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Amino acid sequence of a novel integrin β_4 subunit and primary expression of the mRNA in epithelial cells

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Using the polymerase chain reaction, we have isolated cDNA clones that encode a new integrin β subunit—β_4. Its cDNA, which is 5676 bp in length, has one long coding sequence (5256 bp), a polyadenylation signal and a poly(A) tail. The deduced sequence of 1752 amino acids is unique among the integrin β subunits. It contains a putative signal sequence as well as a transmembrane domain that divides the molecule into an extracellular domain at the N-terminal side and a cytoplasmic domain at the C-terminal side. The extracellular domain exhibits a 4-fold repeat of cysteine-rich motif similar to those of other integrin β subunits. Certain features of the extracellular domain, however, are unique to the β_4 subunit sequence. Of the 56 conserved cysteine residues found within the extracellular domain of other mature β subunits, eight such residues are deleted from the β_4 subunit sequence. The cytoplasmic domain is much larger (~1000 amino acids) than those of other β subunits (~50 amino acids) and has no significant homology with them. A protein homology search revealed that the β_4 subunit cytoplasmic domain has four repeating units that are homologous to the type III repetition exhibited by fibronectin. The β_4 subunit mRNA was expressed primarily in epithelial cells. The restricted expression and the new structural features distinguish the integrin β_4 subunit from other integrin β subunits.

Key words: cloning/fibronectin type III repeat/integrin β_4 subunit/laminin/PCR

Introduction

Cell—substratum interactions play important roles in various biological processes, such as embryogenesis, wound healing, blood coagulation, immunoreaction and metastasis of malignant cells. Recent studies have revealed a family of cell surface receptors, termed 'integrins', that may occupy a central position in such interactions and that appear to be responsible for linking the extracellular matrix with intracellular cytoskeletal proteins (Buck and Horwitz, 1987; Hynes, 1987; Ruoslahti and Pierschbacher, 1987).

Integrins are heterodimer molecules that are composed of one α subunit and one β subunit. Both subunits are transmembrane proteins with small cytoplasmic domains and large extracellular domains. To date, >10 α subunits and three β subunits have been characterized to the extent that the cDNAs for most of them have been cloned. Recent studies from several laboratories suggest that at least two, and possibly three, additional integrin β subunits are expressed (Sonnenberg *et al.*, 1988a; Cheresh *et al.*, 1989; Freed *et al.*, 1989; Hemler *et al.*, 1989; Holzmann *et al.*, 1989; Kajiji *et al.*, 1989). Further studies of these newly-identified β subunits are likely to provide new insight into the functions of integrins. One of the new β subunits, designated β_4, is of considerable interest. It appears that this molecule is expressed primarily in epithelial cells. Moreover, reports have indicated that the β_4 subunit complex is expressed to a high degree in some tumor cells, and that such high expression correlates with metastatic potential of the tumor cells (Falcioni *et al.*, 1986). Furthermore, the two molecular sized (200 kd and 180 kd) of the three identified forms of the β_4 subunit are substantially higher than those of other integrin β subunits.

The polymerase chain reaction (PCR; Saiki *et al.* 1988) facilitates the cloning of cDNAs for proteins homologous to proteins with known sequences (Gould *et al.*, 1989). We utilized PCR to clone cDNA for the unique β_4 subunit. We report here the complete amino acid sequence of this subunit and describe the expression of its mRNA in epithelial cells.

						35						
Sequence 1			Pro	Val	Lys	Ser	Cys	Thr	Glu	Cys	Val	
Primer 1	5′-	GGA TCC (Bam HI)	CCN	GTN	AAR	XN	TGY	ACN	GAR	TGY	GT	- 3′
		Degeneracy 2^{13} or 8192										
		150					155					
Sequence 2		Met	Asp	Leu	Ser*	Tyr	Ser	Met	Lys+	Asp		
Primer 2	3′-	TAC	CTR	RAN	AGN	ATR	AGN	TAC	ZTY	CT	CTT AAG (EcoRI)	- 5′
		Degeneracy 2^{11} or 2048										
		250					255					
Sequence 3		Glu	Gly	Gly	Phe+	Asp	Ala	Ilu	Met	Gln		
Primer 3	3′-	CTY	CCN	CCN	RAR	CTR	CGN	TAN	TAC	GT	CTT AAG (EcoRI)	- 5′
		Degeneracy 2^{12} or 4096										

Fig. 1. Amino acid sequences and corresponding oligonucleotide sequences used as primers for PCR. Amino acid sequence 1 was obtained in accordance with the published N-terminal amino acid sequence of the mature β_4 subunit (Kajiji *et al.*, 1989). Amino acid sequences 2 and 3 were chosen after comparing various integrin β subunits. Numbering of sequences 1–3 was taken from that of corresponding amino acids in the human β_1 subunit (Argraves *et al.*, 1987). The primers contain all the possible combinations of nucleotide sequences that correspond to the respective amino acid, with the exception of the serine residue in primer 2. *The sequences GAR for serine were not included in the development of primer 2, since the known integrin β subunits use only sequences TCN for the serine residues. †The sequences CTN were included, since the amino acid residues at these positions of the β_2 subunit are leucine. The nucleotide sequence is as follow: A, deoxyadenosine; C, deoxycytosine; G, deoxyguanosine; T, deoxythymidine; R, either A or G; X, either AG or TC; Y, either C or T; Z, either G or T; N, either A, C, G or T.

Results

Isolation of cDNA clones for integrin β_4 subunit

In order to isolate partial cDNA clones for the integrin β_4 subunit, we took a PCR approach (Gould *et al.*, 1989). The first primer came from the published N-terminal amino acid sequence of the β_4 subunit (Kajiji *et al.*, 1989); for the second primer, we chose two highly conserved regions of the integrin β subunit by comparing the human β subunit sequences (Argraves *et al.*, 1987; Fitzgerald *et al.*, 1987; Kishimoto *et al.*, 1987) (Figure 1). *Bam*HI and *Eco*RI linkers were added to each primer to facilitate the subsequent analysis. We used cDNAs synthesized of poly(A) RNA preparations from human placenta and retinal pigment epithelium (RPE) cells. PCR carried out with primers 1 and 3 yielded a product of ~600 bp even at 65°C annealing temperature. The combined use of primers 1 and 2, however, yielded an estimated 350 bp product at the lower annealing temperature of 55°C, but poor results at 65°C. Similar results were obtained by using both cDNAs as templates, and the cDNA made from RPE mRNA gave a better result.

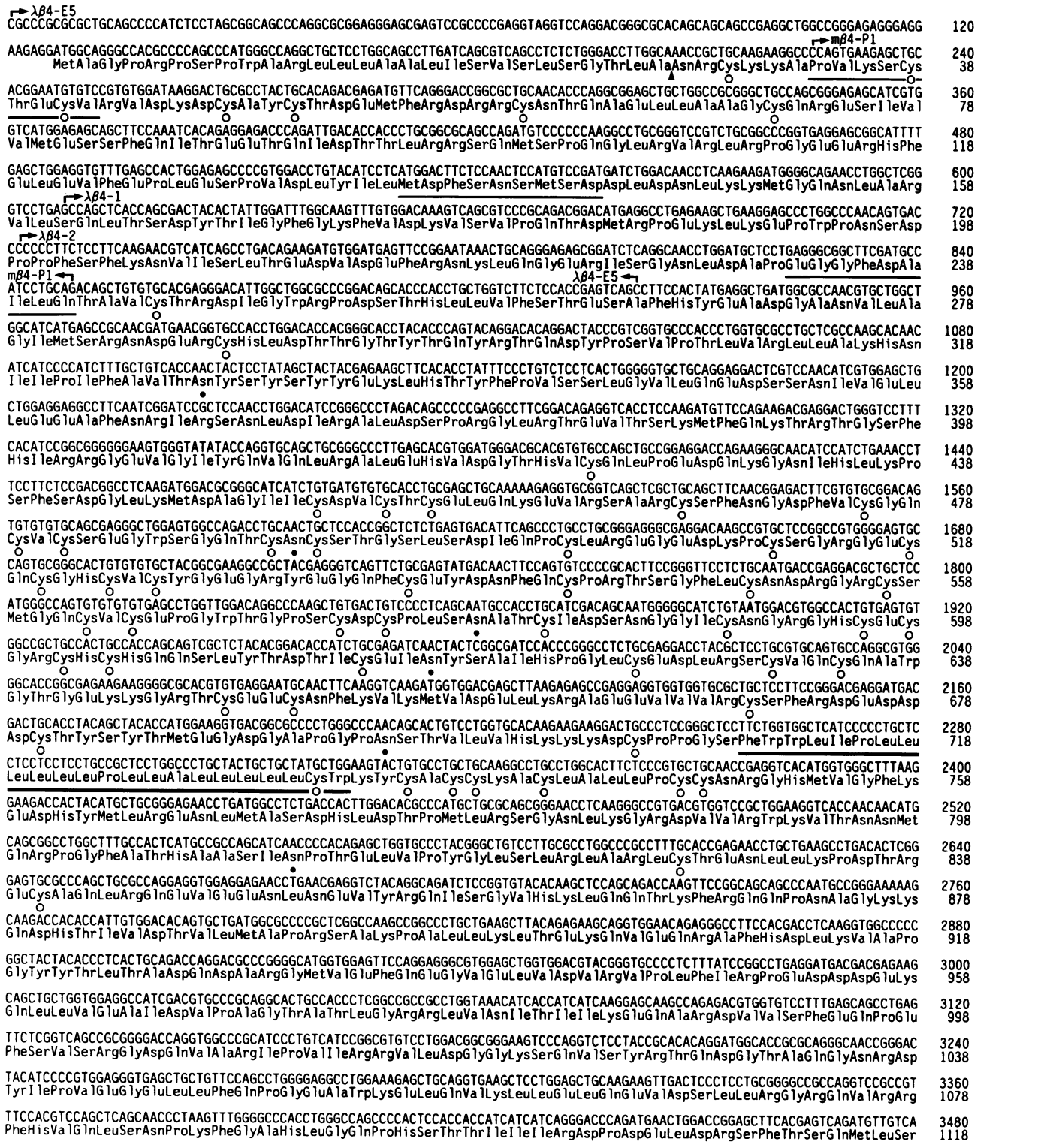

Fig. 2. The cDNA sequence and the deduced amino acid sequence of human β_4 subunit. The ends of the cDNA clones are indicated and the cleavage site of the mature β_4 subunit is shown by an arrowhead. The positions of primers 1–3 and membrane spanning domain are underlined. The cysteine residues are indicated by open circles. The possible N-linked glycosylation sites are marked by closed circles.

The respective sizes of the these products were in keeping with the sizes expected from the β subunit sequences of other integrin β subunits. Moreover, a DNA fragment with the size expected from the sequence (350 bp) was formed when PCR was carried out using the aforementioned 600 bp PCR product as a template in conjunction with primers 1 and 2. These findings suggest strongly that the resultant products contained cDNAs for an integrin β subunit. Subsequently, the 600 bp product was digested with *Bam*HI and *Eco*RI then subcloned into the M13 vector. Four independent clones were then isolated and sequenced. The amino acid sequences deduced from two clones (mB4-P1 and -P2) contained a part of the reported N-terminal amino acid sequence of the mature integrin β_4 subunit and the remaining sequence was homologous to the other integrin β subunits. The deduced amino acid sequence of the remaining two clones failed to display any significant homology to the known integrin β subunit sequences.

In order to isolate the clones that cover the entire coding sequence of the integrin β_4 subunit, we screened a human RPE cDNA library using the aforementioned cDNA (mB4-P1) as a probe. Screening of $\sim 1.0 \times 10^5$ plaques yielded two clones (λB4-1 and -2) that hybridized well with probe mB4-P1. These clones were found to have insert sizes of ~ 5 kbp, but the 5′ end nucleotide sequences revealed that they lacked the translation initiation site. In order to obtain a nucleotide sequence for the missing region at the 5′ end, an extension library was constructed using a primer that complemented a sequence found near the 5′ end of the λB4-1 insert. Four positive clones were isolated by screening the library with the mB4-P1 insert as a probe, and sequenced.

Primary structure of integrin β4 subunit

The nucleotide and deduced amino acid sequences from several overlapping clones are shown in Figure 2. The cDNA is 5676 bp in length and has one long open reading frame of 5256 bp that encodes a 1752 amino acid polypeptide. The open reading frame is followed by ~ 300 bp of a 3′ untranslating flanking sequence that contains a polyadenylation signal and a poly(A) tail. The first ATG and the surrounding sequence match the consensus sequence of the translation initiation site that has been described by Kozak (1984). The putative translation initiation site is followed by a short stretch of hydrophobic amino acid sequence that may serve as the signal sequence of the β_4 subunit. This is followed by the reported N-terminal amino acid sequence of the mature β_4 subunit (Kajiji *et al.*, 1989). The cysteine-rich domain is located before the putative transmembrane domain, which consists of ~ 23 uncharged amino acid residues. As is the case with other β subunits, the cysteine-rich domain is composed of four repeating motifs. Presently, there are no direct data available concerning the topology of the molecule. Based upon the results on other integrin β subunits (Marcantonio and Hynes, 1988; Mueller *et al.*,

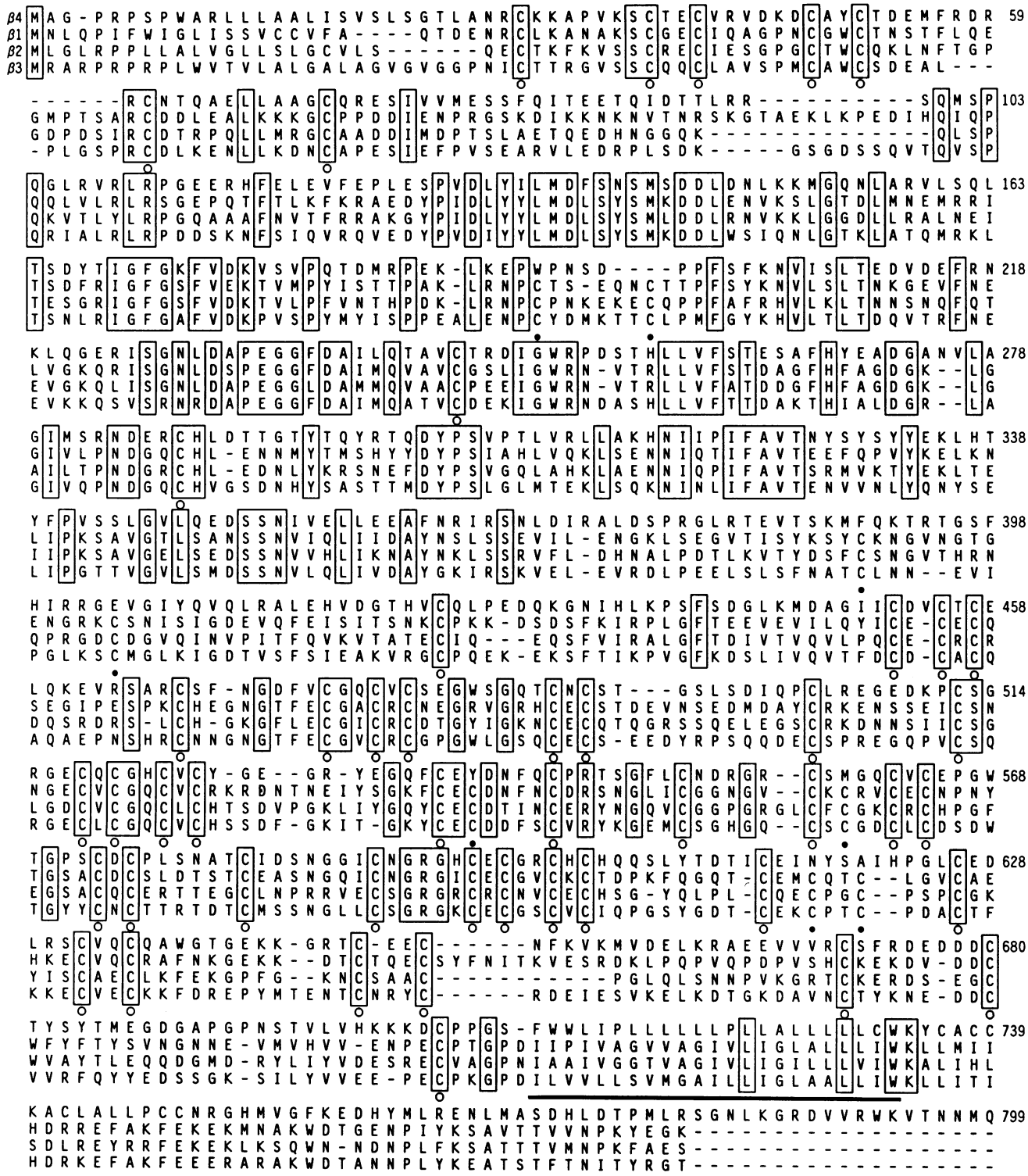

Fig. 3. Comparison of the deduced amino acid sequence of human integrin β subunits. The deduced amino acid sequences of the integrin β_4 subunit are compared with those of human integrin β_1–β_3 subunits (Argraves *et al.*, 1989; Kishimoto *et al.*, 1987; Fitzgerald *et al.*, 1987). Identical residues among the β subunits are boxed. Dashes denote gaps introduced to maximize homology. The positions of conserved cysteine residues among β subunits are indicated by open circles and the position of cysteine residues that are deleted in the β_4 subunit are shown by closed circles. The possible membrane sparing domain is underlined.

1988), however, it appears that the N-terminal side of the transmembrane domain may be the extracellular domain. The putative cytoplasmic domain of the β_4 subunit is large and ~1000 amino acids in size. The resultant amino acid sequence contains five possibe N-linked glycosylation sites in the extracellular domain and three such sites in the cytoplasmic domain.

A comparison of the deduced amino acid sequence with other integrin β subunit sequences revealed that the gross structure of the β_4 subunit is similar to that of other integrin

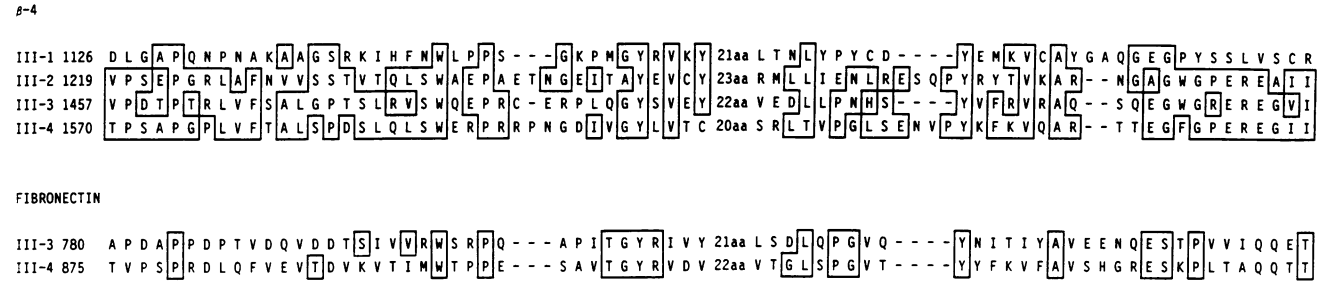

Fig. 4. Alignment of fibronectin type III-like repeats. Four repeating sequences from the cytoplasmic domain of the β_4 subunit and two type III repeats from human fibronectin (Kornblihtt *et al.*, 1985) are compared. Identical amino acid residues in the β_4 subunits appear in boxes. Also in boxes are the amino acid residues that appeared at the same position in more than half of the human fibronectin type III repeats.

β subunits, but possesses two unique features. All cysteine residues (totalling 56) located in the extracellular domain of mature β subunits are conserved among other integrin β subunits from various organisms from *Drosophila* to human (Tamkun *et al.*, 1986; Argraves *et al.*, 1987; Fitzgerald *et al.*, 1987; Kishimoto *et al.*, 1987; DeSimone and Hynes, 1988; MacKrell *et al.*, 1988; Tominaga, 1988). Eight of these cysteines are absent from the β_4 subunit sequence. The other major difference is that the cytoplasmic domain of the β_4 subunit is quite large ($\sim$1000 amino acids) and has no significant homology to the much shorter ($\sim$50 amino acids) cytoplasmic domains of other β subunits. The extracellular domain of the β_4 subunit exhibits an $\sim$38% identity to the human β_1 subunit, 36% to the human β_2 subunit and 37% to the human β_3 subunit (Figure 3). These numbers are slightly lower than those obtained from the comparison between the other human β subunits. However, several areas are also conserved in the β_4 subunit (Argraves *et al.*, 1987; Fitzgerald *et al.*, 1987; Kishimoto *et al.*, 1987; DeSimone and Hynes, 1988; MacKrell *et al.*, 1988; Tominaga, 1988). The sequences corresponding to primers 2 and 3 and their surrounding areas are conserved particularly well.

The comparison of the β_4 subunit sequence with the protein sequences in Bionet protein database revealed the presence of four fibronectin type III-like sequences in the cytoplasmic domain. When these sequences were lined up with human fibronectin type III repeats, $\sim$20–30% of the amino acid residues were matched (Figure 4).

RNA blotting analysis

RNA blotting analysis with an extracellular domain probe (Figure 5A) and a cytoplasmic domain probe (Figure 5B) of the β_4 subunit showed one major band of $\sim$6.0 kb message. Two faint bands of $\sim$7.5 kb and 5 kb were also observed even under stringent washing conditions. These two bands may be alternatively spliced species. The β_4 mRNA was detected primarily in epithelial cells, as reported for the proteins by Kajiji *et al.* (1989) and Hemler *et al.* (1989).

Discussion

We have isolated and sequenced cDNAs that encode a new integrin β subunit. For the following reasons, we believe that the cDNAs correspond to the fourth type of β subunit, β_4, that was described recently by Sonnenberg *et al.* (1988a), Kajiji *et al.* (1989) and Hemler *et al.* (1989).

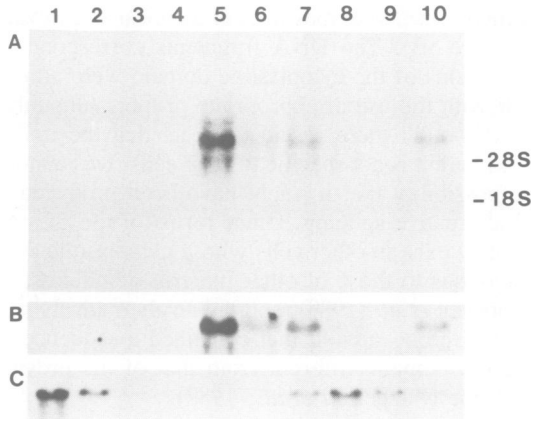

Fig. 5. RNA blot analysis of integrin β_4 subunit. Total RNA preparations from various human cells (10 μg per lane) were separated electrophoretically on 0.8% agarose–formaldehyde gel, transferred to nitrocellulose filters and probed with two different human β_4 subunit cDNAs (A, mB4-P1, 600 bp; B, *Hin*dIII–*Sst*I fragment, 850 bp) and a human β_1 subunit cDNA (C, λI34, 1.1 kb). The size markers were calf liver 28S (4.7 kb) and 18S (1.9 kbp) rRNA. **1**, IMR90 diploid fibroblasts; **2**, MG63 osteosarcoma cells, **3**, K562 myelogenous leukemia cells; **4**, U937 histiocytic lymphoma cells; **5**, Colo205 colon adenocarcinoma cells; **6**, DLD-1 colon adenocarcinoma cells; **7**, A431 epidermoid carcinoma cells; **8**, RPE cells; **9**, SK-N-SH neuroblastoma cells; **10**, U251 glioma cells.

(i) This cDNA contains the sequence that corresponds to the reported N-terminal amino acid sequence of the mature integrin β_4 subunit. (ii) Expression of the mRNA is limited primarily to epithelial cells, which is consistent with the findings of these investigators. (iii) Moreover, the β_4 subunit is larger than the other integrin β subunits and the size predicted from our cDNAs agrees with the size obtained from their protein work.

Although the β_4 subunit possesses basic characteristics and a fundamental molecular structure that are similar to those seen in other integrin β subunits, we have also observed features that are unique to this molecule, such as its large cytoplasmic domain and the omission of eight cysteine residues from its extracellular domain. Clearly, the β_4 subunit defined by the cDNA is a distinctive integrin β subunit. The β_4 subunit cytoplasmic domain has no substantial homology to other integrin β subunit sequences, but it contains fibronectin type III-like repeats of the kind that have been reported in various molecules (Cunningham *et al.*, 1987; Harrelson and Goodman, 1987; Moos *et al.*, 1988; Benian *et al.*, 1989; Streuli *et al.*, 1989). Our data

show that this result did not derive from a cloning artifact. We have isolated two independent cDNA clones that contain the same nucleotide sequence corresponding to the cytoplasmic domain. Moreover, the amino acid sequences deduced from two other reading frames for this region also show no significant homology to other β subunit sequences. This indicates that the unique cytoplasmic domain is not derived from misreading of the coding frame. While one might speculate that the sequence corresponding to the large cytoplasmic domain could have been produced by a special alternative splicing of the mRNA, results from Northern blot and PCR analyses showed that the nucleotide sequence corresponding to the cytoplasmic domain is likely to exist also in the β_4 mRNA of other cells, since the cDNA probes corresponding to the cytoplasmic domain of the β_4 subunit hybridized with messages of identical size in various cells (Figure 5B). The DNA fragments corresponding to several regions of the cytoplasmic domain were amplified via PCR with the use of appropriate primers (unpublished result). Although these results indicate that the molecule described herein is not specific to RPE cells, we cannot rule out the possibility that it might have been produced by a special alternative splicing. Other forms of the β_4 subunit mRNA may exist in other cells whose cytoplasmic domain is homologous to those of other integrin β subunits, since van Kuppevelt *et al.* (1989) reported an alternatively spliced mRNA for the β_3 subunit that contained a sequence for a cytoplasmic domain different from that of the previously reported one (Fitzgerald *et al.*, 1987).

The β_4 subunit protein is expressed by various cells in three forms: 200 kd, 180 kd and 125 kd (Sonnenberg *et al.*, 1988a; Hemler *et al.*, 1989; Kajiji *et al.*, 1989). Sonnenberg and colleagues (1988a) reported that the precursor protein of the β_4 subunit had a large molecular mass of ~195 kd. Subsequently, Kajiji and co-workers (1989) offered evidence that such a large molecular size was due to the extraordinarily high content of sialic acid in the β_4 subunit. However, Hemler *et al.* (1989) reported that the molecular sizes of these molecules decreased only slightly following neuraminidase treatment, and that the 125 kd form had apparently been produced from the 200 kd form via proteolytic release of an 85 kd fragment. Although the N-terminal amino acid sequence of the 200 kd form has not yet been determined, the 180 kd and 125 kd forms are known to have the same N-terminal sequence that is homologous to those of other β subunits (Hemler *et al.*, 1989). This finding suggests that the β_4 subunit contains a very large cytoplasmic domain, and that the 180 kd and 125 kd forms are produced as a result of proteolysis in the cytoplasmic domain of the 200 kd form. The results presented herein are consistent with that conclusion.

Although the physiological function of the β_4 subunit complex is not yet fully understood, there are reasons to believe that this molecule plays an important role within epithelial cells. As mentioned previously, the β_4 subunit is expressed primarily in epithelial cells, whereas the β_1 subunit is expressed in various types of cells. Furthermore, data from other laboratories suggest that the α_6 subunit creates a complex preferentially with the β_4 subunit even in the presence of the β_1 subunit in A431 epidermoid carcinoma cells (Kennel *et al.*, 1989). Judging from the basolateral distribution of the molecule (Sonnenberg *et al.*, 1987; Kajiji *et al.*, 1989), it is possible that the $\alpha_6\beta_4$ complex is involved in cell–extracellular matrix interaction, as is the case with many other integrin molecules. Although the $\alpha_6\beta_1$ complex exhibits laminin binding activity (Sonnenberg *et al.*, 1988b), thus far there is no direct evidence that such activity is generated by the $\alpha_6\beta_4$ complex. It is possible, however, that the $\alpha_6\beta_4$ complex functions as a laminin receptor, as is the case with the $\alpha_v\beta_x$ and $\alpha_v\beta_3$ complexes, which exhibit vitronectin binding activity (Cheresh *et al.*, 1989). If such is the case, validity would be lent to the reported close correlation between high expression of the $\alpha_6\beta_4$ complex and metastatic potential (Falcioni *et al.*, 1986), since many investigators have suggested that laminin is involved in the metastatic process (Martin and Timpl, 1987).

It is believed that the integrin β subunit plays a major role in signal transduction in cell–extracellular matrix interaction, since the cytoplasmic domain of the β_1 subunit is highly conserved in β_1 subunits from various organisms (Tamkun *et al.*, 1986; Argraves *et al.*, 1987; DeSimone and Hynes, 1988; MacKrell *et al.*, 1988: Tominaga, 1988). In addition, the mutated β_1 subunit lacking the cytoplasmic domain does not localize efficiently in focal contacts (Solowska *et al.*, 1989). Horwitz *et al.* (1986) reported that CSAT antigen, a mixture of chicken integrins, could interact with a cytoskeletal protein, talin, although it is not known whether or not this activity is attributable to the β subunit. Argraves and co-workers (1989), on the other hand, discovered a new protein that interacts specifically with the cytoplasmic domain of the β_1 subunit. In this context, the unique, large cytoplasmic domain of the β_4 subunit is of particular interest. The existence of fibronectin type III repeats is especially intriguing. Benian *et al.* (1989) reported recently that twitchin, a large intracellular muscle protein, had fibronectin type III repeats. They mentioned, too, that titin, a protein of striated muscle, also had similar repeats. Since these proteins are thought to interact with other cytoskeletal proteins, it is tempting to speculate that the fibronectin type III repeats in the cytoplasmic domain of the β_4 subunit might interact with some cytoskeletal proteins. Tyrosine phosphorylation within the cytoplasmic domain of integrin β subunits may play an important regulatory role in integrin function (Hirst *et al.*, 1986), although evidence supporting this opinion is limited at present. Kennel *et al.* (1980, 1989) reported that the 200 kd form of the β_4 subunit is phosphorylated possibly at a tyrosine residue located in or about the C-terminal region of the cytoplasmic domain; there are several tyrosine residues in this region, but we could not locate the candidate tyrosine residue. It may be noteworthy that tyrosine 1690 and its surrounding sequence show very little similarity to the short amino acid sequences containing phosphorylated tyrosine residues in the EGF and insulin receptors. In conclusion, the new molecule described herein is a novel β subunit member of the integrin family that possesses truly unique features. Further studies involving this molecule may provide valuable insight into the function of integrin molecules.

Materials and methods

RNA preparation and cDNA synthesis

Total RNA was extracted from various cultured cells and placenta according to a guanidium isothicyanate procedure (Maniatis *et al.*, 1982). Poly(A) RNAs were isolated with oligo(dT)–cellulose spin columns obtained from Pharmacia LKB. Using a Boehringer-Mannheim cDNA synthesis kit, cDNAs were synthesized from the poly(A) RNA preparations.

PCR conditions

PCR was carried out essentially as described (Saiki *et al.*, 1988; Gould *et al.*, 1989). One hundred ng of template cDNA and primers were dissolved in 50 μl of *Taq* polymerase buffer. Both unique and degenerate primers were used, at concentrations of 1 μM and 20 μM, respectively. After the addition of 100 μl of mineral oil, the reaction mixture was incubated at 95°C for 5 min. Subsequently, 2 U of *Taq* polymerase was added and the cycle reaction initiated. Denaturation was performed at 95°C for 1.5 min, annealing at 50–65°C for 2 min and polymerization at 72°C for 3 min. This cycle was repeated 30–60 times, with the addition of another 2 U of *Taq* polymerase after 30 cycles. The resultant products were separated electrophoretically on 1.5% agarose gel and appropriate bands were extracted, precipitated with ethanol and used for subsequent analysis.

Construction and screening of a cDNA library

An RPE cell cDNA library was constructed in λZapII vector from poly(A) RNA prepared from cultured human RPE cells. In accordance with the cDNA synthesis procedure described above, the cDNA was blunt-ended with T4 polymerase, methylated with *Eco*RI methylase and ligated with *Eco*RI linkers. Subsequently, the cDNA was digested with *Eco*RI and ligated into the *Eco*RI site of the λZapII vector obtained from Stratagene. Following the packaging and amplification, the resultant library was screened employing a plaque hybridization method with a ^{32}P-labeled DNA probe, as described previously (Suzuki *et al.*, 1987). The positives were plaque-purified and the inserts were cut out in the form of pBluescript SK plasmid according to the manufacturer's protocol. The plasmid DNAs were purified utilizing an alkaline lysis method (Maniatis *et al.*, 1982) and used for further analysis. In order to construct an extension library, a specific primer [instead of oligo(dT) primer] was used in the first strand cDNA synthesis. Subsequent procedures were essentially the same as those described above.

Other procedures

Cells were cultured in Dulbecco's modified Eagle's medium or RPMI 1640 medium supplemented with 10% fetal calf serum, penicillin and streptomycin. The cultures were maintained at 37°C in a 7% CO_2 atmosphere.

DNAs were labeled with [^{32}P]dCTP using a random primed DNA labeling kit obtained from Boehringer-Mannheim. DNAs were subcloned into the M13 vector and sequenced according to the dideoxynucleotide chain termination method of Sanger *et al.* (1977), using [^{35}S]dATP and a sequenase DNA sequencing kit from United States Biochemical Corporation. All DNA sequences reported herein have resulted from the sequencing of both strands.

Total RNA preparations were electrophoresed on 0.8% agarose gels containing 2.2 M formaldehyde and 0.02 M sodium acetate (Lehrach *et al.*, 1977). RNA transfer to nitrocellulose and hybridization with ^{32}P-labeled probes were carried out according to the method developed by Thomas (1980). Filters were washed at high stringency in 0.2 × SSC containing 0.1% SDS at 65°C for 10 min, or at low stringency in 2 × SSC containing 0.1% SDS at 42°C for 10 min.

The protein homology search was performed by using the Bionet database and the Pearson and Lipman program (1988).

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Note added in proof

After submission of this manuscript, we learned that A.Sonnenberg's group also cloned cDNA for integrin β_4 subunit and we exchanged the sequences with each other. Comparison of the two sequences revealed that the sequence obtained by Sonnenberg and his colleagues contained an insertion of 53 amino acid sequence after histidine 1450. Judging from the nucleotide sequence, we think that this is derived from an alternative splicing of the mRNA.