and IIa identified by a rat monoclonal antibody. *J. Biol. Chem.* 262:10376–10383.

Sonnenberg, A., P. W. Modderman, and F. Hogervorst. 1988. Laminin receptor on platelets is the integrin VLA-6. *Nature (Lond.).* 336:487–489.

Suzuki, S., and Y. Naitoh. 1990. Amino acid sequence of a novel integrin β_4 subunit and primary expression of the mRNA in epithelial cells. *EMBO (Eur. Mol. Biol. Organ.) J.* 9:757–763.

Suzuki, S., W. S. Argraves, H. Arai, L. R. Languino, M. Piershbacher, and E. Ruoslahti. 1987. Amino acid sequence of the vitronectin receptor α subunit and comparative expression of adhesion receptor mRNAs. *J. Biol. Chem.* 262:14080–14085.

Takada, Y., and M. E. Hemler. 1989. The primary structure of the VLA-2/collagen receptor α_2 subunit (platelet gpIa): homology to other integrins and the presence of a possible collagen-binding domain. *J. Cell Biol.* 109:397–407.

Takada, Y., M. J. Elices, C. Crouse, and M. E. Hemler. 1989. The primary structure of the α_4 subunit of VLA-4: homology to other integrins and a possible cell-cell adhesion function. *EMBO (Eur. Mol. Biol. Organ.) J.* 8:1361–1368.

Van Eldik, L. J., J. G. Zendegui, D. R. Marshak, and D. M. Watterson. 1982. Calcium-binding proteins and the molecular basis of calcium action. *Intl. Rev. Cytol.* 77:1–61.

Van Kuppevelt, T. H. M. S. M., L. R. Languino, J. O. Gailit, S. Suzuki, and E. Ruoslahti. 1989. An alternative cytoplasmic domain of the integrin β_3 subunit. *Proc. Natl. Acad. Sci. USA.* 86:5415–5418.

Weinberger, C., S. M. Hollenberg, E. S. Ong, J. M. Harman, S. T. Brower, J. Cidlowski, E. B. Thompson, M. G. Rosenfeld, and R. M. Evans. 1985. Identification of human glucocorticoid receptor complementary DNA clones by epitope selection. *Science (Wash. DC).* 228:740–742.

Wood, W. I., J. Gitschier, L. A. Lasky, and R. M. Lawn. 1985. Base composition-independent hybridization in tetramethylammonium chloride: a method for oligonucleotide screening of highly complex gene libraries. *Proc. Natl. Acad. Sci. USA.* 82:1585–1588.

Young, R. A., and R. W. Davis. 1983. Efficient isolation of genes by using antibody probes. *Proc. Natl. Acad. Sci. USA.* 80:1194–1198.