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The gene organisation of the human β2 integrin subunit (CD18)

Jonathan B. Weitzman, Charmian E. Wells, Ann H. Wright, Paula A. Clark and S.K. Alex Law

The MRC Immunochemistry Unit, Department of Biochemistry, University of Oxford, South Parks Road, Oxford OX1 3QU, UK

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We have studied the gene of the human β2 integrin subunit (CD18) and found it to be organised into 16 exons spanning a region of about 40 kb. All exon/intron boundaries conform to the GT/AG splicing consensus. The exons coding for the cysteine-rich region, which has been postulated to consist of 3 or 4 repeating elements, are not organised correspondingly. Transcription of the gene initiates from multiple sites which may be due to the absence of an upstream TATA box. The polyadenylation site is also heterogeneous. Five different sites were identified over a stretch of 10 bases.

Integrin; Gene structure; Exon/intron boundary

1. INTRODUCTION

The integrins are cell surface antigens that mediate a wide range of cell adhesion activities (for reviews see [1,2]. They are heterodimeric glycoproteins composed of an α and a β subunit. The α subunits characterised to date are similar in their primary structures, as are the β subunits.

The 3 members of the leukocyte integrin subfamily are expressed exclusively on leukocytes and participate in adhesion activities, including the cytolysis of target cells, cross-interaction and cross-stimulation between lymphocytes, phagocytosis of complement-coated targets, and the regulation of leukocyte traffic between the blood stream and tissues [3,4]. The three α subunits, αL (CD11a), αM (CD11b) and αX (CD11c), each associate with the common β2 subunit (CD18) to form, respectively, the antigens LFA-1, Mac-1 and p150,95 [5].

The gene of the β2 subunit (CD18) has been located to chromosome 21 [6] band q22.3 [7,8], and, using radiation hybrid mapping and pulsed-field gel electrophoresis, its location has been refined to about 2000 kb from the q telomere [9]. The β2 gene is extremely polymorphic; at least 10 different haplotypes over 3 loci in 20 healthy, unrelated individuals have been identified by RFLP analysis [10]. Patients suffering from leukocyte adhesion deficiency (LAD) have defects in their β2 gene resulting in the failure to express any of the 3 leukocyte integrins [11]. Heterogeneity of the defect was demonstrated at the phenotypic level [12]. At the molecular level, 5 different mutations have been reported; they are all single base changes resulting in either amino acid substitutions [13,14] or exon deletion due to aberrant splicing [15]. In this paper we report the genomic organisation of the β2 integrin (CD18) gene.

Abbreviations: PCR, polymerase chain reaction; RACE, rapid amplification of cDNA ends; PAGE, polyacrylamide gel electrophoresis; PMA, phorbol myristate acetate; RFLP, restriction fragment length polymorphism.

Correspondence address: S.K.A. Law, The MRC Immunochemistry Unit, Department of Biochemistry, University of Oxford, South Parks Road, Oxford OX1 3QU, UK. Fax: (44) (865) 275 729.

2. MATERIALS AND METHODS

Genomic DNA was prepared from peripheral blood leukocytes using the method of Bell et al. [16]. *Bgl*II-digested genomic DNA was fractionated by ultracentrifugation through a sucrose density gradient. 300 μg of *Bgl*II-digested genomic DNA, in 400 μl of 10 mM Tris, 0.1 mM EDTA, pH 8.0, was layered onto 4 sucrose density gradients made with 2 ml of each of 45, 40, 35, 30, 25 and 20% sucrose in 25 mM Tris, 1 mM EDTA, 0.2% SDS, pH 8.0, in 14 ml Ultra-Clear centrifuge tubes (Beckman). Ultracentrifugation was carried out with an SW40Ti rotor in a Beckman L-8 ultracentrifuge at 20°C, 28 000 rpm, for 20 h. Eighteen fractions were collected from each of the 4 gradients and the corresponding fractions were pooled.

Oligonucleotides were synthesised in an automated synthesiser (Applied Biosystems, Model 381A). CD18 specific oligonucleotides are marked in Fig. 1A. A DNA Thermal Cycler (Perkin Elmer Cetus) was used for PCR: reactions were performed in a standard buffer containing 1.5 mM $MgCl_2$. *Taq* DNA polymerase was obtained from Perkin Elmer Cetus or Promega. Primers were used at 1 μM except in RACE-PCR. Annealing temperature ranged from 50 to 60°C depending on the primer sequences.

A probe for RNase protection analysis was synthesised using the hybrid clone pR5'G (see Fig. 3A). The clone was linearised with *Xho*I and a continuously labelled RNA probe was made using ^{32}P-αCTP and T3 RNA polymerase with other reagents from the Riboprobe system (Promega). About 2×10^5 cpm of the probe was added to 150 μg of RNA from PMA-stimulated U937 cells or tRNA in 40 mM PIPES, 400 mM NaCl, 1 mM EDTA, pH 6.7, containing 80% (v/v) formamide. After 5 min at 85°C, the mixtures were incubated at 45, 55 or 65°C, or subjected to three 65°C (30 min)/45°C (30 min) cycles before incubation at 45°C for 16 h. RNase A (1 μg) and RNase T1 (15 μg) were added and the mixtures were further incubated at 30°C

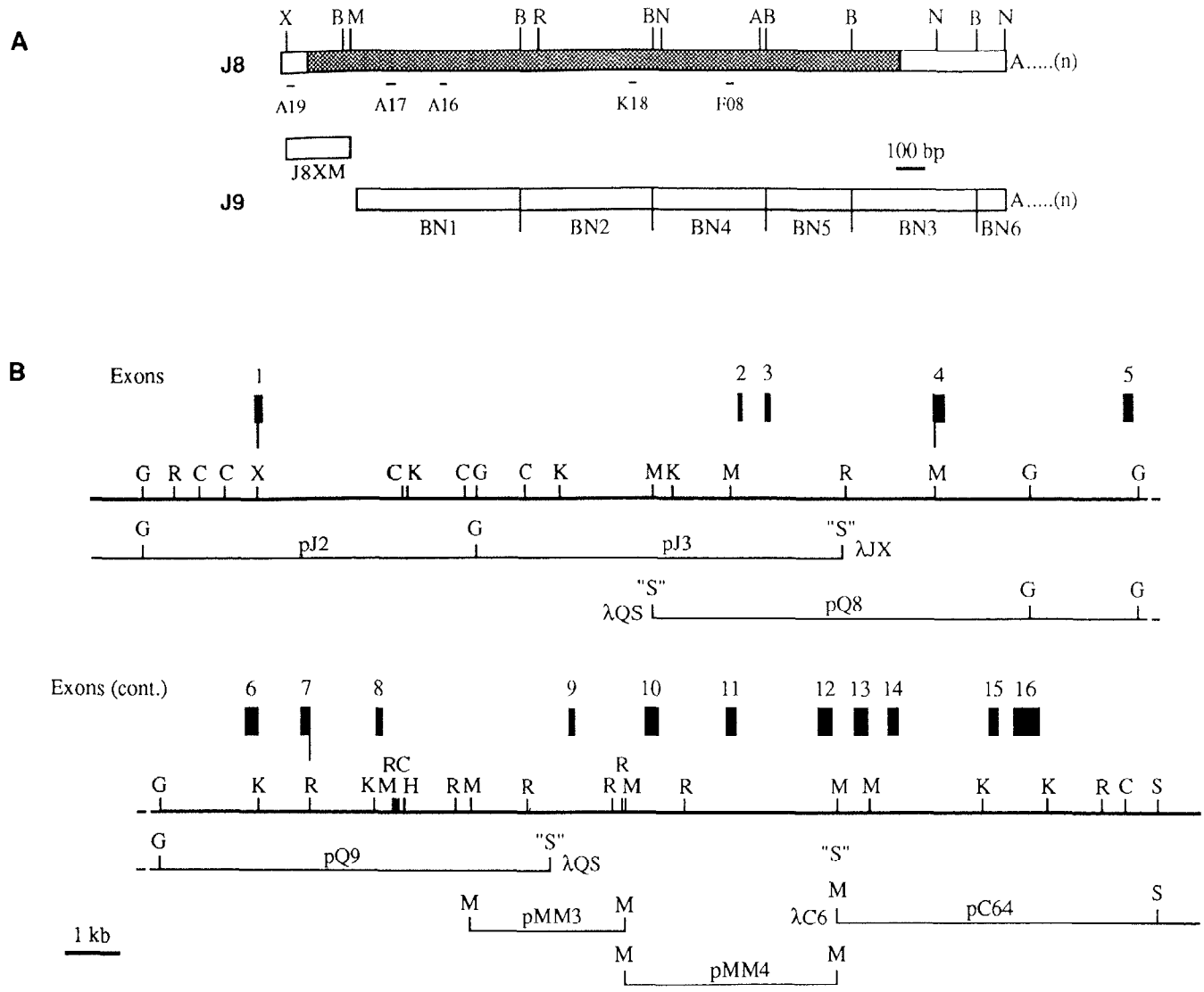


Fig. 1. (A) The cDNA clones of CD18. The cDNA clones J8 and J9 are shown with the restriction sites for *Xba*I (X), *Ban*I (B), *Bam*HI (M), *Eco*RI (R), *Nco*I (N) and *Sac*II (A) marked on J8. The shaded bar marks the position of the coding region. Also indicated are the positions of the sense (A19: 5′-CCAGGGCAGACTGGTAGCAAAGCC-3′, K18: 5′-GCTTCACGGACATAGTGAC-3′, F08: 5′-GCTACAACGGCCAGGTCTG-3′) and antisense (A17: 5′-CTGGTCGCAGGTAAAGC-3′, A16: 5′GCACGGTCTTGTCCACG-3′) CD18-specific oligonucleotides. (B) The restriction map of the CD18 gene and exon locations. The restriction sites for *Bgl*II (G), *Eco*RI (R), *Hin*cII (C), *Xba*I (X), *Kpn*I (K), *Bam*HI (M), *Hin*dIII (H) and *Sal*I (S) are marked and they are complete within the region shown. *Xba*I, *Bam*HI and *Eco*RI sites are found in the transcribed regions in exons 1, 4 and 7 respectively. The *Sal*I cloning sites of the λ clones are marked 'S'.

for 1 h. SDS and proteinase K were added to terminate the RNase digestion. After phenol extraction, the undigested double-strand RNA was precipitated and analysed by urea-PAGE and autoradiography.

Two oligonucleotides, K05 (GGTCGACTCTAGAGGATCC) and K06 (K05 with 17 Ts at the 3′ end), were synthesised for RACE-PCR [17]. For 5′ end analysis, first-strand cDNA was synthesised from poly(A) selected RNA from PMA-stimulated U937 cells using reagents from the cDNA Synthesis System Plus (Amersham). Poly(A) was added to the single-strand cDNA with terminal deoxynucleotide transferase (Bethesda Research Laboratories). The 5′ tailed cDNA was used as template for PCR (30 cycles) using K05 (0.25 μM), K06 (0.1 μM) and the CD18-specific oligonucleotide A16 (0.25 μM) with an annealing temperature of 50°C. The product was further amplified twice, 20 cycles each, using K05 (0.25 μM) and the oligonucleotide A17 (0.25 μM), with a higher annealing temperature of 55°C. A similar strategy was used for 3′ end analysis except that double-stranded cDNA was used as the first-round template making use of the naturally occurring poly(A) tail at the 3′ end.

Lambda-DNA was prepared using QIAGEN-pack 100 (supplied by Hybaid Ltd., UK) according to the manufacturer's instructions. Plasmid cloning was performed with pBluescript KS (Stratagene) linearised with different enzymes (indicated in brackets) in the polylinker region. Restriction and other nucleic acid modification enzymes were purchased from Amersham, Boehringer-Mannheim, Bethesda Research Laboratories, New England Biolabs or Pharmacia. Sequencing reactions were performed either with the double-stranded plasmid clones using the ^{T7}Sequencing kit (Pharmacia) or with single-strand DNA made by transfecting pBluescript clones in *E. coli* (strain TG-1) with the helper phage M13/K07, using Sequenase version 2.0 or TA-Quence DNA sequencing kits (US Biochemical Co.). The M13 and T3 primers, as well as CD18-specific oligonucleotides, were used as primers for sequencing.

Details of other standard techniques for molecular biology can be found in Sambrook et al. [18].

3. RESULTS

3.1. *cDNA clones*

Several cDNA clones, including J9 and J19, of CD18 have been previously reported [19]. Subsequently, a clone (J8) containing 101 bases 5′ to the initiating ATG codon was obtained. The six *Ban*I fragments of clone J9, BN1 to BN6, were individually subcloned into the vector pATX (*Pvu*II). The cDNA clones and their subcloned fragments are shown in Fig. 1A.

3.2. *Genomic clones of the CD18 gene*

λEMBL3 genomic libraries containing partially digested *Mbo*I fragments, generously provided by Dr. D.R. Bentley, were screened with the J8XM and J9 cDNA probes. A number of CD18 clones were obtained. Southern blot analysis using the probes J8XM, and BN1–6 established that 3 of the clones, λJX, λQS and λC6, gave maximal coverage of the gene (Fig. 1B). Extensive screening of other genomic libraries, in λ or cosmids, failed to yield clones that covered the gap between λQS and λC6.

The gap was found, by Southern blot analysis of genomic DNA, to lie within a 29 kb *Bgl*II fragment. *Bgl*II digests of whole genomic DNA was size-fractionated on a sucrose density gradient. Fractions containing the highest specific abundance of the 29 kb fragment were further digested with *Bam*HI. A library was made in pBluescript (*Bam*HI). Of approximately 8000 colonies, 3 were positive with the BN2 probe and another was positive with both BN2 and BN4 probes. One of the BN2-positive clones (pMM3) and the BN2/BN4-positive clone (pMM4) (Fig. 1B) were analysed further.

3.3. *Mapping of the CD18 gene*

Preliminary restriction maps of the CD18 gene were established by Southern blot analysis of genomic DNA. The λ clones were digested with *Sal*I and *Bgl*II and fragments of 5–7 kb were cloned into pBluescript (*Sal*I; *Bam*HI; or *Sal*I/*Bam*HI). Five clones, pJ2, pJ3, pQ8, pQ9 and pC64 were obtained. A restriction map for the enzymes *Bgl*II, *Eco*RI, *Hin*cII, *Xba*I, *Kpn*I, *Bam*HI, *Hin*dIII and *Sal*I (Fig. 1B) was generated from analysis of the 5 plasmid subclones as well as the plasmid clones pMM3 and pMM4. Attempts to clone the 2 kb *Bgl*II fragment of λQS in pBluescript were unsuccessful.

The overlap between pQ9 and pMM3 was established by sequencing. Oligonucleotides in exons on either side of the junctions between pMM3 and pMM4 and between pMM4 and pC64 were made and DNA fragments were amplified from the genomic DNA template by PCR. These fragments were cloned and the sequences obtained established the continuity of the clones pMM3, pMM4 and pC64 in the CD 18 gene.

3.4. *Exon/intron boundaries*

Exon/intron boundaries were established by sequencing using one of the following 2 approaches: (1) Oligonucleotides based on the CD18 cDNA sequence were used as primers to sequence various genomic plasmid clones directly. (2) The genomic clones were digested with either *Alu*I, *Ava*II, *Hin*fI or *Nco*I, end-filled, and cloned into pBluescript (*Hin*cII). Clones containing exonic fragments were identified by screening, and sequenced. Sixteen exons were found. The exon/intron boundaries, including the phase and size of the introns, are shown in Fig. 2. All were found to conform to the GT/AG splicing consensus [20].

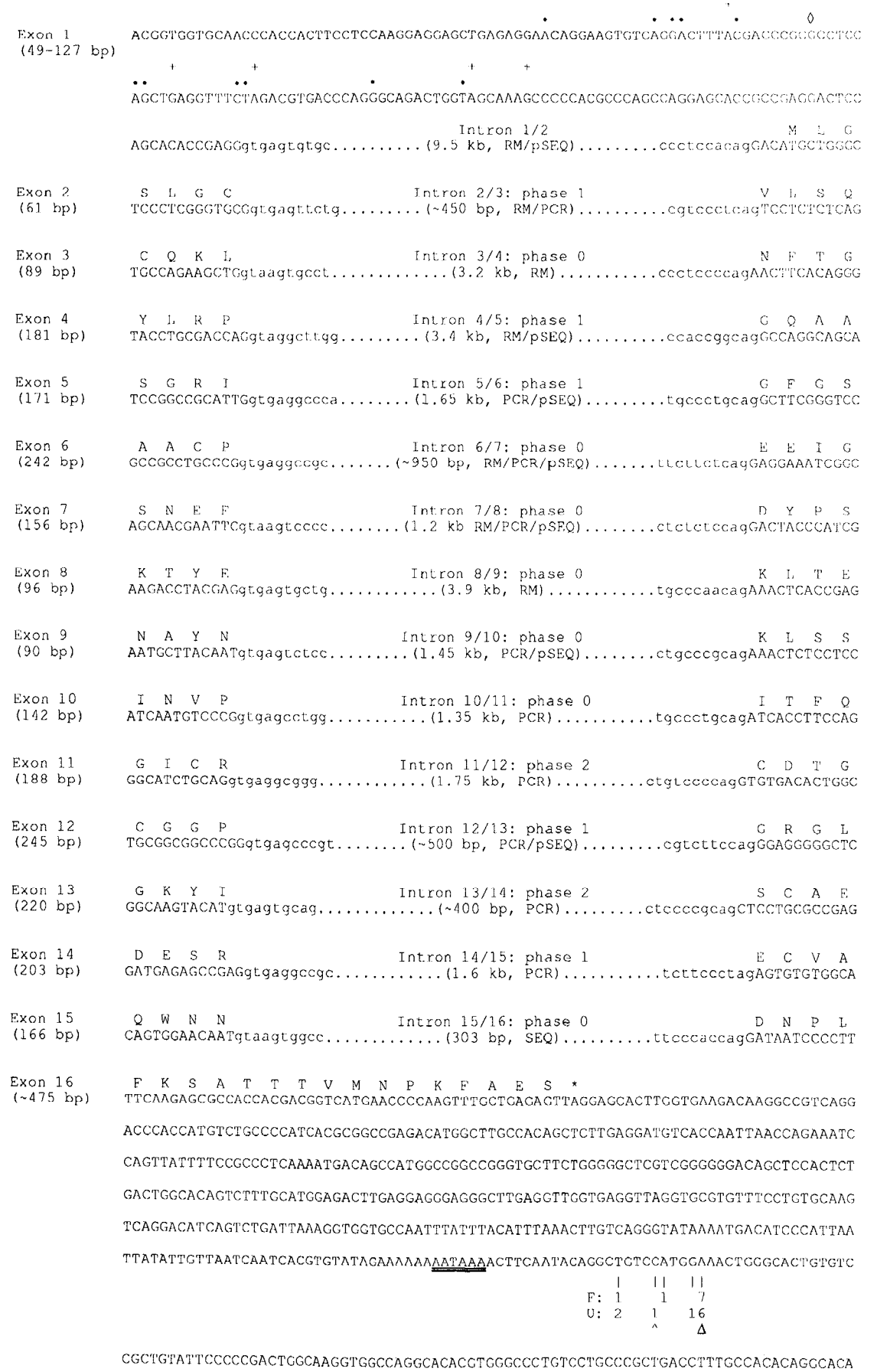
3.5. *Intron sizes and exon positions*

The size of the introns and the position of the exons with respect to the restriction map were established by one of 4 methods: (1) Restriction mapping (RM); (2) PCR between flanking exons; (3) partial sequencing (pSEQ) where sequences to the nearest restriction enzyme site were determined; and (4) sequencing (SEQ) of the entire intron (Fig. 2). The locations of the exons are shown in Fig. 1B.

3.6. *The 5′ end*

J8 is the longest cDNA clone obtained and it includes 101 bp of 5′ untranslated sequence. The 5′ 98 bp were found in a single stretch in pJ2. Making use of an *Xba*I site in this stretch, a hybrid genomic/cDNA clone, pR5′ G, containing an *Ava*II/*Xba*I genomic fragment and an *Xba*I/*Bam*HI cDNA fragment (J8XM) was constructed in the pBluescript SK+ vector (Fig. 3A). A radiolabelled RNA probe was synthesised using T3 RNA polymerase and hybridised to total RNA from PMA-stimulated U937 cells under various conditions. After digestion of unprotected regions with RNase A and RNase T1, the protected regions of the probe were analysed by urea-PAGE. Five major bands of 222, 228, 252, 261 and 280 bases were found when the probe was hybridised to U937 RNA but not when hybridised with tRNA (Fig. 3B). These results suggested that there are multiple initiation sites of transcription and the major ones lie 222–280 bases 5′ to the *Bam*HI site in the cDNA, corresponding to 49–127 bases for the first exon.

The rapid amplification of cDNA ends (RACE) protocol was used to determine the sequence of the 5′ cDNA ends. The first round PCR products, using K05, K06 and A16 primers, were further amplified twice for 20 cycles each with A17 and K05. The resultant products, which appeared as a smear of heterogeneous products on agarose gels, were digested with *Bam*HI and cloned into pBluescript (*Bam*HI). Eight CD18-specific clones were obtained by screening with oligonucleotide A19 and analysed by sequencing. They contain 80–127 nucleotides 5′ from the putative Exon 1/Exon 2 junction, all with sequences identical to the genomic sequence. Similar results were obtained using other


Exon 1
(49-127 bp)
ACGGTGGTGCAACCCACCACTTCCTCCAAGGAGGAGCTGAGAGGAACAGGAAGTGTCAGGACTTTACGACCCGGGCCTCC
AGCTGAGGTTTCTAGACGTGACCCAGGGCAGACTGGTAGCAAAGCCCCCACGCCCAGCCAGGAGCACCGCCGAGGACTCC
Intron 1/2
M L G
AGCACACCGAGGtgagtgtgc.....(9.5 kb, RM/pSEQ).....ccctccacagGACATGCTGGCC
Exon 2
(61 bp)
S L G C
Intron 2/3: phase 1
V L S Q
TCCCTCGGGTGCGgtgagttctg.....(~450 bp, RM/PCR).....cgtccctcagTCCTCTCTCAG
Exon 3
(89 bp)
C Q K L
Intron 3/4: phase 0
N F T G
TGCCAGAAGCTGgtaagtgcct.....(3.2 kb, RM).....ccctccccagAACTTCACAGGG
Exon 4
(181 bp)
Y L R P
Intron 4/5: phase 1
G Q A A
TACCTGCGACCAGgtaggcttgg.....(3.4 kb, RM/pSEQ).....ccaccggcagGCCAGGCAGCA
Exon 5
(171 bp)
S G R I
Intron 5/6: phase 1
G F G S
TCCGGCCGCATTGgtgaggccca.....(1.65 kb, PCR/pSEQ).....tgccctgcagGCTTCGGGTCC
Exon 6
(242 bp)
A A C P
Intron 6/7: phase 0
E E I G
GCCGCCTGCCCGgtgaggccgc.....(~950 bp, RM/PCR/pSEQ).....ttcttctcagGAGGAAATCGGC
Exon 7
(156 bp)
S N E F
Intron 7/8: phase 0
D Y P S
AGCAACGAATTCgtaagtcccc.....(1.2 kb RM/PCR/pSEQ).....ctctctccagGACTACCCATCG
Exon 8
(96 bp)
K T Y E
Intron 8/9: phase 0
K L T E
AAGACCTACGAGgtgagtgctg.....(3.9 kb, RM).....tgcccaacagAAACTCACCGAG
Exon 9
(90 bp)
N A Y N
Intron 9/10: phase 0
K L S S
AATGCTTACAATgtgagtctcc.....(1.45 kb, PCR/pSEQ).....ctgcccgcagAAACTCTCCTCC
Exon 10
(142 bp)
I N V P
Intron 10/11: phase 0
I T F Q
ATCAATGTCCCGgtgagcctgg.....(1.35 kb, PCR).....tgccctgcagATCACCTTCCAG
Exon 11
(188 bp)
G I C R
Intron 11/12: phase 2
C D T G
GGCATCTGCAGgtgaggcggg.....(1.75 kb, PCR).....ctgtccccagGTGTGACACTGGC
Exon 12
(245 bp)
C G G P
Intron 12/13: phase 1
G R G L
TGCGGCGGCCCGGgtgagcccgt.....(~500 bp, PCR/pSEQ).....cgtcttccagGGAGGGGGCTC
Exon 13
(220 bp)
G K Y I
Intron 13/14: phase 2
S C A E
GGCAAGTACATgtgagtgcag.....(~400 bp, PCR).....ctccccgcagCTCCTGCGCCGAG
Exon 14
(203 bp)
D E S R
Intron 14/15: phase 1
E C V A
GATGAGAGCCGAGgtgaggccgc.....(1.6 kb, PCR).....tcttccctagAGTGTGTGGCA
Exon 15
(166 bp)
Q W N N
Intron 15/16: phase 0
D N P L
CAGTGGAACAATgtaagtggcc.....(303 bp, SEQ).....ttcccaccagGATAATCCCCTT
Exon 16
(~475 bp)
F K S A T T T V M N P K F A E S *
TTCAAGAGCGCCACCACGACGGTCATGAACCCCAAGTTTGCTGAGAGTTAGGAGCACTTGGTGAAGACAAGGCCGTCAGG
ACCCACCATGTCTGCCCCATCACGCGGCCGAGACATGGCTTGCCACAGCTCTTGAGGATGTCACCAATTAACCAGAAATC
CAGTTATTTTCCGCCCTCAAAATGACAGCCATGGCCGGCCGGGTGCTTCTGGGGGCTCGTCGGGGGGACAGCTCCACTCT
GACTGGCACAGTCTTTGCATGGAGACTTGAGGAGGGAGGGCTTGAGGTTGGTGAGGTTAGGTGCGTGTTTCCTGTGCAAG
TCAGGACATCAGTCTGATTAAAGGTGGTGCCAATTTATTTACATTTAAACTTGTCAGGGTATAAAATGACATCCCATTAA
TTATATTGTTAATCAATCACGTGTATAGAAAAAAAATAAAACTTCAATACAGGCTGTCCATGGAAACTGGGCACTGTGTC
F: 1 1 7
U: 2 1 16
CGCTGTATTCCCCCGACTGGCAAGGTGGCCAGGCACACGTGGGCCCTGTCCTGCCCGCTGACCTTTGCCACACAGGCACA

CD18-specific oligonucleotides as primers at the amplification stages. Since these clones were screened with the oligonucleotide A19, they are biased for clones extended 5′ to A19. The initiation sites, as determined by the 2 methods, are shown in Fig. 2 (Exon 1).

3.7. *The 3′ end*

In the course of our studies on the cDNA clones of CD18, 2 types of clone were found; those with an *Nco*I site at the 3′ end, such as J9 and J8, and those without, such as J19. Sequencing revealed that the 6 bases immediately preceding the poly(A) tail are CCATGG, which account for the presence of the *Nco*I site. On the other hand, the last 5 bases are not found in J19. This phenomenon was investigated further using RACE at the 3′ end. cDNA prepared from poly(A) selected RNA of PMA-stimulated U937 cells (U) or an EBV-transformed cell line (F) were used as templates. PCR was first performed with the oligonucleotides K18 and a mixture of K05 and K06 for 30 cycles. The product was further amplified for 20 cycles using the oligonucleotides F08 and K05. The fragment between the *Sac*II site within the cDNA sequence and the *Bam*HI site within K05 was cloned into pBluescript (*Sac*II/*Bam*HI). Nine clones from F and 10 from U were analysed by sequencing. There are at least 5 different poly(A) addition sites but 7 out of 10 clones from F and 6 of 9 clones from U have the same site as J8 and J9 (Fig. 2, Exon 16).

4. DISCUSSION

We have obtained genomic clones which cover the transcribed region of the CD18 gene. The gene spans approximately 40 kb and is organised into 16 exons. All the exonic segments of the genomic clones have been sequenced to establish formally their continuity. Two exon/intron boundaries of the CD18 gene have been previously reported. In the study of a patient with leukocyte adhesion deficiency, the defect was caused by a mutation in a splice site leading to the deletion of Exon 9 in the mRNA [15]. In addition, an aberrant cDNA clone of the mouse CD18 led Zeger et al. [21] to propose an exon/intron boundary which is equivalent to the 5′ boundary of Exon 13.

We have used both hybridisation/protection and primer extension type experiments to study the 5′ end of the CD18 gene. Five major initiation sites were found with RNA protection using an RNA probe. Primer extension experiments confirmed the existence of multiple

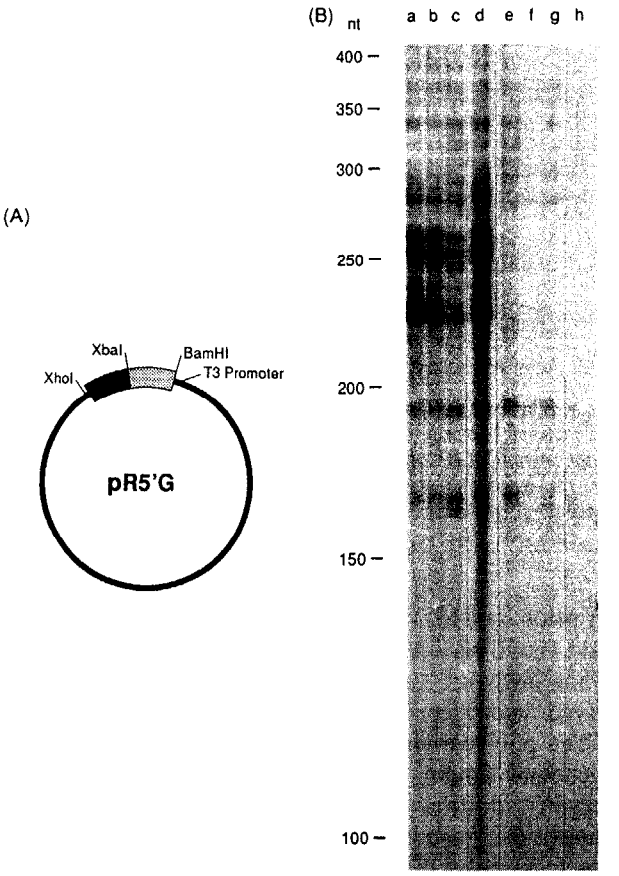


Fig. 3. The initiation sites of transcription of the CD18 gene. (A) The genomic-cDNA hybrid clone (pR5′G) in pBluescript (SK+) constructed for the purpose of making RNA probes. The dark-shaded region is from the genomic clone pJ2 whereas the light-shaded region is from the cDNA clone J8. (B). RNase protection studies. The RNA probe made from pR5′G was hybridised to total RNA from U937 (tracks a–d) and tRNA (tracks e–h) followed by digestion with RNase A and RNase T1. The hybridisation was at 45°C (tracks a and e), 55°C (tracks b and f) and 65°C (tracks c and g). Samples in tracks d and h were subjected to three 65°C/45°C cycles before incubation at 45°C for 16 h. The size markers (in nucleotides, nt) were from a sequencing reaction run on the same gel (not shown).

transcription initiation sites (data not shown), which were found to be more scattered and in general indicative of a more 3′ start. This may be caused by the high G+C content of the region (>60%) affecting the efficiency of the reverse transcriptase. The RACE-PCR cloning experiments, on the other hand, were biased towards longer clones since they were screened with the oligonucleotide A19. In addition, the RACE-PCR clones have sequences 5′ extended along the genomic sequence. Taking these results together, we may conclude that (i) there are multiple initiation sites of tran-

Fig. 2. The exon/intron boundaries of the CD18 gene. The phase of the introns, their size, and the method used for size determination are indicated. Genomic sequence running into Exon 1 is shown. Initiation sites of transcription determined by RNase protection (+) and those by RACE-PCR (•) are marked. The first base of the cDNA clone J8 is indicated (◇). The sequence of Exon 16 is shown and the first A of the poly(A) tails are marked by (|). Indicated below the markers are the number of clones found with RNA from F (an EBV-transformed cell line) and U (the cell line U937). The polyadenylation sites of the cDNA clones J19 (^) and J8 and J9 (△) are also indicated.

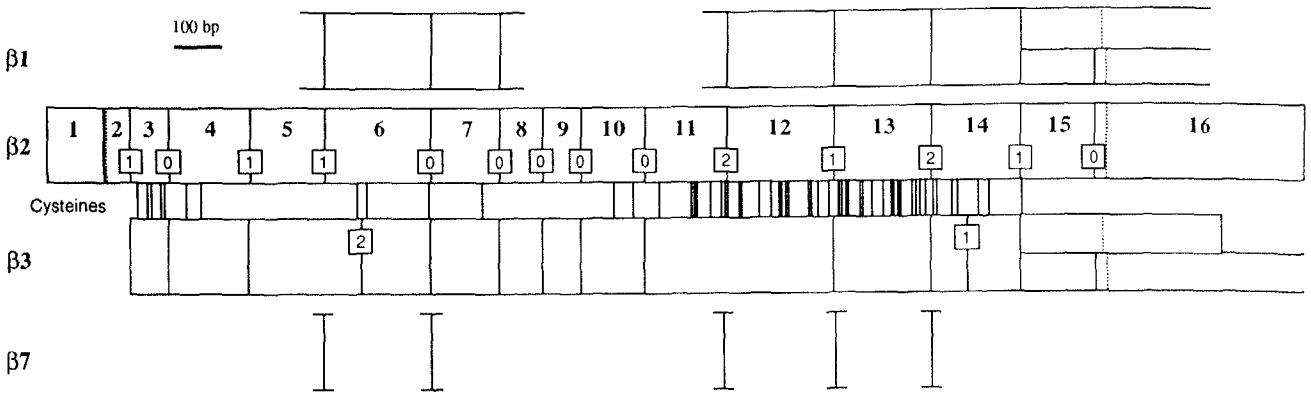


Fig. 4. The exons of the $\beta 2$ integrin subunit (CD18) aligned with those of the $\beta 1$ [37], $\beta 3$ [37,38] and $\beta 7$ integrin subunits [36]. The coding regions are enclosed between the vertical dotted lines in Exons 2 and 16. The phases of the introns are shown in square boxes between exons. The phases of the introns of $\beta 3$, where different from those of $\beta 2$, are also shown. Both $\beta 1$ [39] and $\beta 3$ [40] have 2 alternative transcripts resulting from the non-splicing of the intron equivalent to that between E15 and E16 in $\beta 2$. The boundaries of $\beta 7$ were determined from incompletely processed and alternatively spliced cDNA clones; hence the exons in between were not defined formally [36]. Also marked are the position of the 56 cysteine residues in the extracellular domains of the $\beta 2$ subunit.

scription, and (ii) the region studied is the first exon. We have found no TATA box motif immediately upstream of the initiation site cluster which may explain the observed heterogeneity.

The polyadenylation site at the 3′ end is also heterogeneous. There is only one AATAAA signal, but poly(A) addition is found at several sites around 20 nucleotides from the AATAAA signal, with one major site accounting for about 70% of the clones studied. It is generally accepted that a GT-rich region 20–30 nucleotides 3′ to the site is required for efficient polyadenylation [22–24]. The absence of a pronounced GT-rich element in this region may account for the lack of a single polyadenylation site of the CD18 message (Fig. 2).

The CD18 antigen is the $\beta 2$ subunit of the integrin superfamily. To date, the primary structures of at least 6 other β subunits have been determined [19,25–36]. They share similar sequence features, with highly conserved cysteine residues in their extracellular domains. Fourteen exon/intron boundaries of the $\beta 3$ integrin subunit (gpIIIa) have been determined [37,38], 3 of which are different from those of the $\beta 2$ gene (Fig. 4). The locations and phases of the introns between Exons 5 and 6 of $\beta 2$, and their counterparts in $\beta 3$ are different. The boundary between Exon 11 and Exon 12 is not found in $\beta 3$, and there are 2 exons in $\beta 3$ corresponding to Exon 14 of $\beta 2$. Eight exon boundaries of $\beta 1$ [37] and 5 of $\beta 7$ [36] have also been reported. They are identical to those of $\beta 2$ but not necessarily to those of $\beta 3$, suggesting that $\beta 1$, $\beta 2$ and $\beta 7$ are more closely related, in evolutionary terms, to each other than any is to $\beta 3$.

Alternative messages arising from the non-splicing of the intron between Exons 15 and 16 have been described both for the $\beta 1$ [39] and $\beta 3$ [40] integrin subunits. In both cases, the resultant proteins would have a variant cytoplasmic segment. In the analysis of the CD18 3′ end by RACE-PCR, we have not been able to detect the presence of variant products, suggesting that alternative splicing of the $\beta 2$ message does not occur at a significant level, at least not in PMA-stimulated U937 or EBV-transformed cells.

Primary structure analysis of the $\beta 2$ and other β integrin subunits invariably shows repeating elements in the cysteine-rich region. Although it is generally agreed that each repeating element contains 8 cysteine residues, the units themselves have not been defined since it is not clear where the boundaries lie. It is not uncommon to find structural motifs in proteins encoded by separate, or by a discrete number of exons [41]. However, Exons 11 to 14, which encode the cysteine-rich region, contain 7, 13, 16 and 6 cysteine residues, respectively, and therefore provide no information on the putative repeating elements. Thus the definition of these motifs must await further work at the protein level.

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