Proc. Natl. Acad. Sci. USA
Vol. 88, pp. 10183–10187 November 1991
Developmental Biology

Cell type-specific integrin variants with alternative α chain cytoplasmic domains

RICHARD N. TAMURA, HELEN M. COOPER, GINETTA COLLO, AND VITO QUARANTA

The Scripps Research Institute, IMM-8, 10666 North Torrey Pines Road, La Jolla, CA 92037

Communicated by Frank J. Dixon, August 13, 1991

ABSTRACT **The integrin heterodimers composed of the α6 subunit with the β1 or β4 subunit (α6β1 and α6β4) are receptors for laminin and basement membrane components, respectively. The α3β1 integrin recognizes laminin, collagen, fibronectin, or epiligrin. We report the identification of structural variants (A and B) of the α6 and α3 subunits, containing distinct cytoplasmic domains. The expression of one cytoplasmic domain or the other, based probably on alternative exon usage, is cell-type dependent. Most transformed cell lines express both α6A and α6B isoforms, as determined by mRNA amplification or antibody immunoprecipitation. In contrast, embryonic fibroblasts express exclusively α6A, and embryonic stem cells express exclusively α6B. In most normal tissues, both α6 isoforms are detectable, but several tissues express either α6A or α6B. The α3B mRNA was amplified from heart and brain, while all other tissues and cell lines tested contained only α3A mRNA. Alternative cytoplasmic domains may provide a means for varying the cellular responses to the ligands of α6 and α3 integrins according to the cell type.**

Interactions of cells with the extracellular matrix and neighboring cells are important to numerous biological processes, including cell migration, morphogenesis, growth control, and wound repair (1, 2). These interactions are mediated, in part, by the integrin family of adhesion receptors.

Integrins are cell surface heterodimers composed of noncovalently associated transmembrane subunits α and β (3, 4). More than 15 members of this family have been identified and are generally expressed in a tissue-specific fashion. They bind to extracellular matrix, plasma, or cell surface ligands, in some cases via recognition of the amino acid sequence Arg-Gly-Asp (4). Along with mediating cellular adhesion and cytoskeletal reorganization upon engagement of ligands, integrins may also induce gene expression (5) and changes in intracellular pH (6) or Ca^{2+} flux (7). Integrin avidity for ligand may also be regulated by cell "activation" (8) or differentiation (9).

The integrin extracellular domains form the ligand-binding sites, while the cytoplasmic domains interact with the intracellular environment and are likely to be critical mediators of integrin functions. The cytoplasmic domains of the β subunits associate with cytoskeletal components such as talin, vinculin, or α-actinin (10) and thereby mediate the formation of transmembrane links between the cytoskeleton and the extracellular matrix (10). Structural changes in the β-chain cytoplasmic domains may also affect integrin avidity for ligand (11), presumably by influencing the conformation of the extracellular domains. The molecular interactions and potential functions of the α-subunit cytoplasmic domains have not been determined.

We recently described the primary structure of the integrin α6 subunit (12), which can associate with two β subunits, either β1 or β4. The α6β1 integrin is a receptor for laminin in many cell types, including platelets (13), lymphocytes (14), macrophages (15), microvascular endothelial cells (16), developing kidney (17), and retinal neurons (18), whereas the α6β4 integrin is a component of hemidesmosome adhesion complexes in epithelial cells (19). α6 is most similar (37% identity) to the integrin α3 subunit, which pairs with the β1 subunit to form a receptor for laminin (20), collagen (21), fibronectin (21), or epiligrin (22) and also is involved in cell–cell interactions (23).

In this study, we report the identification of α6 and α3 structural variants with alternative cytoplasmic domains. These cytoplasmic domain isoforms are expressed in a cell type-specific fashion and may be relevant to the regulation of integrin function.

MATERIALS AND METHODS

mRNA Amplification, Cloning, and Sequencing. mRNA amplification was carried out by a reverse transcription–polymerase chain reaction assay (RT-PCR). Briefly, poly(A)$^+$ RNA from cultured cell lines or tissues was isolated by using the Micro-FastTrack mRNA isolation kit (Invitrogen, San Diego). First-strand cDNA was prepared by using random hexamer primers (Pharmacia) and the cDNA Cycle kit (Invitrogen). Oligonucleotides were synthesized with a Gene Assembler (Pharmacia). Primers for α6 (12) were: 1156, 5′-ATCTCTCGCTCTTCTTTCCG-3′; 1157, 5′-GACTCTTAACTGTAGCGTGA-3′; 1681, 5′-GAACTGTGTGAACATCAGA-3′; 2002, 5′-ATCCTTACAGCATGGTATCG-3′; and 1937, 5′-AGTTTGGGTACTGTGAAGCT-3′ (see Fig. 1 for position). Primers for α3 were: 2032, 5′-AAGCCAAATCTGAGACTGTG-3′; and 2033, 5′-GTAGTATCGGTCCCGAATCT-3′, corresponding to nucleotides 3233–3252 and 3872–3891, respectively, of the hamster α3 sequence (24). PCR assays were carried out as described (12) with primer concentrations of 0.1 μM. Two-round PCR consisted of a first round of 40 cycles with the oligonucleotide pair 1156/1157, after which one-fiftieth of the first-round products was amplified (25 cycles) with a nested pair of primers, 1681/2002 (see Fig. 1 for location). The PCR hybridizing temperature was changed from 55°C to 50°C for the assay of the mouse tissues. The final PCR products (1/10th) were analyzed on 2% agarose/TBE buffer (90 mm Tris/64.6 mM boric acid/2.5 mM EDTA, pH 8.3) gels. For cloning, half of the PCR products was purified and blunt-ended according to the Double Gene Clean protocol (Bio 101, San Diego), ligated into the *Eco*RV site of pBluescript II pKS(+) (Stratagene), and transformed into *Escherichia coli* XL1-Blue. White colonies were selected for "minipreps" and restriction digestion analysis. Clones containing an insert corresponding in size to the PCR products were sequenced using either the T3 or the T7 polymerase primers. Sequences were analyzed on a VAX-VMS version 5.4-2 computer, with the programs of the University of Wisconsin Genetics Computer Group (25).

Cell Labeling, Immunoprecipitation, and Antibodies. Cultured cells (6×10^7) were surface-labeled with 2 mCi (1 Ci =

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: PCR, polymerase chain reaction(s); RT-PCR, reverse transcription–PCR; UT, untranslated region.

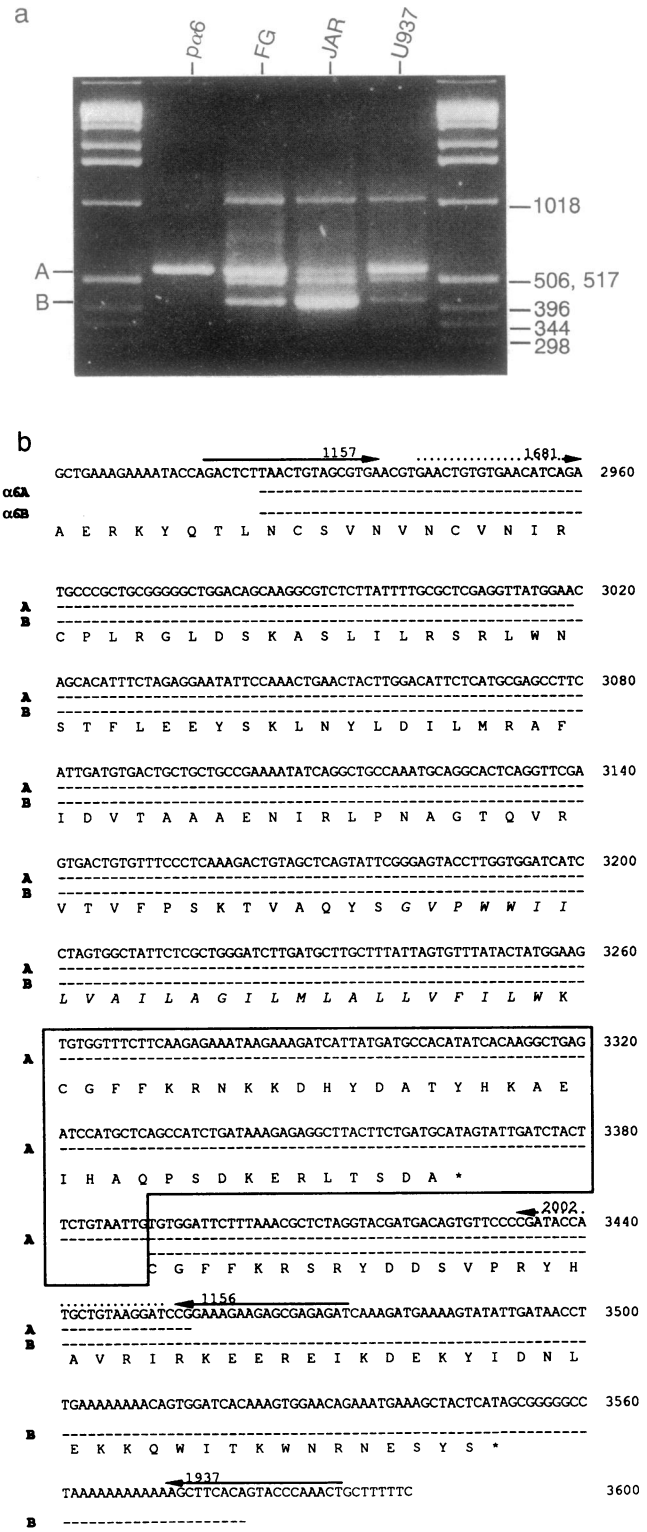


FIG. 1. Identification of α6A and α6B mRNA isoforms by RT-PCR. (*a*) RT-PCR amplification was performed on a human α6 cDNA clone (pα6A) or cDNA from the human cell lines FG (pancreatic carcinoma), U937 (monocytoid), and JAR (choriocarcinoma). The location of the oligonucleotide primers, 1156/1157, is shown in *b* by arrows. The 550-bp fragment in the pα6 lane corresponds to the A cytoplasmic domain isoform. The 420-bp fragment in the other lanes corresponds to the B isoform. The band visible at 520 bp (lanes FG, JAR, and U937) is a hybrid resulting from reannealing single strands of the 550- and 420-bp products (Lisa Starr, personal communication) (see also Fig. 3). The band visible at 1050 bp is, instead, an occasional RT-PCR artifact (absent in Fig. 3). (*b*) Nucleotide sequence of the A and B RT-PCR fragments (dashes in the second and third lines), compared to our previously published (12) sequence for α6 (top line). Dashes indicate identical nucleotides. A 130-bp segment present in the A fragment but not in the B fragment is boxed. The open reading frame is shown beneath the nucleotide sequences. The predicted α6 transmembrane domain is shown in italics. Arrows indicate the positions of RT-PCR primers, identified by four digit numbers. Cloning and sequencing were also performed on RT-PCR fragments generated with the pair 1157/1937 for the purpose of verifying the 3′ end of the α6B coding region.

37 GBq) of $Na^{125}I$ (17 Ci/mg) and lactoperoxidase as described (26). The preparation of nonionic detergent lysates, immunoprecipitations, and SDS/PAGE have been described (26). The 6844 antisera was raised to a synthetic peptide (Cys-Ile-His-Ala-Gln-Ser-Asp-Lys-Glu-Arg-Leu-Thr-Ser-Asp-Ala) corresponding to the last 15 amino acids of α6A, and the 382 antisera was raised to a synthetic peptide from the carboxyl terminus of α6B (Cys-Lys-Asp-Glu-Lys-Tyr-Ile-Asp-Asn-Leu-Glu-Lys-Lys-Gln-Trp-Ile-Thr-Lys-Trp-Asn-Arg-Asn-Glu-Ser-Tyr-Ser) as described before (12). Cysteine residues at the amino terminus were included to facilitate coupling to a protein carrier (12). The rat monoclonal anti-α6 antibody GoH3 (13) was a gift of Arnoud Sonnenberg (Central Laboratory of The Netherlands Red Cross, University of Amsterdam, The Netherlands).

RESULTS

Using oligonucleotide primers flanking the 3′ end of the integrin α6 coding region (Fig. 1) in RT-PCR (27), we obtained two products of 550 and 420 base pairs (bp) from three cell lines. The nucleotide sequence of the 550-bp fragment (Fig. 1) matched exactly the published α6 cDNA sequence (12) and encoded the end of the extracellular domain, the transmembrane, and the cytoplasmic domains, followed by the initial part of the 3′ untranslated region (3′ UT). The sequence of the 420-bp band matched the 550-bp sequence except for a 130-bp gap (Fig. 1), which encoded the entire cytoplasmic domain and 25 bp of the 3′ UT. This gap modifies the reading frame, resulting in an α6 protein with an alternative cytoplasmic domain (Fig. 1*b*) that is 17 amino acids longer than (and bears no sequence homology with) the reported α6 cytoplasmic domain. The alternative cytoplasmic domain contains the sequence Gly-Phe-Phe-Lys-Arg, a motif conserved among integrin α chains (12, 18, 28). For convenience, we refer to the published form of α6 as α6A and to the α6 with the alternate cytoplasmic domain as α6B.

To verify that the α6B mRNA encoded a protein product, we prepared a rabbit antiserum (382) to a synthetic peptide corresponding to the last 25 residues of the deduced α6B amino acid sequence. This antiserum immunoprecipitated, from radiolabeled detergent lysates of the human choriocarcinoma cell line JAR, a pattern of bands similar or identical to those obtained with 6844, an anti-peptide antiserum to the α6A cytoplasmic domain, and GoH3 (29), a monoclonal antibody to the α6 extracellular domain (Fig. 2). The bands had molecular masses that corresponded to those of α6, β1, and β4 and were positively identified as those subunits with specific antibodies (not shown). Sequential immunoprecipitations (Fig. 2) showed that the monoclonal antibody GoH3 completely depleted the JAR lysates of antigen reactive with antisera 382 (anti-α6B) or 6844 (anti-α6A). The 382 antiserum did not remove material reactive with 6844, and 6844 did not remove 382-reactive material. These results indicate that JAR cells express both α6A and α6B proteins, each of which is associated with either β1 or β4, and are consistent with the RT-PCR detection of both α6A and α6B mRNA isoforms (Fig. 3).

The distribution of the α6 isoforms in cultured cell lines was assessed by RT-PCR (Fig. 3). The majority of the cells tested contained both α6A and α6B mRNA at ratios characteristic of a cell line. Interestingly, two carcinoma cell lines

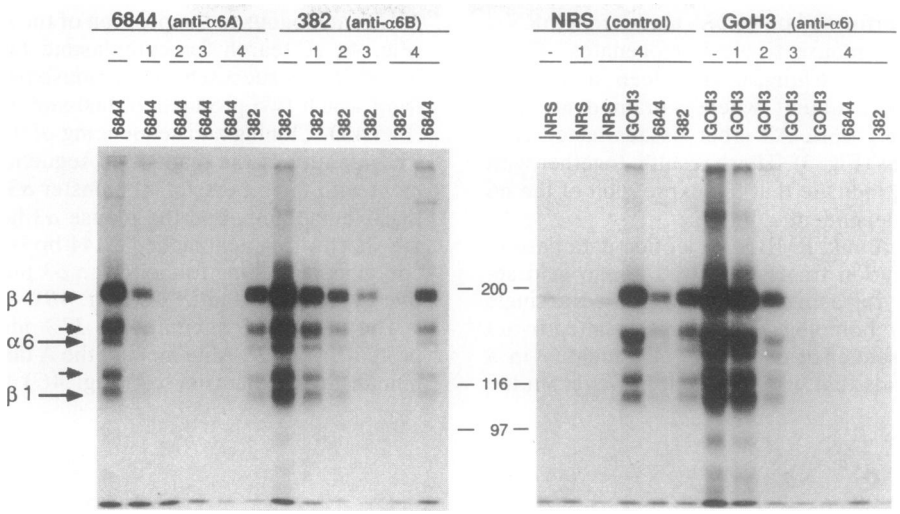


FIG. 2. Detection of α6A and α6B protein isoforms by antibody immunoprecipitation. Detergent lysates of ^{125}I-surface-labeled JAR cells were depleted by four sequential cycles of immunoprecipitation with one of the depleting antibodies indicated in the top line. Depleted lysates from cycles 1–4 were divided into equal aliquots as shown in the second line and immunoprecipitated with either the same antibody or other antibodies as indicated above each lane. The antibodies used were: anti-peptide antisera to the cytoplasmic domains of either α6A (6844) or α6B (382), a rat monoclonal antibody (GoH3) to an epitope on the extracellular domain (29) of α6, and normal rabbit serum (NRS) as control. The material precipitated at intermediate or final rounds of depletion was analyzed by SDS/PAGE under nonreducing conditions and autoradiography. Molecular mass markers are in kilodaltons.

(A-431 and 804G) and three lines of immortalized mouse embryonic fibroblasts (3T3, STO, L) expressed exclusively α6A. In contrast, mouse embryonic stem cells and F9 teratocarcinoma cells contained exclusively α6B (Fig. 3). Immunoprecipitations with 6844 and 382 demonstrated that normal human keratinocytes expressed only α6A, while Caco-2, FG, and 804G expressed α6 protein isoforms consistent with the RT-PCR assays (data not shown).

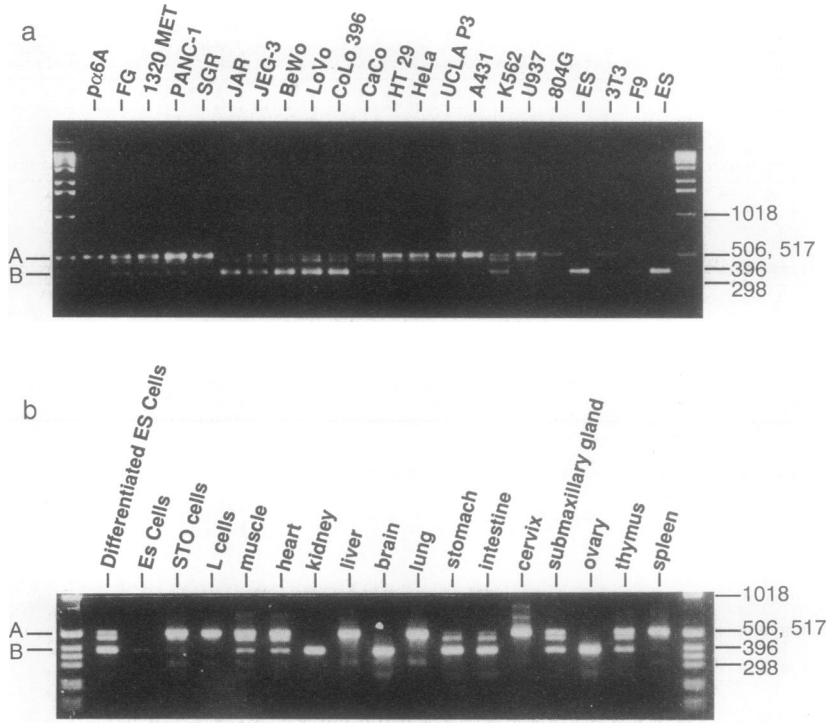


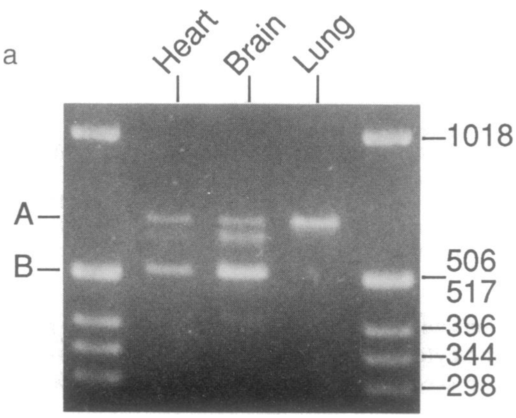
FIG. 3. Expression of α6A and α6B mRNA in cultured cells and tissues. α6 mRNA isoforms were detected by two rounds of RT-PCR with a nested set of oligonucleotide primers. With these primers (1681/2002 in Fig. 1), the A and B isoforms give rise to products of about 500 and 370 bp, respectively. In *a*, the following cultured cell lines are shown: human pancreatic carcinoma (FG, 1320 MET, PANC-1, and SGR), choriocarcinoma (JAR, JEG-3, and BeWo), colon carcinoma (LoVo, COLO 396, Caco-2, and HT-29), cervix carcinoma (HeLa), lung carcinoma (UCLA P3), epidermoid carcinoma (A-431), monocytoid (K-562, U-937), rat bladder carcinoma (804G), and mouse embryonic stem cells CCE (ES), embryonic fibroblasts (3T3) and teratocarcinoma (F9). In *b*, the indicated tissues are from normal mouse. Murine cell lines are as follows: embryonic fibroblasts (STO and L) and embryonic stem cells CCE (ES). Both α6A and α6B isoform bands are visible in most cell and tissue samples, with one band often more intense than the other. Only the α6A band is seen in five cell lines (A-431, 804G, 3T3, STO, and L cells) and four tissues (lung, liver, spleen, and cervix). In contrast, only α6B is seen in brain, ovary, kidney, and the pluripotent cell lines ES and F9. *In vitro* differentiated ES cells (*b*) display the α6B band and a smaller amount of α6A isoform, while nondifferentiated ES cells (*a*) show only α6B (compare ES samples with α6B bands of approximately equal intensity).

RT-PCR was also performed on cDNA made from mRNA extracted from normal mouse tissue homogenates. These assays (Fig. 3) revealed that lung, liver, spleen, and cervix expressed solely the α6A subunit, whereas brain, ovary, and kidney expressed solely α6B. All other tissues tested contained both α6 isoforms (Fig. 3). These results, together with the cultured cell data, indicate that the expression of the α6 isoforms is cell-type dependent.

A search of the GenBank/EMBL nucleotide data base in all six possible translation frames with the amino acid sequence of the α6B cytoplasmic domain identified a single entry with significant homology, the hamster integrin α3 sequence (24). This sequence displayed 44% identity in a region of 52 amino acids that was encoded, however, in the 3′ UT, analogous to the position of the α6B tail coding region (Fig. 4). A search for cytoplasmic tail isoforms of α3 by RT-PCR was successful with mouse heart and brain tissues, from which two products of 660 and 516 bp were amplified (Fig. 4*A*). Cloning and sequencing of these products showed 95% identity of the nucleotide sequence of the 660-bp fragment with the reported (24) hamster α3 sequence, indicating that we had amplified the mouse α3 homologue. However, the shorter fragment lacked a 144-bp segment and, like α6B, contained a reading frame for an α3 protein with an alternative cytoplasmic tail (α3B) (Fig. 4*B*).

The α6 and α3 subunits are 37% identical overall at the protein level (12). Alignment of the A tails and the B tails from human α6 vs. hamster α3 (Fig. 4*C*) revealed even higher

a



b

```
                  a   c       a tg c                       g                                  g
α3A  TGTGTGTGGCTGGAGTGCCCCCTTCCAGACACCTCCAACATTACCAATGTGACCGTGAAAGCACGGGTGTGGAACAGCACCTTCATTGAGGACTACAAAGACTTTGACAGAGTCAGGGTA   3400
α3B  ------------------------------------------------------------------------------------------------------------------------
α3A  C  V  W  L  E  C  P  L  P  D  T  S  N  I  T  N  V  T  V  K  A  R  V  W  N  S  T  F  I  E  D  Y  K  D  F  D  R  V  R  V
α3B  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -

                c tt       c                                            g g    a  g
A    GATGGCTGGGCTACCCTGTTCCTGAGAACCAGCATCCCTACCATCAACATGGAGAACAAGACCACATGTTTCTCTGTGAACATTGACTCAGAGCTGGTGGAGGAGCTGCCCGCTGAGATT   3520
B    ------------------------------------------------------------------------------------------------------------------------
A    D  G  W  A  T  L  F  L  R  T  S  I  P  T  I  N  M  E  N  K  T  T  W  F  S  V  D  I  D  S  E  L  V  E  E  L  P  A  E  I
B    -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -

                                   c  c                          a   gc                                     c
A    GAGCTGTGGTTGGTGCTTGTGGCCGTGAGTGCTGGGTTGCTGCTGCTGGGGCTCATCATCCTCCTCTTGTGGAAG|TGTGACTTCTTTAAGCCGACCCGCTACTACCGGATTATGCCCAAG   3739
B    ---------------------------------------------------------------------------|---------------------------------------------
A    E  L  W  L  V  L  V  A  V  S  A  G  L  L  L  L  G  L  I  I  L  L  L  W  K  |
B    -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  |C  D  F  F  K  P  T  R  Y  Y  R  I  M  P  K

                                                                                                                                   c
A  | TGCGGCTTCTTCAAGCGAGCCCGCACTCGTGCCCTGTATGAAGCTAAGAGGCAGAAGGCTGAGATGAAGAGCCAGCCGTCAGAGACAGAAAGGCTGACCGACGACTACTGAGGGGGGTCACCCCCCCTGCCCCGGCCCACCCGG |
A  | C  G  F  F  K  R  A  R  T  R  A  L  Y  E  A  K  R  Q  K  A  E  M  K  S  Q  P  S  E  T  E  R  L  T  D  D  Y  *                                                      |

                                      g        g    g  a     c
A    TACCATGCAGTGCGTATCCGGGAGGAGGACCGCTACCCACCTCCAGGGAGCACGCTACCCACCAAGAAGCACTGGG                                              3860
B    ----------------------------------------------------------------------------

B    Y  H  A  V  R  I  R  E  E  E  R  Y  P  P  P  G  S  T  L  P  T  K  K  H  W
```

c

```
α6A (Human)      C G F F K R N K . K D H Y D A T Y H K A E I H A Q P S D K E R L T S D A *
                 | | | | | |   :   :     | : |       : | | | :   | | | :   | | | |   |
α3A (Hamster)    C G F F K R A R T R A L Y E A K R Q K A E M K S Q P S E T E R L T D D Y *

α6B (Human)      C G F F K R S R Y D D S V P R Y H A V R I R K E E R E I K D E K Y I D N L E K K Q W I T K W N R N E S Y S *
                 | : | | | : : | |       : | : | | | | | | | : | | |         : :   :       : | | : | : | : | :   : : : |
α3B (Hamster)    C D F F K P T R Y Y R I M P K Y H A V R I R E E E R Y P P P G S T L P . . T K K H W V T S W Q I R D R Y Y *
```

FIG. 4. Identification and sequence of murine α3A and α3B isoforms by RT-PCR. (*a*) RT-PCR assays of heart, brain, and lung mouse tissues resulted in a product consistent in size (660 bp) with an α3A isoform and an additional product of 516 bp in heart and brain consistent in size with an α3B isoform. The PCR products from these two tissues were cloned and sequenced. (*b*) The nucleotide sequence of the mouse α3A fragment is aligned with that of the α3B fragment. Dashes indicate identical nucleotides. Nucleotide positions that differ in the α3 hamster sequence are noted in lowercase letters above the mouse α3A sequence. Numbers along the right margin correspond to the numbering of the published hamster α3 sequence (24). The open reading frame of both α3 isoforms is shown beneath the nucleotide sequence, with dashes designating identical residues. The position of the putative alternatively spliced exon is also shown. (*c*) Optimized alignment of the A and B isoform cytoplasmic domains of α6 (human) and α3 (hamster). Vertical dashes indicate identical residues, while double dots indicate conservative substitutions. Asterisks indicate stop codons.

conservation (54% and 45%, respectively). Of possible relevance in the B tails may be a conserved stretch of 14 amino acids, of which 8 are charged. Such interspecies, intersubunit conservation suggests that these regions may be functionally significant.

DISCUSSION

We have identified structural variants (A and B), or isoforms, of the integrin subunits $\alpha 6$ and $\alpha 3$, which are distinguished by the presence of distinct cytoplasmic domains. These variants are expressed in a cell type-specific manner and can be precipitated by specific antibodies.

The cytoplasmic domains are important functional parts of the integrin molecule. The cytoplasmic domains of the β chains are involved in establishing physical interactions with the cytoskeleton (10) and, in $\beta 2$ integrins, may regulate ligand avidity of the extracellular domain (11). No functional role for the α-chain cytoplasmic domains has emerged as of yet. While our findings do not provide direct clues to the potential roles of α-chain cytoplasmic domains, they suggest that, in the case of $\alpha 6$ and $\alpha 3$, such roles could be linked to cell differentiation. We speculate that a structural change in the α-chain cytoplasmic domain could be an efficient means for modifying cellular responses to an integrin ligand as a function of differentiation.

In many cell types including platelets (13), lymphocytes (14), macrophages (15), microvascular endothelial cells (16), developing kidney (17), and retinal neurons (18), $\alpha 6\beta 1$ is a receptor for laminin, a widespread extracellular matrix protein implicated in neurite outgrowth, morphogenesis, differentiation, and cell proliferation (30). The avidity for laminin of $\alpha 6\beta 1$ is upregulated by activation in lymphocytes (14) while, in retinal neurons, it can be downregulated during development (18). Activation-dependent adhesion of macrophages to laminin is accompanied by phosphorylation of the $\alpha 6$ cytoplasmic domain and receptor association with the cytoskeleton (15). Whether or not the alternate $\alpha 6$ cytoplasmic domains are involved in the regulation of some of these phenomena should be determined.

The structure of the $\alpha 6$ and $\alpha 3$ mRNA A and B isoforms suggests that they may be generated by alternative splicing. Such a mechanism requires an exon–intron boundary near the transmembrane–cytoplasmic domain junction. A boundary at this position is present, in fact, in the integrin α-chain genomic structures that have been characterized to date (αX, αPS2, and αIIb) (31–33). Therefore, it is possible that other integrin α chains will be found to undergo alternative splicing of their cytoplasmic domain.

Alternative exon splicing has been described (34) for many genes, including integrins (32, 35, 36), and is a strategy for potentially modifying the functions of encoded products (34). A developmentally regulated alternative splicing of the *Drosophila* αPS2 integrin was proposed to affect receptor function because it occurred near the predicted ligand-binding site (32). With this precedent, it will be important to determine the possible functional role of the $\alpha 6$ and $\alpha 3$ A and B isoforms during development and differentiation. Suggestive, in this respect, is that mouse embryonic stem cells express solely $\alpha 6$B when kept undifferentiated, whereas they start expressing $\alpha 6$A when allowed to differentiate *in vitro* (Fig. 3) (37).

Screening of tissues at the mRNA level revealed no obvious pattern in the expression of the A vs. the B forms of either $\alpha 6$ or $\alpha 3$. However, before any conclusions can be reached, it is necessary to reassess the normal tissue distribution patterns of these integrin isoforms with assays that, unlike RT-PCR, can assess expression at both the protein and the single-cell level, e.g., immunohistochemistry with antibodies 382 and 6844.

In conclusion, our findings add to the structural complexity and diversity of integrins. They also raise the possibility of a novel means of regulating integrin functions, by suggesting that cellular responses evoked by ligand engagement of $\alpha 6$ and $\alpha 3$ integrins may vary in development and differentiation, according to whether the A or the B cytoplasmic domain is expressed.

Note Added in Proof. The cytoplasmic domain of the integrin αIIB subunit has been shown to modulate the ligand binding affinity of the integrin $\alpha IIB\beta 3$ (38).

We thank Anita Holmsen and Lisa Starr for outstanding technical help, Donna Uranowski for excellent secretarial expertise, and Michael Jackson and Anders Brunmark for advice. This work was supported by National Institutes of Health Grants CA47858, CA50286, and RR00833 for computer services.

1. Ekblom, P., Vestweber, D. & Kemler, R. (1986) *Annu. Rev. Cell Biol.* **2**, 27–47.
2. Buck, C. A. & Horwitz, A. F. (1987) *Annu. Rev. Cell Biol.* **3**, 179–205.
3. Hynes, R. O. (1987) *Cell* **48**, 549–554.
4. Ruoslahti, E. & Pierschbacher, M. D. (1987) *Science* **238**, 491–497.
5. Werb, Z., Tremble, P. M., Behrendtsen, O., Crowley, E. & Damsky, C. H. (1989) *J. Cell Biol.* **109**, 877–889.
6. Schwartz, M. A., Ingber, D. E., Lawrence, M., Springer, T. A. & Lechene, C. (1991) *Exp. Cell Res.* **195**, 533–535.
7. Wacholtz, M. C., Patel, S. S. & Lipsky, P. E. (1989) *J. Exp. Med.* **170**, 431–448.
8. Springer, T. A. (1990) *Nature (London)* **346**, 425–434.
9. Adams, J. C. & Watt, F. M. (1990) *Cell* **63**, 425–435.
10. Otey, C. A., Pavalko, F. M. & Burridge, K. (1990) *J. Cell Biol.* **111**, 721–729.
11. Hibbs, M. L., Xu, H., Stacker, S. A. & Springer, T. A. (1991) *Science* **251**, 1611–1613.
12. Tamura, R. N., Rozzo, C., Starr, L., Chambers, J., Reichardt, L. F., Cooper, H. M. & Quaranta, V. (1990) *J. Cell Biol.* **111**, 1593–1604.
13. Sonnenberg, A., Modderman, P. W. & Hogervorst, F. (1988) *Nature (London)* **336**, 487–489.
14. Shimizu, Y., Van Seventer, G. A., Horgan, K. J. & Shaw, S. (1990) *Nature (London)* **345**, 250–253.
15. Shaw, L. M., Messier, J. M. & Mercurio, A. M. (1990) *J. Cell Biol.* **110**, 2167–2174.
16. Kramer, R. H., Cheng, Y.-F. & Clyman, R. J. (1990) *J. Cell Biol.* **111**, 1233–1243.
17. Sorokin, L., Sonnenberg, A., Aumailley, M., Timpl, R. & Ekblom, P. (1990) *J. Cell Biol.* **111**, 1265–1273.
18. deCurtis, I., Quaranta, V., Tamura, R. N. & Reichardt, L. F. (1991) *J. Cell Biol.* **113**, 405–416.
19. Quaranta, V. & Jones, J. C. R. (1991) *Trends Cell Biol.* **1**, 2–4.
20. Gehlsen, K. R., Dickerson, K., Argraves, W. S., Engvall, E. & Ruoslahti, E. (1989) *J. Biol. Chem.* **264**, 19034–19038.
21. Wayner, E. A. & Carter, W. G. (1987) *J. Cell Biol.* **105**, 1873–1884.
22. Carter, W. G., Ryan, M. C. & Gahr, P. J. (1991) *Cell* **65**, 599–610.
23. Larjava, H., Peltonen, J., Akiyama, S. K., Yamada, S. S., Gralnick, H. R., Uitto, J. & Yamada, K. M. (1990) *J. Cell Biol.* **110**, 803–815.
24. Tsuji, T., Yamamoto, F.-i., Miura, Y., Takio, K., Titani, K., Pawar, S., Osawa, T. & Hakomori, S.-i. (1990) *J. Biol. Chem.* **265**, 7016–7021.
25. Devereux, J., Haeberli, P. & Smithies, O. (1984) *Nucleic Acids Res.* **12**, 387–395.
26. Kajiji, S., Tamura, R. N. & Quaranta, V. (1989) *EMBO J.* **3**, 673–680.
27. Mullis, K. B. & Faloona, F. A. (1987) *Methods Enzymol.* **155**, 335–350.
28. Ignatius, M. J., Large, T. H., Houde, M., Tawil, J. W., Barton, A., Esch, F., Carbonetto, S. & Reichardt, L. F. (1990) *J. Cell Biol.* **111**, 709–720.
29. Hemler, M. E., Crouse, C., Takada, Y. & Sonnenberg, A. (1988) *J. Biol. Chem.* **263**, 7660–7665.
30. Beck, K., Hunter, I. & Engel, J. (1990) *FASEB J.* **4**, 148–160.
31. Corbi, A. L., Garcia Aguilar, J. & Springer, T. A. (1990) *J. Biol. Chem.* **265**, 2782–2788.
32. Brown, N. H., King, D. L., Wilcox, M. & Kafatos, F. C. (1989) *Cell* **59**, 185–195.
33. Prandini, M. H., Denarier, E., Frachet, P., Uzan, G. & Marguerie, G. (1988) *Biochem. Biophys. Res. Commun.* **156**, 595–601.
34. Smith, C. W. J., Patton, J. G. & Nadal-Ginard, B. (1989) *Annu. Rev. Genet.* **23**, 527–577.
35. van Kuppevelt, T. H. M. S. M., Languino, L. R., Gailit, J. O., Suzuki, S. & Ruoslahti, E. (1989) *Proc. Natl. Acad. Sci. USA* **86**, 5415–5418.
36. Bray, P. F., Leung, C. S.-I. & Shuman, M. A. (1990) *J. Biol. Chem.* **265**, 9587–9590.
37. Cooper, H. M., Tamura, R. N. & Quaranta, V. (1991) *J. Cell Biol.*, in press.
38. O'Toole, T. E., Mandelman, D., Forsyth, J., Shattil, S.J., Plow, E. F. & Ginsberg, M. H. (1991) *Science*, in press.