Molecular Cloning and Expression of the cDNA for α_3 Subunit of Human $\alpha_3\beta_1$ (VLA-3), an Integrin Receptor for Fibronectin, Laminin, and Collagen

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Abstract. $\alpha_3\beta_1$ (VLA-3), a member of the integrin family of cell adhesion receptors, may function as a receptor for fibronectin, laminin, and collagen. A partial cDNA clone (2.4 kb) for the human α_3 subunit was selected from an endothelial cell λgt11 cDNA library by specific antibody screening. Several overlapping cDNA clones were subsequently obtained, of a total length of 4.6 kb from various cDNA libraries. The reconstructed α_3 cDNA was expressed on the surface of chinese hamster ovary cells as detected by an α_3-specific mAb after transfection, suggesting that the cDNA is authentic. Within this sequence was an open reading frame, encoding for 1,051 amino acids, including a signal peptide of 32 residues, a long extracellular domain (959 residues), a transmembrane domain (28 residues), and a short cytoplasmic segment (32 residues). Overall, the α_3 amino acid sequence was 25–37% similar to the other integrin α subunits that are cleaved, with most similarity to the α_6 sequence (37%), and less similarity to those α subunits that have I domains (15–20%, excluding the I domain sequence itself). Features most like those in other α subunits are (*a*) the positions of 18/19 cysteine residues, (*b*) three potential metal binding domains of the general structure DX(D/N)X(D/N)GXXD, and (*c*) the predicted transmembrane domain. The mass of α_3 calculated from its amino acid sequence is 113,505. The human α_3 sequence was 89% identical to hamster galactoprotein b3, and 70% similar to the chicken CSAT antigen band 2 protein partial sequence, suggesting that these two polypeptides are homologues of human α_3.

$\alpha_3\beta_1$ (VLA-3), a cell surface heterodimer composed of α_3 and β_1 subunits, is an integrin (1, 6, 19, 23, 27) that has been implicated as a receptor for collagen, laminin, and fibronectin (13, 17, 18, 49, 54). The role of $\alpha_3\beta_1$ has been somewhat difficult to assess, partly because its adhesive functions are often obscured by other collagen, laminin, and fibronectin receptors such as $\alpha_2\beta_1$, $\alpha_6\beta_1$ and $\alpha_5\beta_1$ (13). The binding of $\alpha_3\beta_1$ to its different ligands appears to be accomplished by multiple binding mechanisms. For example, $\alpha_3\beta_1$ binding to fibronectin differs substantially from binding to collagen and laminin with respect to both the divalent cation requirements and the influence of RGD peptides (13). In addition to being a receptor for extracellular matrix ligands, $\alpha_3\beta_1$ could possibly also bind to cell surface ligands, as inferred from $\alpha_3\beta_1$ localization to cell–cell contact sites (7, 31, 35).

In normal tissue, $\alpha_3\beta_1$ expression is limited to a few cell types, including kidney glomeruli, and the basal cells of epidermis and other epithelia (7, 16, 34, 35). In contrast, nearly all cultured cell lines express $\alpha_3\beta_1$ except for lymphoid cells (16). Underscoring the likely importance of $\alpha_3\beta_1$-mediated cell adhesion, its expression levels have been noted to vary on different cell types and with different growth conditions. For example, $\alpha_3\beta_1$ levels diminished in response to TGFβ in one case (22), but were increased in other examples (21). Also, $\alpha_3\beta_1$ can be induced by attachment of some cultured cells to extracellular matrix (40) while $\alpha_3\beta_1$ levels decrease upon shifting of fibroblasts from exponential growth to quiescence (14).

On rat (37) and human (34) transformed cells, $\alpha_3\beta_1$ levels were elevated, suggesting that $\alpha_3\beta_1$ could possibly contribute to tumorigenicity and/or invasiveness. However, in several other studies, $\alpha_3\beta_1$ levels were either unchanged or decreased on malignant cells (11, 35, 36), emphasizing that regulation of $\alpha_3\beta_1$ expression on cancer cells is complex.

In this paper we have cloned, sequenced, and expressed in eukaryotic cells the cDNA for the α_3 subunit of $\alpha_3\beta_1$. We have found that α_3 resembles other integrin α subunits in having a signal peptide, a long extracellular domain (959 residues) and a putative transmembrane domain and a short cytoplasmic segment (32 residues). The extracellular domain includes 18 conserved cysteine residues and three metal binding domains of the general structure DX(D/N)-X(D/N)GXXD. The α_3 subunit amino acid sequence was

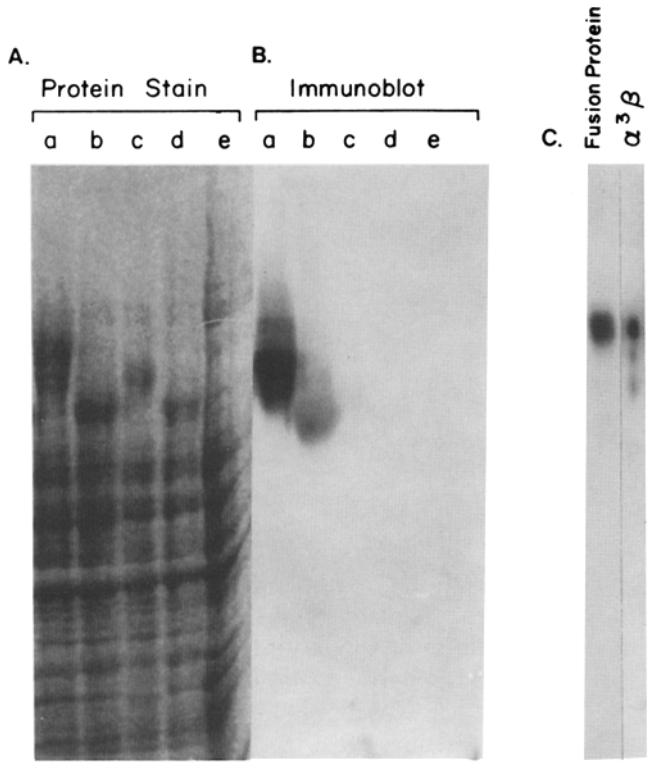


Figure 1. Immunological crossreactivity between purified α_3 and α_3 fusion proteins. β-Galactosidase fusion proteins were produced from clones 3.22 (lane *a*), 3.24 (lane *b*), 3.25 (lane *c*), and 3.26 (lane *d*) that had been immunoselected using anti-α_3 antiserum. NP-40-insoluble material from these fusion protein preparations and from uninfected Y1089 *E. coli* (lane *e*) was analyzed by SDS-PAGE and stained for protein using Coomassie blue as shown in *A*. After blotting of these proteins onto nitrocellulose, immunostaining with enriched anti-α_3 antibodies (1/500–1/1,000 final dilution) was carried out (*B*). For *C*, purified fusion protein from clone 3.22 was used to prepare fusion protein-enriched antiserum as described in Materials and Methods, and this antibody stained both the fusion protein from clone 3.22 and purified α_3 protein. The comigration of the fusion protein and α_3 protein at ~150,000 M_r is fortuitous.

most similar to the integrin α_6 subunit (37% identity), and had 25–30% identity to other integrin α subunits. Also, the α_3 subunit was 89% identical to hamster galactoprotein b3, a protein reportedly upregulated in oncogene-transformed fibroblasts (52), and was 70% similar to the avian CSAT antigen band 2 partial amino acid sequence (43), suggesting that they are homologues of human α_3 subunit.

Materials and Methods

Purification of $\alpha_3\beta_1$ and Preparation of α_3-specific Antiserum

The anti-α_3 mAb J143 (16) was coupled to CNBr-Sepharose (Pharmacia Fine Chemicals, Piscataway, NJ) according to the manufacturer's instructions and then used for immunoaffinity purification of $\alpha_3\beta_1$ heterodimer from placental cell extracts as previously described (49). The α_3 and β_1 subunits from purified $\alpha_3\beta_1$ complex (containing 30–50 μg of α_3 subunit) were then separated using preparative 5% SDS-PAGE and transferred to nitrocellulose. After localization of the α_3 band relative to ^{125}I-labeled α_3 subunit by autoradiography, that band was cut out and solubilized in the minimum amount of DMSO. After multiple (5–10 μg) injections of purified α_3 material in Freund's adjuvant, rabbit anti-α_3 antiserum was obtained.

To obtain antibodies enriched for anti-α_3 binding activity, purified $\alpha_3\beta_1$ complex (isolated using the J143 mAb column as described above) was used to prepare an immunoaffinity column. First, purified $\alpha_3\beta_1$ was denatured by boiling for 5 min in the presence of 1% SDS, and coupled to CNBr-Sepharose according to the manufacturer's instructions (except that coupling buffer included 0.1% SDS). Then crude rabbit anti-α_3 serum was passed over the $\alpha_3\beta_1$-Sepharose affinity column, and the column was washed successively with 3 column vol each of PBST[1] (0.14 M NaCl, 10 mM sodium phosphate, 0.2% Tween 20), 0.2 M KSCN, and then PBST again. For elution of anti-α_3 antibodies, 3.5 M KSCN was added, and then the eluate (with 0.1% hemoglobin carrier protein) was desalted using a Sephadex G-25 column equilibrated with PBST.

Several putative α_3 cDNA clones (isolated as described below) were used to produce β-galactosidase fusion proteins in lysogenic Y1089 *Escherichia coli* as described (26). The insoluble fusion proteins from these clones were purified by repeated washing of the insoluble pellet with 1% NP-40, 0.14 M NaCl and 10 mM sodium phosphate, pH 7.4. The NP-40-insoluble fusion protein was further separated by SDS-PAGE and the stained band was cut out from the gel, electroeluted, coupled to Sepharose and then used to positively select anti-fusion protein antibodies from crude anti α_3 serum.

Isolation of α_3 cDNA

An oligo dT primed λgt11 expression cDNA library, made from endothelial cells was the kind gift of Dr. Tucker Collins (Brigham and Women's Hospital, Boston, MA). That library was screened using affinity-purified α_3 antibodies according to the method of Young and Davis (55). Positive clones from phage screening were plaque purified and the phage DNA was prepared by the plate lysate method (33). The insert cDNA was cut out from the phage DNA by EcoRI digestion and subcloned into either pGEM-3 (Promega Biotec, Madison, WI) or Bluescript KS+ plasmid (Stratagene Corp., La Jolla, CA) as previously described (46, 50).

The initially selected clone 3.24 included only the 3′ noncoding region and ~100 COOH-terminal amino acid residues. Using ^{32}P-labeled 3.24 cDNA as a probe, additional clones (3.122, 3.285) were selected from a λgt11 cDNA library made from the hepatocarcinoma cell line Hep G2; a kind gift from Drs. M. Muckler (Massachusetts Institute of Technology, Boston) and M. Krangel (Dana Farber Cancer Institute, Boston). Using ^{32}P-labeled 3.122 cDNA as probe, further screening was carried out to finally select clones (3.410 and 3.520) from a different human endothelial cell λgt11 cDNA library (from Dr. J. E. Sadler, Washington University, St. Louis, MO). The insert cDNA was cut out from the phage DNA by SalI digestion in this case. Clone 3.410 included the translation initiation site and 5′ noncoding region of the α_3 mRNA (see Fig. 3, below).

Immunoprecipitation of α_3 Subunit

A polyclonal anti-peptide serum was prepared in the laboratory of Dr. J. McDonald (Washington University, St. Louis) using the synthetic peptide CRIQPSETERLTDDY. This peptide contains the COOH-terminal 14 amino acids predicted for a putative chicken CSAT antigen band 2 sequence (28), and the COOH-terminal 12 amino acids are identical to those predicted for human α_3 (see Fig. 4, below). The rabbit anti-α_3 COOH-terminal peptide serum and the anti-α_3 mAb J143 (16) were used to immunoprecipitate α_3 protein from detergent lysate of LOX cells (a melanoma line), and these were analyzed by SDS-PAGE as previously described (24).

DNA Sequencing

The DNA sequences were determined by the dideoxy nucleotide chain-termination method of Sanger et al. (41), using adenosine [^{35}S]5′-[α-thio]-triphosphate. To facilitate complete sequencing of both cDNA strands, a series of overlapping deletion clones was made in both directions as described (25) by using the Erase-a-base system (Promega Biotec). Alternatively, synthetic oligonucleotides (~20 bases) corresponding to known α_3 sequence regions were used as sequencing primers. Clones 3.24 and 3.285, and 5′-1 kb of clone 3.410 were sequenced in both directions.

1. *Abbreviations used in this paper*: PBST, PBS plus 0.2% Tween-20.

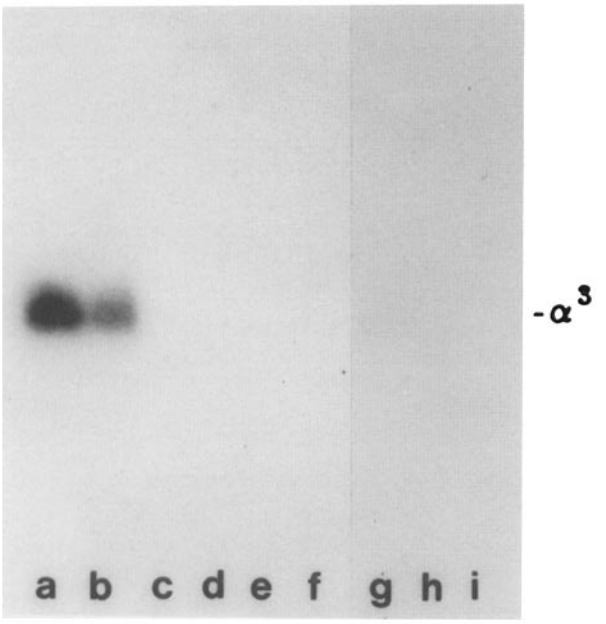


Figure 2. Immunoprecipitation of the α_3 subunit. Immunoprecipitation was carried out using extract from the surface ^{125}I-radiolabeled melanoma cell line LOX. After immunodepletion of labeled material reacting with negative control antibody (lanes *a–c*), the anti-α_3 MAb J143 (lanes *d–f*), or rabbit anti-α_3 peptide serum (lanes *g–i*), the remaining LOX extract was then subjected to a second round of immunoprecipitation using J143 (lanes *a, d, g*), rabbit anti-α_3 peptide (lanes *b, e, h*), or a negative control antibody (lanes *c, f, i*).

Construction of the α_3 cDNA Expression Vector and Transfection of CHO Cells

The BamHI (in the vector polycloning site)/NdeI fragment (0.9 kb) of clone 3.410 and the NdeI/EcoRI fragment (1.66 kb) of clone 3.285 were ligated to the BamHI/EcoRI fragment (3 kb) of Bluescript KSII+ vector. The resulting pBSKSII+ (α_3; 1-2465) was digested with HindIII and SalI and the large SalI (in the vector polycloning site)/HindIII fragment (5.46 kb) was ligated with HindIII/SalI fragment (1.08 kb) of cDNA clone 3.520 (SalI site derives from a linker used for ligation of cDNA to phage vector). The XhoI site in the resulting pBSKSII+ (α_3; 1-3470) was converted to XbaI site by digesting with XhoI, filling in reaction with Klenow enzyme, and insertion of XbaI linker. The resulting pBSKSII+(α_3;X/X) was digested with XbaI and the XbaI fragment (3.47 kb) containing α_3 coding region was ligated to the XbaI-digested, calf intestinal alkaline phosphatase-treated CDM8 vector. 10 μg of the purified plasmid was transfected to 10^7 CHO cells by electrophoration (8). 72 h after transfection, cells were harvested with 3.5 mM EDTA in 0.14 M NaCl and 10 mM sodium phosphate (pH 7.4), incubated with primary mouse monoclonal antibody and then with FITC-labeled goat anti-mouse IgG antibody, and subjected to a fluorescence-activated cell sorter (FACS IV,[2] Becton Dickinson Co., Oxnard, CA) analysis.

Results

Immunoscreening of α_3 cDNA Clones

An antiserum preparation made against purified placental α_3 protein was enriched for α_3 specific antibodies using an $\alpha_3\beta_1$ Sepharose affinity column. The purified anti-α_3 antibody preparation strongly recognized a band (M_r 150,000) from crude cellular extracts and purified $\alpha_3\beta_1$ preparation with very little background (not shown). We then used the purified antibody for immunoselection of α_3 cDNA clones from a λgt11 cDNA library from endothelial cells which are known to express substantial amount of α_3 protein (2). From a few representative isolates, cDNA clones 3.22 and 3.24 were found to cross-hybridize to each other by Southern blotting. Then β-galactosidase fusion proteins (Fig. 1, *a–e*) were prepared from several clones, and of these, the fusion proteins from clones 3.22 and 3.24 were recognized by antiserum enriched for anti-α_3 antibodies (Fig. 1 *B*, lanes *a*, *b*). Two other fusion proteins (lanes *c*, *d*) and *E. coli* strain Y1089 control protein (lane *e*) were not recognized by anti-α_3 antibodies. In a reciprocal experiment, clone 3.22 fusion protein was coupled to Sepharose and then used to enrich for anti-fusion protein antibodies. This antibody preparation (derived from the original crude antiserum) was immunologically reactive with both the fusion protein itself and with purified α_3 (Fig. 1 *C*). The cDNA clone 3.24 was subcloned into a plasmid and sequenced, and found to have an amino acid sequence (about 100 residues) similar to the COOH-terminal regions of other known integrin α subunits.

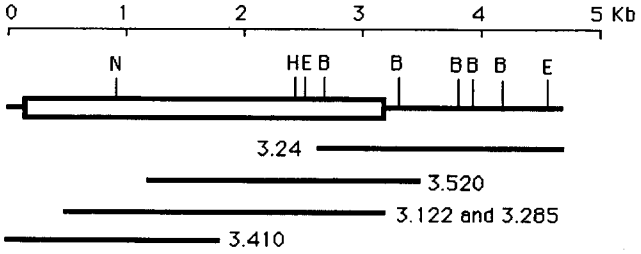


Figure 3. Overlapping α_3 cDNA fragments. cDNA clone 3.24 was obtained from an endothelial cell λgt11 cDNA library, clones 3.122 and 3.285 from that of HepG2 hepatocarcinoma cell line, and 3.410 and 3.520 from another endothelial cell cDNA library. Sites of restriction enzymes used for reconstruction of α_3 cDNA coding region are shown. *B*, BamHI; *E*, EcoRI; *H*, HindIII; *N*, NdeI.

Immunological Evidence for the Identity of the α_3 Clone

To further assess the identity of the putative α_3 cDNA, rabbit antiserum against a synthetic peptide containing the COOH-terminal 12 amino acids from the predicted α_3 amino acid sequence was used to immunoprecipitate a 150,000-M_r protein from radiolabeled LOX cells (Fig. 2, lane *b*) which comigrates with authentic α_3 immunoprecipitated using the mAb J143 (lane *a*). In addition, prior immunodepletion of the LOX cell extract with either the mAb J143 (lanes *d–f*), or with anti-peptide rabbit serum (lanes *g–i*), almost completely eliminated subsequent immunoprecipitation by either J143 (lanes *d*, *g*) or by the anti-peptide serum (lanes *e*, *h*).

Isolation of Clones Corresponding to Full-Length α_3 cDNA

When a human Hep G2 λgt11 cDNA library was screened using the cDNA clone 3.24 as a probe, additional clones (3.285 and 3.122) were selected which extended 1.8 kb beyond the 5′ end of clone 3.24. Finally, a clone (3.410) was isolated from another endothelial λgt11 cDNA library that

2. FACS is a registered trademark of Becton Dickinson and Company.

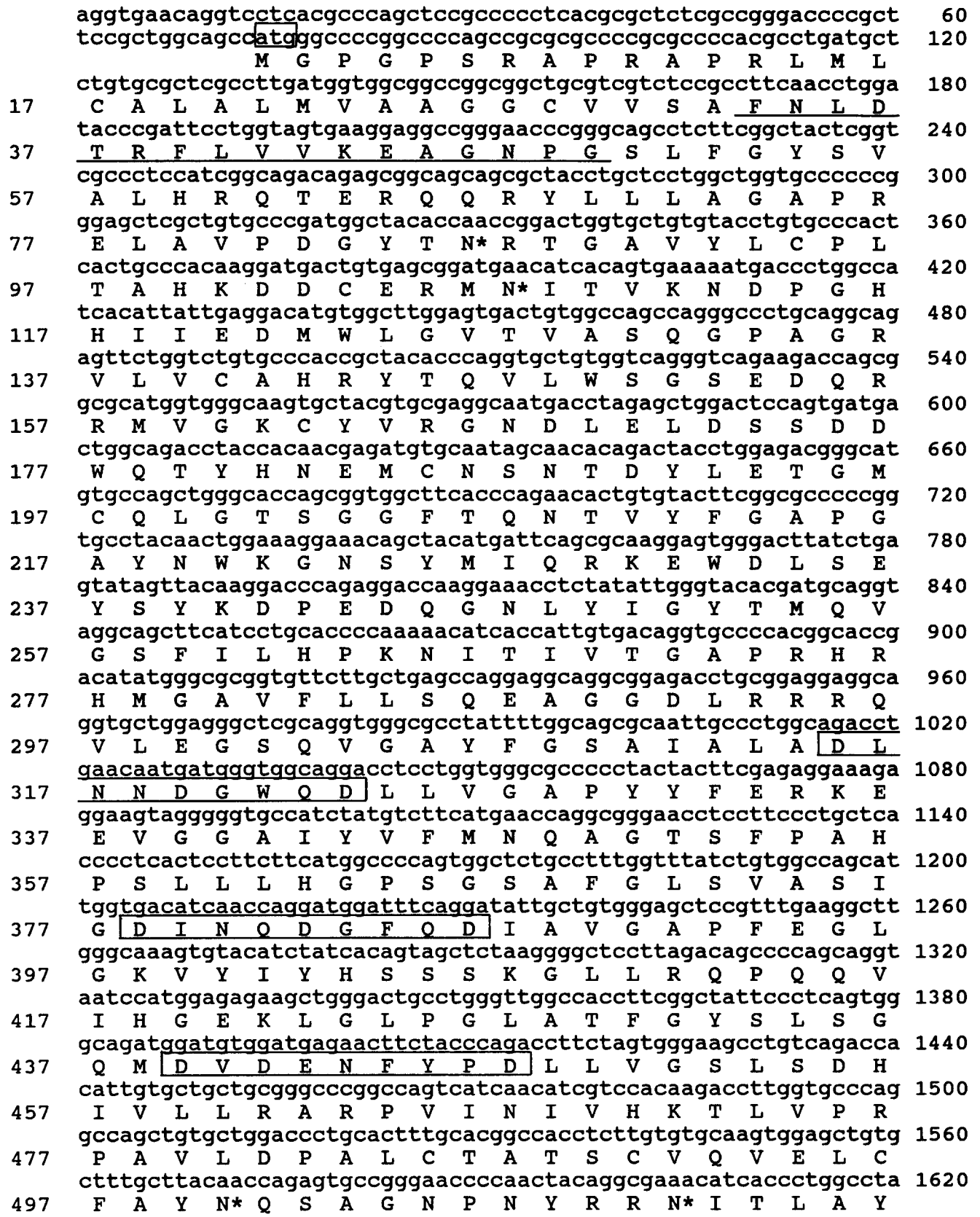
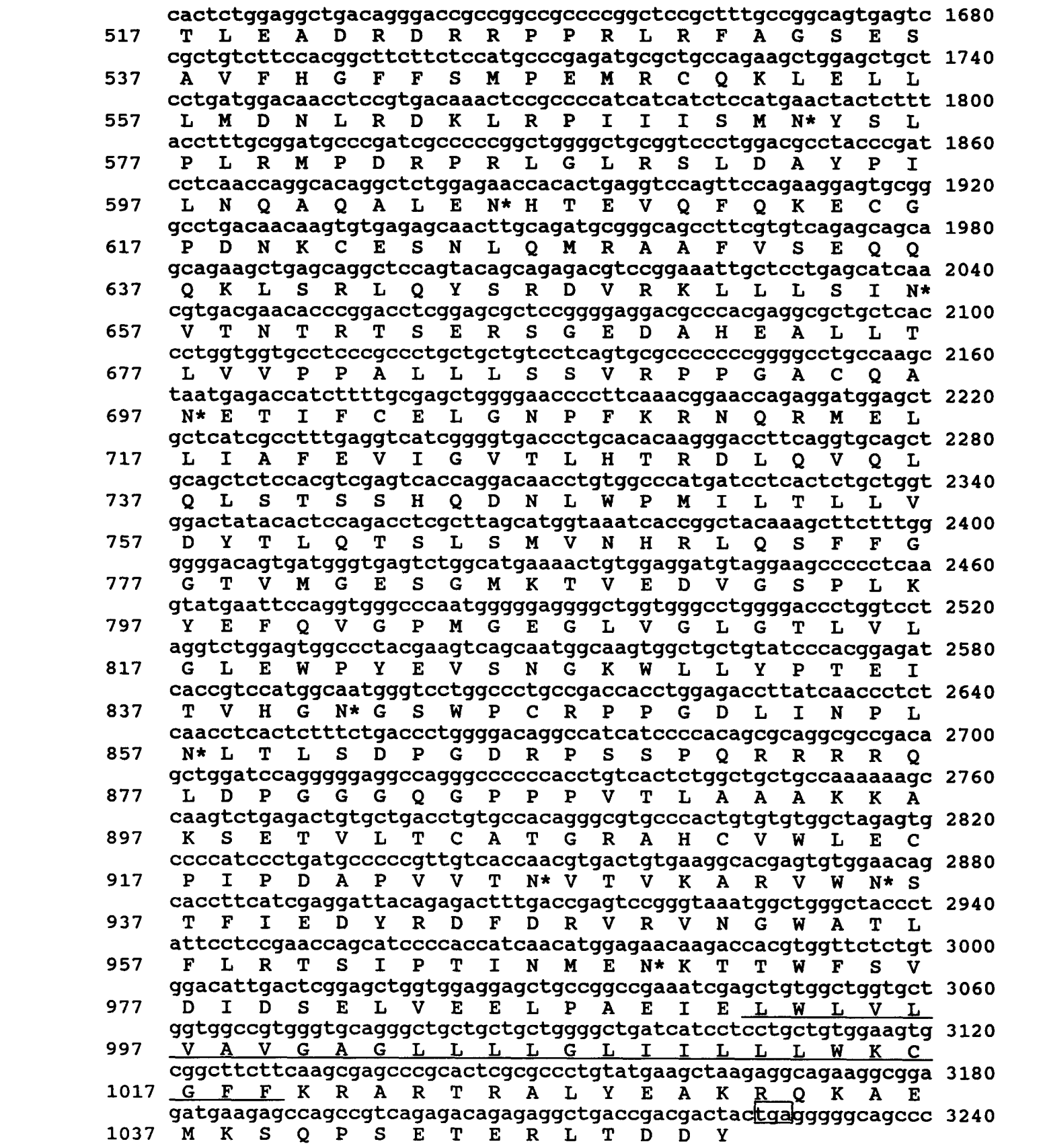


Figure 4. Complete nucleotide sequence of α_3 subunit cDNA and deduced amino acid sequence. The initiation codon, stop codon, and potential metal binding domains are boxed. The NH_2-terminal amino acid sequence, putative transmembrane domain, and polyadenylation signal (*AATAAA*) are underlined. *N**, a potential *N*-glycosylation site. These sequence data are available from EMBL/GenBank/DDBJ under accession number M59911.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537 A V F H G F F S M P E M R C Q K L E L L
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557 L M D N L R D K L R P I I I S M N* Y S L
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577 P L R M P D R P R L G L R S L D A Y P I
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597 L N Q A Q A L E N* H T E V Q F Q K E C G
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657 V T N T R T S E R S G E D A H E A L L T
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917 P I P D A P V V T N* V T V K A R V W N* S
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997 V A V G A G L L L L G L I I L L L W K C
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1017 G F F K R A R T R A L Y E A K R Q K A E
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1037 M K S Q P S E T E R L T D D Y

Figure 4.

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aacagctcccagtttgtaaaaaaaaaaaaaa 4652
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Figure 4.

encoded an NH_2-terminal sequence (FNLDTRFLVVKEAG) which exactly corresponds to the NH_2-terminal sequence of the mature α_3 subunit (48). The clone 3.410 also contained a putative ATG start site and a region of 5′ noncoding sequence.

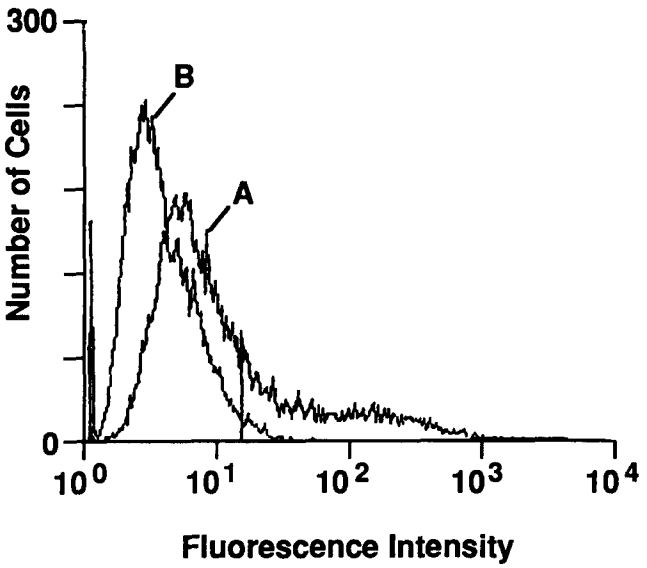

Figure 5. Transient expression of human α_3 subunit in CHO cells. Human α_3 subunit cDNA expression plasmid was constructed as described in Materials and Methods. 10 μg of the plasmid was transfected into 10^7 CHO cells by electroporation. After 72 h in medium with 10% FCS, cells were harvested, stained with mAb J143 followed by FITC-labeled goat anti-mouse IgG antibody and subjected to FACS analysis. Also a FACS profile of a typical negative control is shown (e.g., cells transfected with CDM-8 vector only and stained with mAb J143).

Nucleotide and Amino Acid Sequence for Human α_3 cDNA

From the various overlapping cDNA clones (Fig. 3), the complete sequence for the α_3 coding region, and some of the 3′ and 5′ untranslated regions was obtained (Fig. 4). The 3′ untranslated region contained the poly A addition signal AATAAA. Translation of the α_3 cDNA yielded 1,051 amino acids including a signal sequence (32 residues). The predicted mass of mature α_3 subunit peptide is calculated as 113,505. The α_3 subunit sequence has 13 potential N-glycosylation sites (Asn-X-Ser/Thr, where X is not Pro). If carbohydrate chains with an average molecular weight of 2,500 are assumed to attach to all 13 putative glycosylation sites, the total molecular weight of the mature α_3 subunit would be 146,000. This value is consistent with the estimated size from the relative mobility on SDS-PAGE (M_r 145,000–150,000). The α_3 subunit has been reported to be cleaved to heavy and light chains upon reduction (M_r 110,000 and 30,000, respectively). Consistent with this, there are two potential cleavage sites (QRRRR) and (AKKAK), characterized by dibasic residues, which are located in the same region (residues 839–864) as cleavage sites predicted for the integrin α_5, α_v, α_{IIb} subunits. As noted for other published integrin α subunits (3–5, 9, 10, 15, 29, 32, 38, 39, 45–47, 51), the NH_2-terminal portion of the α_3 sequence contains seven similar repeating units (I, residue 18–60; II, 99–131; III, 165–193; IV, 295–329; V, 352–390; VI, 415–447; VII, 475–512), which are each 28–42 amino acids in length, and are 20–30% similar. Repeating domains V, VI, and VII each contain a putative divalent cation binding region, of the general structure of DX(D/N)X(D/N)GXXD. This sequence is found among a variety of Ca^{2+} and Mg^{2+} binding proteins including integrins

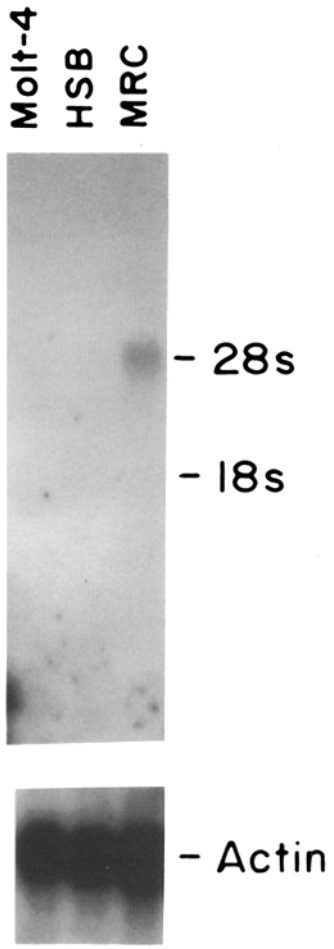

Figure 6. RNA hybridization analysis. From the leukemic cell lines MOLT-4 and HSB and from the fibroblast cell line MRC, total RNA (5 μg per lane) was electrophoretically separated on 1% agarose-formaldehyde gels, transferred to nylon membrane (Nytran; Schleicher & Schuell, Inc., Keene, NH), and then probed with ^{32}P-labeled α_3 cDNA clone 3.24. In a control experiment (below), the same RNA samples were probed with actin cDNA.

(3–5, 9, 10, 15, 29, 32, 38, 39, 45, 46, 50, 51, 53). Whereas several other integrin α subunits contain a 180-200 amino acid insert (called I domain) between repeating domains II and III (4, 9, 10, 29, 32, 39, 47), α_3 subunit has no such domain.

Transient Expression of Human α_3 Subunit in CHO Cells

The α_3 expression plasmid was constructed as described in Materials and Methods and was transfected into CHO cells by electroporation. After 72 h, the human α_3 subunit was expressed on the surface of 20% of the transfected cells as detected by α_3-specific mAb J143 (Fig. 5), further indicating that the α_3 cDNA is authentic. Because integrin α subunit expression at the cell surface is known to require an associated β subunit, and because human α_3 has previously been found to associate with rodent β_1 (30), it is presumed that transfected α_3 in this experiment is associated with hamster β_1 at the cell surface.

Northern Blotting Analysis

Upon probing of total RNA with α_3 clone 3.24, a single band (5 kb) was obtained from the fibroblast cell line MRC (Fig. 6, lane *c*). In contrast, only a faint band was seen from the leukemic T cell lines Molt-4 and HSB. These results are consistent with the relative levels of surface expression of

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Human_a3                    GGgQGpPPVTLAAAKKAKSET 900
HamGap_b3                   GGdQGsPPVTLAAAKKAKSET
Chicken_CSAT_band_2         GaepGePPiTLAtgKKAKSEv

VLTCAtGRAhCVWLECPIPDApvVTNVTVKARVWNSTFIEDYRDFDRVRV 950
VLTCAsGRArCVWLECPIPDtsnVTNVTVKARVWNSTFIEDYRDFDRVRV
lLsCsqGtArCiWfECPIPaAqhpatfrVrARVWNSTFIEeYRsFDRVkV

nGWATLFLRTSIPTINMENKTTWFSVDIDSELVEELPAEIELWLVLVAVg 1000
dGWATLFLRTSIPTINMENKTTWFSVDIDSELVEELPAEIELWLVLVAVs
dGtATLFLRThIPTINMrNhT..FSVDvDSELtEEqPpqvaLWLVLVAaa

AGLLLLGLIIlLLWKCGFFKRARTRALYEAKRQKAEMKSQPSETERLTDDY 1051
AGLLLLGLIIiLLWKCGFFKRARTRALYEAKRQKAEMKSQPSETERLTDDY
AGLLLLGLIIvLLWKCGFFrRAsTgAmYEAKgQKAEMriQPSETERLTDDY
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Figure 7. Alignment of human α_3, hamster Gapb3 and chicken CSAT antigen band 2 sequences. CSAT band 2 (43) partial amino acid sequence (170 residues) was aligned with the corresponding part of the human α_3 and hamster Gap b3 (52) sequences by using the GAP program of UWGCG (12).

α_3 protein on these cells (23). Hybridization of the same blot with cDNA probe for the human actin gene gave comparable signals in all lanes.

Discussion

This paper describes the cloning, sequencing, and expression in eukaryotic cells of cDNA for the human integrin α_3 subunit of the VLA-3 ($\alpha_3\beta_1$) heterodimer. The α_3 cDNA is authentic because (*a*) the NH_2-terminal amino acid sequence deduced from the nucleotide sequence is identical to that from purified α_3 peptides (48), (*b*) α_3 fusion proteins were immunologically cross-reactive with purified α_3 protein, (*c*) rabbit antiserum against a predicted α_3 COOH-terminal synthetic peptide recognized the α_3 subunit, and (*d*) the anti α_3 mAb J143 recognized CHO cells transfected with α_3 cDNA in CDM8 vector.

This paper shows that hamster galactoprotein b3 (Gapb3) is 89% homologous to the human α_3 subunit with uniform similarity throughout the two molecules. There is high conservation (100%) of the 40 COOH-terminal residues (52). Also a partial amino acid sequence of chicken CSAT band 2 protein (28) is 70% similar to the corresponding region (COOH-terminal 170 residues) of the human α_3 sequence (Fig. 7). Thus, it is proposed that Gapb3 and CSAT antigen band 2 are homologues of the human α_3 subunit. In this regard, CSAT protein and $\alpha_3\beta_1$ were previously shown to be immunologically cross-reactive (48), and CSAT band 2 cDNA cross-hybridized with α_3 cDNA in Southern blots (not shown).

Whereas Tsuji et al. (1990) (52) showed the homology of Gapb3 to human α_3 subunit from only a very limited NH_2-terminal amino acid sequence comparison (14 residues), this work provides compelling evidence of the homology of human and hamster protein.

Alignment of α_3 with α_1, α_2, α_4, α_5, α_6, α_v shows that the essential features of integrin α subunits are conserved in the α_3 subunit (Fig. 8). From the alignment of these α subunits, along with the other integrin α subunits α_{IIb}, α_L, α_M, α_X, PS2α, an α subunit similarity tree was constructed (Fig. 9). Overall, the α_3 amino acid sequence was 25–37% similar to the other integrin α subunits that are cleaved into disulfide-linked fragments, and less similar to those α subunits that have I domains (15–20%, excluding the I domain sequence itself). The similarity of the α_3 subunit

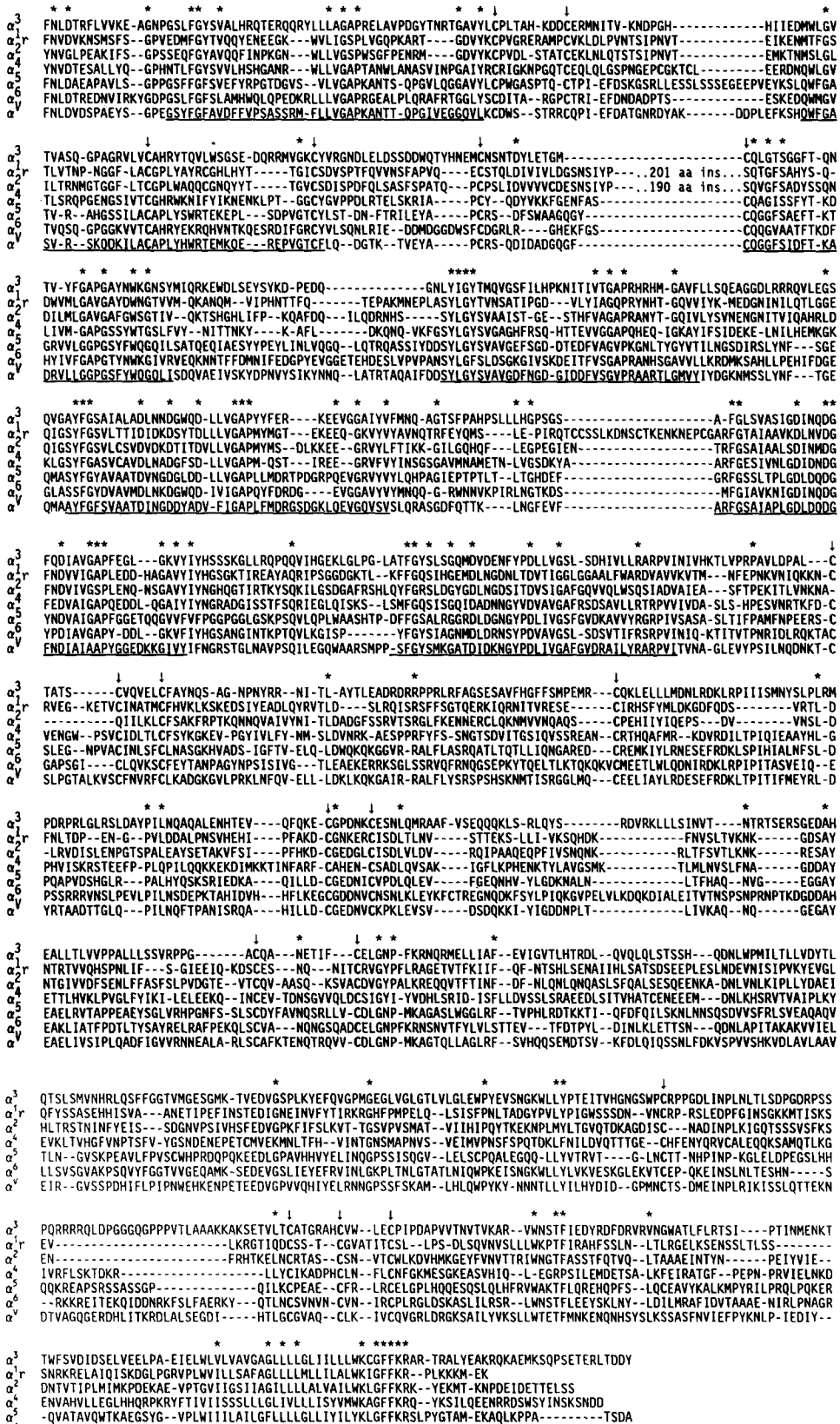

Figure 8. Alignment of α_3 protein sequence with other integrin α subunits that associate with the β_1 subunit. Amino acid sequences for human α_3, α_2, (46), α_4 (50), α_5 (3, 15), α_6 (51), α_v (45), and rat α_1 (29) are aligned, and residues conserved in at least six of the sequences are marked with "*," and conserved cysteines are marked with "↓." Regions corresponding to the seven repeating domains are underlined, and regions corresponding to the I-domains in α_1 and α_2 (201 and 190 amino acids, respectively) have been omitted. Initial alignments were carried out using the computer program of Smith and Smith (42), and then minor adjustments were made by eye to improve the alignment, with emphasis on maintaining conserved cysteines.

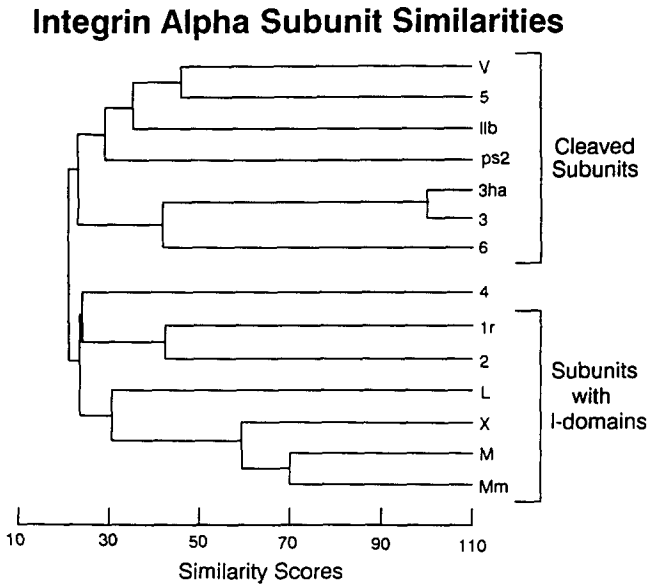

Figure 9. Integrin α subunit similarity tree. Amino acid sequences for the human subunits α_v (45), α_5 (3, 15), α_{IIb} (38), α_3, α_6 (51), α_4 (46), α_2 (47), α_L (32), α_M (4, 9), α_X (10), the *Drosophila* PS2α (5), hamster Gapb3 (52), rat α_1r (29), and mouse α_Mm (39) were aligned using the program of Smith and Smith (42), and similarity scores were generated, and then averaged similarity scores were used to construct the similarity tree.

(37%) to the α_6 subunit is much higher than the average similarity (~25%) between α_3 and other integrin α subunits, suggesting that α_3 is evolutionarily closer to α_6 than the others. This pair of α subunits also has an unusually high degree of similarity within their short cytoplasmic domains (13/30 = 43% identity). No other pair of α subunits is that similar in their cytoplasmic domains. For example, even though α_M and α_X are 60% similar overall, and α_V and α_5 are 43% similar overall, these pairs are only 25 and 24% similar, respectively, in their cytoplasmic domains. Recently, both $\alpha_3\beta_1$ and $\alpha_6\beta_1$ have been shown to recognize the E8 fragment of laminin, which is located COOH-terminal of the laminin cross (17, 20, 44). It is possible, based on the evolutionary similarity of the two subunits, that they recognize the same site of laminin by a similar mechanism.

The present human α_3 subunit cDNA clone will be useful in future studies (*a*) to directly evaluate the role of $\alpha_3\beta_1$ in migration, invasion and metastasis and (*b*) to study the mechanism of recognition of multiple ligands by $\alpha_3\beta_1$. For example, it will be particularly interesting to examine the behavior in vitro and in vivo of the transfected CHO cells or other cells overproducing human $\alpha_3\beta_1$.

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