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A novel β1 integrin isoform produced by alternative splicing: unique expression in cardiac and skeletal muscle

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Abstract The mRNA's of several integrin subunits are alternatively spliced in the region encoding cytoplasmic domains, that may potentially provide alternative integrin–cytoskeleton interactions and transmembrane signaling pathways. We identified a novel cytoplasmic tail variant of the human β1 subunit by reverse transcriptase polymerase chain reaction. This fourth β1 variant, named β1D, is specific for skeletal and cardiac muscle. The determined genomic organization of the 3′-region of the human β1 gene reveals that β1D is produced by alternative splicing of mRNA. In addition, we show that the expression of β1D is developmentally regulated during murine myoblast differentiation, suggesting a role for β1D in myogenesis.

Key words: Integrin variant; Splicing; Myogenesis; Differentiation

1. Introduction

Integrins constitute a family of heterodimeric transmembrane glycoproteins, consisting of noncovalently associated α and β chains. The extracellular domains of both the α and β subunits are involved in cell–extracellular matrix or cell–cell interactions and their cytoplasmic domains link the receptors to cytoskeletal proteins. Thus, integrins provide a link between the extracellular matrix and the cytoskeleton. In addition to adhesion, integrins have been shown to mediate signal transduction [1].

Sixteen α and eight β subunits have thus far been identified and more than 20 combinations of these subunits have been reported. The diversity of the integrin family is further increased by alternative splicing of mRNAs, involving both the extracellular and cytoplasmic domains of integrin subunits. Splice variants of the cytoplasmic domain of both α and β subunits have been reported: i.e. α3, α6, α7, β1, β3, and β4 [1,2]. In both the β1 and β3 subunits, alternative mRNA splicing leads to the production of different cytoplasmic domains after the conserved KWDT sequence, while in the α3, α6 and α7 subunits the alternative cytoplasmic domains deviate after the conserved cytoplasmic GFFKR amino acid sequence [3]. This alternative mRNA splicing is often tissue-specific. For example, the β1B variant, which is produced by retention of intron sequences downstream of exon 6, carrying a new stop codon [4], is expressed only in skin and liver tissue, whereas the β1C variant, which results from the insertion of an exon in the encoding mRNA, is expressed in haematopoietic cells [5]. Furthermore, alternative mRNA splicing is developmentally regulated as in the case of α6. The α6B variant is already expressed in the preimplantated mouse embryo, whereas α6A is expressed later on during embryogenesis [6,7].

Alternative splicing of the mRNA for the α7 integrin is interesting with regard to myogenesis. Developmentally regulated splicing of the mRNA for the α7 subunit occurs during the fusion of mononucleated myoblasts into multinucleated myotubes [8–10], a characteristic process in skeletal muscle differentiation. During this fusion process, the terminally differentiated muscle cells are withdrawn from the cell cycle and a set of muscle-specific genes is expressed. Subsequently, the myotubes mature to muscle fibers. In terminally differentiated skeletal muscle the major α7A and α7B and C integrin variants are expressed, whereas only the α7B variant is present in myoblasts and heart [8].

In this study we have identified a new muscle-specific cytoplasmic splice variant of the β1 subunit, β1D. Human β1D cDNA was cloned from various muscle tissues, and the genomic organization of the 3′-region of the β1 gene was determined. Furthermore, we demonstrate that the expression of the β1D variant is developmentally regulated during murine C2C12 myoblast differentiation.

2. Materials and methods

2.1. Cells

The murine myoblast cell line C2C12 (ATCC CRL 1772) was propagated in Dulbecco's modified Eagle's medium (DMEM) supplemented with 20% fetal calf serum, high glucose (4.5 g/l), penicillin and streptomycin. Terminal differentiation of confluent C2C12 monolayers to multinuclear myotubes was induced by changing the culture medium to DMEM containing 2% horse serum.

2.2. cDNA synthesis and PCR

First strand cDNA was prepared from 5 μg total RNA of various human tissues using an oligo d(T)15 primer and the riboclone cDNA synthesis system (Promega, Madison, WI). To amplify the transmembrane and cytoplasmic encoding regions of the human β_1 integrin subunit a sense primer (5′-GAACAACGAGGTCATGG-3′; positions 2230–2246) and an antisense primer (5′-GCCCTAAAGCTACTACCTAACTGTGAC-3′; positions 2553–2576 [11]) were used. All polymerase chain reactions (PCR) were performed in a final volume of 25 μl containing: 20 mM Tris (pH 8.4), 50 mM NaCl, 1.5 mM $MgCl_2$, 1 mM of each dNTP, 80 ng of each primer and 1.0 U Taq polymerase (Life Technology). The PCR conditions were: 35 cycles of 1 min at 94 °C, 1 min 30 s at 55°C and 30 s at 72°C. All PCR reactions described in this study had an initial denaturing step of 5 min at 94 °C and a terminal extension step of 5 min at 72 °C. The PCR products were digested with *Hin*dIII and *Sca*I. Subsequently, the fragments were isolated from a 1.5% agarose gel and cloned into *Hin*dIII/*Sma*I digested pUC18. Sequencing of the subclones was done by using the dideoxy chain termination method (Sequenase version 2.0 kit, USB, Cleveland, OH).

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Total RNA from C2C12 cells was isolated at various time points after myotube induction, using the Ultraspec RNA kit (Biotecx Laboratories Inc, Houston, USA) as recommended by the manufacturer. Subsequently, cDNA was prepared as described above using Superscript II reverse transcriptase (Life Technology). Primers designed for identification of murine β1A and β1D were a sense primer (5'cggaattcGGCAACAATGAAGCTATCGT-3'; positions 2219–2238) and an antisense primer (5'-TGTCAGTCCCTGGCATG-3'; positions 2661–2677 [12]). Primers for the detection of murine α3 were: a sense primer (5'-CAAGTGGCTGCTGTATCCCACG-3'; positions 2554–2574 [13]) and an antisense primer (5'-gaatctagaTGCTCCCTGGAGGT-3' positions 3824–3836 [14]). The α3 products were further amplified by nested PCR with a sense primer (5'-CTCGGAGCTGGTGGAGGAGCT-3'; positions 3487–3507) and an antisense primer (5'-TACTTGGGCATAATCCGGTAGTAG-3'; positions 3762–3786 [14]). Murine α5 was amplified by using a sense primer (5'-CTGCAGCTCCATTTCCGAGTCTGG-3'; positions 880–903) and an antisense primer (5'-GAAGCCGAGCTTGTAGAGGACGTA-3'; positions 1132–1155 [15]); murine α6: a sense primer (5'-GTCAGGTGTGTGAACATCAGG-3'; positions 2975–2995) and an antisense primer (5'-CTGGAAAAAATAAGGGGGGGC-3'; positions 3627–3647 [7]) and murine α7: a sense primer (5'-GTTGTGGAAGGAGTCCC-3'; positions 184–200) and an antisense primer (5'-GTCTTCCCGAGGGATCTT-3'; positions 447–464 [9]). The additional nucleotides containing restriction-sites for cloning of the PCR products are indicated in lower-case letters.

The PCR conditions for the amplification of murine β1A and β1D, α5 and the first PCR's for murine α3 consisted of: 35 cycles of 1 min at 94 °C, 1 min at 50°C, and 1 min at 72 °C. The nested PCR for α3 was 35 cycles of 1 min at 94 °C, 1 min at 55 °C and 1 min at 72 °C. For murine α6, 35 cycles of 45 s at 94 °C, 45 s at 50°C, 1 min at 72°C were used. Murine α7 cDNA was amplified by 35 cycles of 45 s at 94 °C, 45 sec at 50°C and 30 s at 72°C. PCR products were analyzed on 2% agarose gels.

2.3. Genomic organization of the 3' terminus of the β1 gene

Human genomic DNA was isolated by a standard procedure [15] and subjected to PCR. The following primer sets were used to define the exon/intron boundaries of the 3' part of the β1 gene: a sense primer in the untranslated 3' end of β1B (5'-cggaattcTGGGGTAACCAAATGTTGGC-3'; positions 1040–1059 [4]) and an antisense primer in exon D, (5'-cggaattcTCTTGAAATTATTAATAGGAC-3'); a sense primer in exon D, (5'-cggaattcGTCCTATTAATAATTTCAAGA-3') and an antisense primer in exon C (5'-cggaattcCTGGAAGTCAGAGGTTGC-3'; positions 66–83 [5]). The additional nucleotides containing an *Eco*RI restriction-site are indicated in lower-case letters. An antisense primer in exon 7 (positions 2553–2576 [11]) and sense primer in exon C (positions 66–83 [5]) were included in the initial screening for the genomic organization (see also Fig. 3A).

PCR conditions were 35 cycles of 1 min at 94 °C, 90 s at 49°C and 90 s at 72 °C. After *Eco*RI digestion of the 5' *Eco*RI flags the PCR products were subcloned into pUC18 and sequenced as described above.

3. Results

3.1. Identification of a β1 isoform specific for skeletal and cardiac muscle containing an alternative cytoplasmic domain

Amplification by the reverse transcription polymerase chain reaction (RT-PCR) of mRNA extracted from various human tissues, with primers flanking the transmembrane and cytoplasmic encoding sequences of the β1 cDNA, revealed a band of 347 bp in all tissues tested (Fig. 1). The size of this band corresponds to that predicted for the β1A cDNA sequence and sequence analysis confirmed that this PCR product encoded β1A [11]. An additional band of 428 bp was detected in skeletal and cardiac muscles. The PCR products from these tissues were cloned and sequenced. The sequence data obtained from three independent clones of both cardiac and skeletal muscles revealed the existence of a new, fourth β1 cytoplasmic domain variant (Fig. 2), which we named β1D according to the established nomenclature. The β1D specific sequence (81 bp) contains a translation stop codon and encodes the 24 C-terminal amino acids of the integrin subunit. Like the β1A variant, β1D contains three highly conserved cyto-sequences, cyto-1,-2 and -3, that are required for the localization of several β-subunits in focal adhesions [16,17]. Comparison with the three previously described β1 splice variants, β1A, β1B and β1C, shows that the β1D is most similar to β1A (Fig. 2B). In addition to the sequences encoding β1A and β1D, another minor PCR product of 284 bp was detected in stomach tissue. It encodes human pepsinogen C (98.6% identity) as was determined by sequencing (Fig. 1).

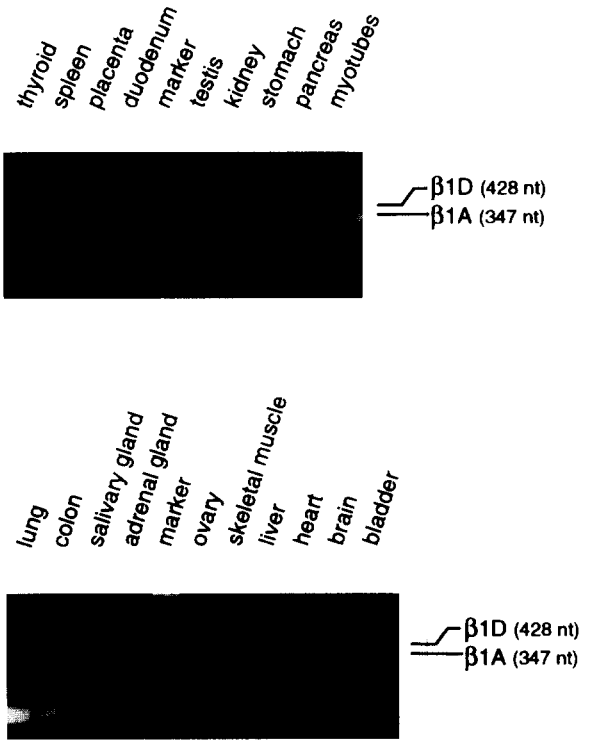

Fig. 1. Expression of β1A and β1D mRNA in various tissues. RT-PCR was performed on RNA extracted from tissues, using primers designed to amplify the β1 cytoplasmic domain. The 347 bp product, corresponding to β1A, is present in all samples tested. The 428 bp product represents the novel β1D variant and is exclusively expressed in skeletal muscle, heart muscle and myotubes.

3.2. Genomic organization of β1D

In order to localize the β1D specific sequence within the β1 gene we determined the exon/intron boundaries of the 3'-region of this gene. Using genomic DNA as a template, we amplified the introns adjacent to the β1D exon. Primers located in the 3'-untranslated end of β1B cDNA and in the β1D-specific cDNA sequence (Fig. 3A, primers 1 and 2, respectively) revealed a 323 bp product. Primers located in the β1D specific cDNA sequence and in the β1C specific cDNA sequence (Fig. 3A, primers 3 and 4, respectively), yielded a 624 bp product. The PCR products were subcloned and sequenced as described in section 2.

Sequence analysis revealed the localization of the β1D specific exon, exon D, between exon 6 and exon C (Fig. 3A). Figure 3B shows the characterized exon/intron boundaries in the cyto-

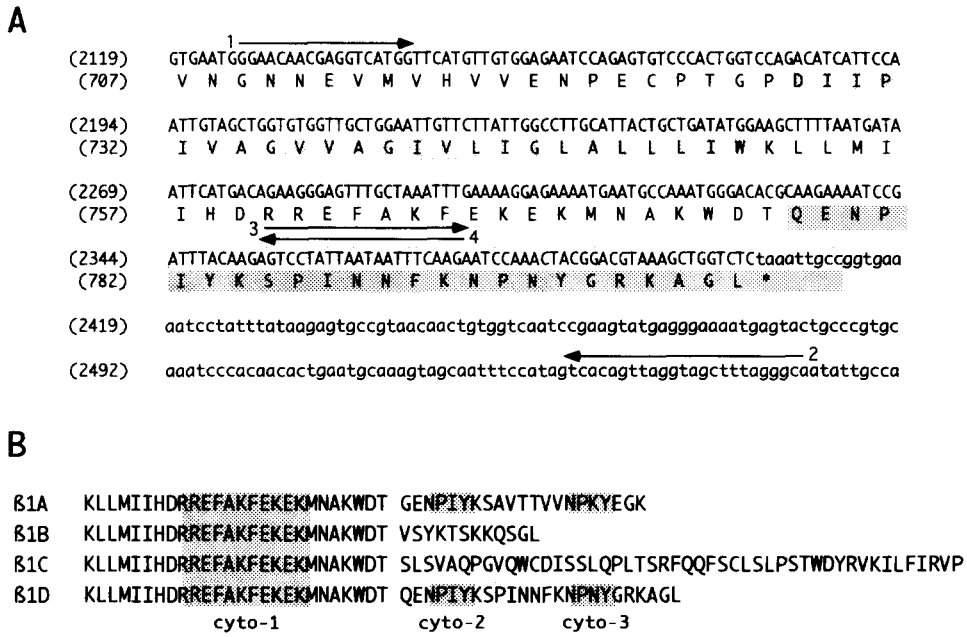

Fig. 2. (A) The partial cDNA sequence and predicted amino acid sequence of the human β1D cytoplasmic variant. The putative trans-membrane region is lightly shaded and the β1D specific insertion (81 bp) is boxed and darkly shaded. Arrows indicate positions of primers used for cloning (primers 1 and 2), and those used for the localization of exon D in the β1 gene (primers 3 and 4). (Numbering of the nucleotide and deduced amino acid sequence starts at the reported translation start codon of β1A [11]). (B) Alignment of alternative human β1 cytoplasmic domains. The cytoplasmic domain amino acid sequences of β1A [11], β1B [4], β1C [5] and β1D are shown. The sequences diverge from the KWDT sequence after the first 26 cytoplasmic amino acids. The conserved cyto-sequences are shaded [17]. The nucleotide sequence data are available from the EMBL Nucleotide Sequence Database under accession number U28252.

plasmic domain region of the β1 gene; which all match the consensus splice donor and acceptor sequences, GT and AG, respectively [18]. Furthermore, Fig. 3A also shows the position of a polyadenylation sequence (AATAAA), situated 90 bp upstream of exon D, which may function as a polyadenylation signal for the β1B variant.

3.3. Expression of β1D during muscle differentiation

Splice variants of α7 that are exclusively expressed in muscle have been described [8,10]. The appearance of the α7A splice variant in murine C2C12 myoblasts has been reported to coincide with differentiation to myotubes [9]. To analyze whether the expression of β1D is also correlated with muscle differenti-

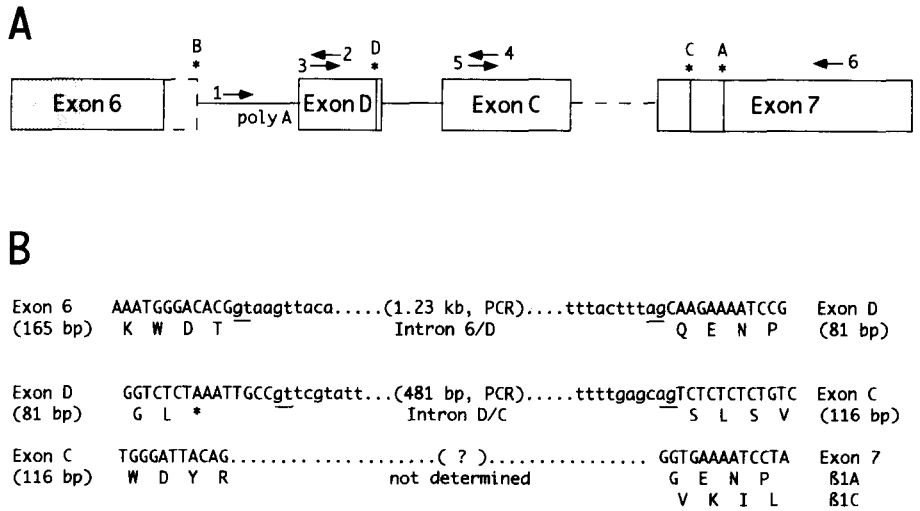

Fig. 3. Genomic organization of the 3'-region of the β1 gene. (A) Schematic drawing of the 3'-region of the β1 gene. Exons are boxed and numbered according to Lanza et al. [19]. In the β1A variant exon 6 is spliced to exon 7. β1B is not spliced, but arises from a skin and liver-specific readthrough. The β1B polyadenylation signal (AATAAA) that we located 90 bp upstream of exon D is indicated. The haematopoietic variant, β1C, contains a 116-bp insert (exon C), which induces a frame shift and a β1C-specific translation stop in exon 7. The skeletal and cardiac muscle-specific β1D variant has exon D inserted at the same splice junction between exon 6 and exon 7. Arrows indicate the positions of oligonucleotides used for PCR analysis of the genomic organization. The putative transmembrane region is shaded and the translational stop codons of β1B, D, C, and A, respectively, are indicated by asterisks. (B) The exon/intron boundaries in the 3' terminus of the β1 gene. Sizes of exons and introns are indicated between brackets. The consensus splice donor and acceptor sequences, GT/AG of each exon/intron border are underlined.

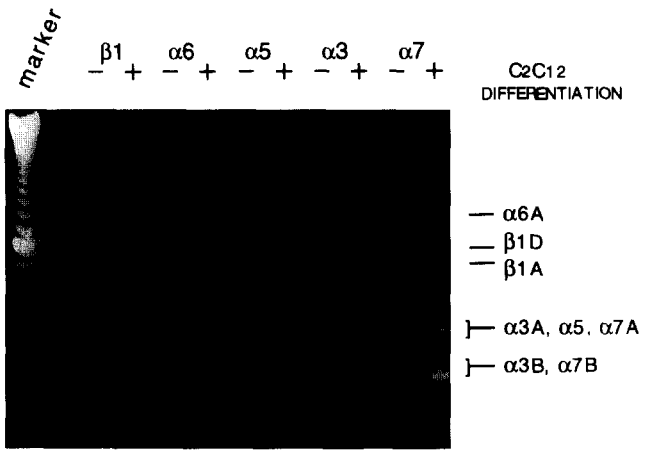

Fig. 4. RT-PCR amplification of β1A, β1D, α3, α5, α6, and α7 in undifferentiated and differentiated (3 days) C2C12 cultures. The expression of β1D (540 bp), α3B (156 bp) and α7A (281 bp) is differentiation stage-dependent, since β1D, α3B and α7A specific PCR products are detectable in differentiated C2C12 cultures only. The mRNA's of β1A (459 bp), α3A (300 bp), α5 (275 bp), α6A (673), and α7B (168 bp) subunits are expressed in both differentiated and undifferentiated C2C12 cells.

ation we used murine C2C12 myoblast cells that forms myotubes in 2% horse serum. We were able to use murine cells in culture as a model, since the β1D variant is also expressed in murine muscle (C. Baudoin, unpublished results). We also analyzed, by RT-PCR, the expression of β1A and of various α- subunits that putatively form heterodimers with β1D (i.e. α3, α5, α6, and α7) using cDNA prepared from both myoblasts and myotubes (3-day differentiated myoblasts). In undifferentiated C2C12 myoblasts, β1A as well as the α3A, α5, α6A and α7B cytoplasmic splice variants were detected (Fig. 4). Differentiated myoblasts additionally expressed the β1D, α3B and α7A variants, while the expression of the other α-subunits seemed to be unaltered. Analysis of these integrin subunits in C2C12 cells, grown under differentiation-inducing conditions, showed that during muscle differentiation the decrease in β1A expression coincides with an increase in β1D and α7A expression (not shown).

4. Discussion

In this report a new splice variant of the integrin β1 subunit (β1D) is described which has an alternative cytoplasmic domain and is specifically expressed in cardiac and skeletal muscle. This variant was detected by RT-PCR analysis in myotubes, skeletal and cardiac muscle tissue, but not in smooth muscle or other tissues. β1D appears to be the prominent β1 isoform in adult skeletal and cardiac muscle. Since it is the fourth β1 splice variant to date, the others being the ubiquitously expressed β1A and the much less widely expressed β1B and β1C variants, we named this novel variant β1D. Furthermore, expression of the β1D splice variant is induced during C2C12 myoblast differentiation into myotubes and this is accompanied by induction of α3B and the previously reported α7A variant [9].

Analysis of the genomic organization of the 3′ terminus of the β1 gene by RT-PCR revealed an exon, named exon D, that encodes the specifically inserted sequence (81 bp) in β1D cDNA. This exon is located between exon 6 [19] and the β1C-specific exon [5]. In the β1A variant three regions have been identified, named cyto-domain 1, 2 and 3 which contribute to integrin localization in focal contacts [17]. These cyto-domain sequences are highly conserved among the β1A, β2, β3A, β5, β6 and β7 subunits. In addition, the observation that β1B and β3B splice variants, which both lack the cyto-2 and cyto-3 domains, are not present in focal contacts supports the notion that the cyto-domains are important for integrin localization in focal contacts [20,21]. β1D contains all three cyto-sequences suggesting that β1D may also be localized in focal contact-like structures, e.g. in myotendinous and neuromuscular junctions or in intercalated discs in skeletal or cardiac muscle, respectively. Consistent with its assumed cellular localization, the cytoplasmic domain of β1D contains potential binding sites for α-actinin and talin. These cytoplasmic proteins are co-localized in focal contacts in cultured cells and they have been reported to bind to β1A in vitro [22,23]. The β1D cytoplasmic domain differs from the β1A, β2, and β3 in sequences between the two NPXY sequences. This part contains three related amino acids (VTT, TTT and TST) that have recently been implicated in the regulation of ligand affinity of integrins [24–27].

It has been reported that the function of the cytoplasmic domain of β1B, resulting from alternative splicing, is different from that of β1A. Cell adhesion mediated by β1B integrin to fibronectin and laminin is reduced as compared to that mediated by β1A, and cells expressing β1B have a reduced motility [20,28]. The observation that β1D is upregulated during myoblast differentiation points to a role in myogenesis. At this stage of investigation the function of the skeletal and cardiac muscle-specific β1 variant is only hypothetical. A crucial role for β1D in myoblast fusion is unlikely since β1D is also expressed in cardiac muscle in which, in contrast to skeletal muscle, multinucleated myotubes are not formed. In addition, the inhibitory effect of the CSAT antibody, directed against the extracellular part of chicken β1, on myotube fusion is likely to be due to a disturbance of adhesion before the actual fusion process [29,30]. However, the suggestion by Rosen et al. [31] that α4β1 and its ligand VCAM-1 are involved in secondary myoblast fusion still allows a role for β1D in that stage of the process.

Presumably, β1D plays a role in the organization of the sarcomeric cytoarchitecture: its cytoplasmic domain may be involved in striated muscle-specific, myofibrillar protein-protein interactions. This hypothesis is supported by the observation that the loss of β_{PS}, the β1-subunit homologue in *Drosophila*, in the *myospheriod* mutant, leads to a disrupted Z-band formation [32]. In addition, several immunohistochemical studies [33–36] have shown that the localization of $\alpha\beta$1 heterodimers at the myotendinous junction, which connects the myofibrils at the cellular end to the tendon, and at costameres, structures that link the Z-disks to the sarcolemma in banded patterns, is regulated by α-subunits (e.g. α3, α5, α7 and αv). However, no β1 variants have so far been implicated in these studies.

Association of β1D with α-subunits is probably not affected, since the heterodimer formation is determined by the extracellular and transmembrane domains of the β subunit [37–39]. Association of β1D with the simultaneously expressed α7A and α3B subunit could result in muscle specific integrins with unique cytoplasmic domains. The association of β1D with a variant α-subunit, resulting from alternative splicing, could

thus lead to the acquisition of signal transduction pathways in addition to the pp125^{FAK} phosphorylation cascade [40]. Antibodies against β1D are currently being generated to test these hypotheses.

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References

[1] Hynes, R.O. (1992) Cell 69, 11–25.
[2] Sonnenberg, A. (1993) Curr. Top. Microbiol. Immunol. 184, 7–35.
[3] Williams, M.J., Hughes, P.E., O'Toole, T.E. and Ginsberg, M.H. (1994) Trends Cell. Biol. 4, 109–112.
[4] Altruda, F., Cervella, P., Tarone, G., Botta, C., Balzac, F., Stefanuto, G. and Silengo, L. (1990) Gene 95, 261–266.
[5] Languino, L.R. and Ruoslahti, E. (1992) J. Biol. Chem. 267, 7116–7120.
[6] Sutherland, A.E., Calarco, P.G. and Damsky, C.H. (1993) Development 119, 1175–1186.
[7] Hierck, B.P., Thorsteinsdottir, S., Niessen, C.M., Freund, E., Iperen, L., Feyen, A., Hogervorst, F., Poelman, R.E., Mummery, C.L. and Sonnenberg, A. (1993) Cell Adhes. Commun. 1, 1–21.
[8] Ziober, B.L., Vu, M.P., Waleh, N., Crawford, J., Lin, C.S. and Kramer, R.H. (1993) J. Biol. Chem. 268, 26773–26783.
[9] Collo, G., Starr, L. and Quaranta, V. (1993) J. Biol. Chem. 268, 19019–19024.
[10] Song, W.K., Wang, W., Sato, H., Bielser, D.A. and Kaufman, S.J. (1993) J. Cell Sci. 106, 1139–1152.
[11] Argraves, W.S., Suzuki, S., Arai, H., Thompson, K., Pierschbacher, M.D., Ruoslahti, E. (1987) J. Cell Biol. 105, 1183–1190.
[12] Tominaga, S. (1988) FEBS Lett. 238, 315–319.
[13] Tamura, R.N., Cooper, H.M., Collo, G. and Quaranta, V. (1991) Proc. Natl. Acad. Sci. USA 88, 10183–10187.
[14] Takada, Y., Murphy, E., Pil, P., Chen, C., Ginsberg, M.H. and Hemler, M.E. (1991) J. Cell Biol. 115, 257–266.
[15] Sambrook, J., Fritsch, E.F. and Maniatis, T. (1989) Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory Press, New York.
[16] McArthur-Lewis, J. and Schwartz, M.A. (1995) Mol. Biol. Cell. 6, 151–160.
[17] Reszka, A.A., Hayashi, Y. and Horwitz, A.F. (1992) J. Cell Biol. 117, 1321–1330.
[18] Breathnach, R. and Chambon, P. (1981) Annu. Rev. Biochem. 50, 349–383.
[19] Lanza, F., Kiefer, N., Phillips, D.R. and Fitzgerald, L.A. (1990) J. Biol. Chem. 265, 18098–18103.
[20] Balzac, F., Belkin, A.M., Koteliansky, V.E., Balabanov, Y.V., Altruda, F., Silengo, L. and Tarone, G. (1993) J. Cell Biol. 121, 171–178.
[21] Laflamme, S.E., Thomas, L.A., Yamada, S.S. and Yamada, K.M. (1994) J. Cell Biol. 126, 1287–1298.
[22] Burridge, K., Fath, K., Kelly, T., Nuckolls, G. and Turner, C. (1988) Annu. Rev. Cell. Biol. 4, 487–523.
[23] Turner, C.E. and Burridge, K. (1991) Curr. Opin. Cell. Biol. 3, 849–853.
[24] O'Toole, T.E., Ylanne, J. and Culley, B.M. (1995) J. Biol. Chem. 270, 8553–8558.
[25] Peter, K. and O'Toole, T.E. (1995) J. Exp. Med. 181, 315–326.
[26] Hibbs, M.L., Jakes, S., Stacker, S.A., Wallace, R.W. and Springer, T.A. (1991) J. Exp. Med. 174, 1227–1238.
[27] Chen, Y.P., Djaffar, I., Pidard, D., Steiner, B., Cieutat, A.M., Caen, J.P. and Rosa, J.P. (1992) Proc. Natl. Acad. Sci. USA 89, 10169–10173.
[28] Balzac, F., Retta, S.F., Albini, A., Melchiorri, A., Koteliansky, V.E., Geuna, M., Silengo, L. and Tarone, G. (1994) J. Cell Biol. 127, 557–565.
[29] Menko, A.S. and Boettiger, D. (1987) Cell 51, 51–57.
[30] Neff, N.T., Lowrey, C., Decker, A., Tovar, A., Damsky, C., Buck, C. and Horwitz, A.F. (1982) J. Cell Biol. 95, 654–666.
[31] Rosen, G.D., Sanes, J.R., LaChance, R., Cunningham, J.M., Roman, J. and Dean, D.C. (1992) Cell 69, 1107–1119.
[32] Volk, T., Fessler, L.I. and Fessler, J.H. (1990) Cell 63, 525–536.
[33] Bao, Z., Z., Lakonishok, M., Kaufman, S. and Horwitz, A.F. (1993) J. Cell Sci. 106, 579–590.
[34] Bozyczko, D., Decker, C., Muschler, J. and Horwitz, A.F. (1989) Exp. Cell Res. 183, 72–91.
[35] Enomoto, M.I., Boettiger, D. and Menko, A.S. (1993) Dev. Biol. 155, 180–197.
[36] McDonald, K.A., Lakonishok, M. and Horwitz, A.F. (1995) J. Cell Sci. 108, 975- 983.
[37] Hayashi, Y., Haimovich, B., Reszka, A., Boettiger, D. and Horwitz, A. (1990) J. Cell Biol. 110, 175–184.
[38] Marcantonio, E.E., Guan, J.L., Trevithick, J.E. and Hynes, R.O. (1990) Cell. Regul. 1, 597–604.
[39] Solowska, J., Guan, J.L., Marcantonio, F.E., Trevithick, J.E., Buck, C.A. and Hynes, R.O. (1989) J. Cell Biol. 109, 853–861.
[40] Schaller, M.D. and Parsons, J.T. (1994) Curr. Opin. Cell Biol. 6, 705–710.