

BRIEF REPORT

Characterization of the Murine Platelet α IIB Gene and Encoded cDNA

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The α IIB/ β 3 receptor is central to platelet aggregation. Biological studies of this receptor have been limited by the inability to reproduce α IIB/ β 3 function in a cell system. Increasingly, efforts are being directed at studies of this receptor in mice models. The structure of murine (m) β 3 has been reported. We now have sequenced the m α IIB gene and found that it has the same size and organization as the human gene. The exon/intron borders are reported here, as are the distances between exons. m α IIB protein is 1,033

amino acids (aa), 7 and 5 aa shorter than human (h) and rodent (r) α IIB, respectively, with 79% and 90% homology, respectively. As part of the comparative analysis of the 3 known α IIB chains included in this report, we found that a particular region of the α IIB N-terminal β -propeller is highly conserved and speculate that it directly participates in ligand binding.

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THE PLATELET-SPECIFIC integrin α IIB/ β 3 (GPIIb-IIIa, CD41, CD62) binds fibrinogen and other ligands after platelet activation.^{1,2} This receptor is densely packed on the surface of the platelet with 80,000 copies per platelet³ and also does not bind its ligand with high affinity until the platelet is activated and the receptors are "turned on."⁴ Given the central role played by this receptor in thrombosis and the successful clinical application of anti- α IIB/ β 3-directed strategies to prevent thrombotic complications,⁵ a great deal of effort has been carried out to establish a functional assay system to study the α IIB/ β 3 receptor system *ex vivo*. These studies have focused on stable transfections in cell lines such as Chinese hamster ovary (CHO) or in lymphoid cells.^{6,7} Both lines suffer from the limited number of receptors on the surface and the artificial fashion in which these cells need to be activated. At best, these lines show binding in the nonactivated state and only modest increases after activation.

Attention has, therefore, focused on setting up more vigorous systems in *in vivo* models with an emphasis on murine studies. The m β 3 cDNA has been described⁸ and, subsequently, the m β 3 knockout has been produced with a phenotype similar to Glanzmann thrombasthenia.⁹ Furthermore, studies have been done with the first m β 3 knock-in, introducing a mutation into the m β 3 cytoplasmic tail (Y⁷⁴⁷F) and showing a mild decrease in platelet aggregation after agonist activation.¹⁰ Additionally, studies have begun introducing human α IIB and β 3 chains into primary megakaryocytic progenitors. For example, h β 3 constructs were introduced into m β 3 knockout marrow cells using a retroviral system, and the resulting megakaryocytes were rescued with regard to α IIB/ β 3 properties such as clot retraction, presumably secondary to m α IIB/h β 3 receptor complexes on the surface of the megakaryocytes.¹¹ Such studies show that cross-species heterodimers readily form and are functional, although there are going to be important limitations in such studies, as it is known that α IIB/ β 3 receptors appear to have different species related properties such as in their sensitivity to RGD peptides.^{12,13}

To carry out similar studies for m α IIB, we have cloned the gene and derived the encoded cDNA sequence. The gene organization is remarkably well preserved. The encoded protein is described and compared with the known h α IIB and r α IIB.^{14,15}

MATERIALS AND METHODS

Isolation of the m α IIB λ and bacterial artificial chromosome (BAC) clones. Full-length r α IIB cDNA was random-primer labeled¹⁶ with ³²P-dCTP and used to screen a λ FIX mouse 129SV genomic library

(Stratagene, La Jolla, CA) using nitrocellulose filter lifts of the plated phage. Individual positive colonies were obtained using repeat rounds of dilutional plating, and these were grown in large scale and purified using a cesium gradient as previously described.¹⁶

DNA from the genomic clones was characterized by restriction digest and Southern blotting¹⁶ using again the rat α IIB cDNA as probe. A 6-kb *Bam*HI fragment was subcloned from the original positive λ FIX clone and subcloned into *Bam*HI cut pBSK⁺ library (Stratagene).

The sequence in m α IIB Intron 12 was used to polymerase chain reaction (PCR)-screen a BAC mouse 129SV genomic library (Genome Systems, St Louis, MO), using the primers forward: 5'-ATGGACTTAC-CCCATAGAT-3' and reverse: 3'-ACTTCCCCGGGATTCTGCGC-3' to give a 0.5-kb band. The BAC clone was then used to obtain the sequence through exon 15, and then a PCR-amplified region for exons 15 and 16 was randomly primer labeled and used to rescreen the original λ FIX library to complete the characterization of the m α IIB gene.

m α IIB gene characterization. The 5'-*Bam*HI pBSK clone, the 5'- and 3'- λ FIX clones, and the BAC clone described above were end sequenced with the appropriate primers (eg, T₇, SP6, and T₃ primers). Subsequent primers were generated based on the new data and used to prime the next round of sequencing reaction. All sequencing used fluorescencelabeled dNTPs, and Sequenase (USB, Cleveland, OH) and an ABI 373A automated sequencer (PE Applied Biosystems, Foster City, CA). Sequences were stored and analyzed using MacDNAsis (Hitachi Software, San Diego, CA) and the BLAST program at the National Center for Biotechnology, which hosts an internet site at URL: <http://www.nlm.nih.gov/>.

RESULTS AND DISCUSSION

Characterization of the m α IIB gene. The only previously characterized α IIB cDNAs were those of human and rat.^{14,15} Using the rat α IIB cDNA, we screened a 129SV murine genomic λ FIX library. Sequencing of overlapping α IIB⁺ clones helped to provide the majority of sequence of the m α IIB gene

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Table 1. Exon/Intron Organization of the *m α 11b* Gene

Splice Donor	Intron No. (size)	Splice Acceptor
acatggaag <u>GTGAGCGCTAAAGGACATATGGGCG</u> H G S	1 (1,623 bp)	GTGCTCATCCAGCTTTCCTACACAG <u>cggtgtccat</u> V S I
tcgacctca <u>GTGAGTCTCAAGAATGAAGGGGAAA</u> F D L	2 (89 bp)	CTCCACCTTTACTTGTGCCCTCCAG <u>gggatgaga</u> R D E
gtcattgtg <u>GTGGTACTGTGGACAAGTCAAAGG</u> V I V	3 (97 bp)	CTACTCTCCTGTGGTCTGCTCTAG <u>gcctgtgcc</u> A C A
agagttt <u>cGTAAGCTCTGGTTTGGCCATTCGCTC</u> E S F	4 (222 bp)	GCTGATCCCCGCTTGCCCTCTTGTAG <u>gcggagaca</u> R G D
gtgaccag <u>GCAAGTCACAGGCAAAAGCAAACAA</u> V T Q	5 (535 bp)	TTCACGACCAGGACTTCTTTTTCAG <u>gctggggag</u> A G E
tttttttag <u>GTAGTGCCAGGAATCCAACCCACT</u> F F L	6 (91 bp)	AGCCCTCTCCCTGTGCCCTTCTAG <u>gtctcctgg</u> G L L
gttaccggg <u>GTAACGCTGGCAGCTCCTTTCCAAG</u> G Y R	7 (312 bp)	CGGTAAACTGTGCTGTGCTTTTCAG <u>gatattcgg</u> G Y S
gcactacag <u>GCAAGGAATGCAGGGGGCAGGGGG</u> S T T	8 (135 bp)	ATTTGCTGACCCCTTCTCTCCCA <u>gagtaacgat</u> E Y V
ttgggagcg <u>GTGAGTAGTGGCTCTCTACCCGCTT</u> L G A	9 (160 bp)	CATTTTGGTGTGCCCTCCATGCAG <u>gttgaatt</u> V E I
Ggagaacag <u>GTGGGGCGGTACCCCATGGGCAT</u> G E Q	10 (142 bp)	AATGGAAGTGCTATTCTCTTGTAG <u>tggttca</u> M A S
cggggacgg <u>GTGAGAAGAGGAATGTCCCACCCT</u> G D G	11 (153 bp)	CTAACCTTACCCATCTGGTCCACAG <u>gagggatga</u> R H D
gctataatg <u>GTGAGTGGGGGAGCTGCATTTGGCC</u> G Y N	12 (3,121 bp)	CCACACTCTTTCTCTCTCTTCTAG <u>atattgtg</u> D I A
gataccag <u>GTGACTATGGTTACAGCCAGCCAG</u> G Y P	13 (273 bp)	TTGACCCCTCTCCCTATGCCTATAG <u>acctgattg</u> D L I
tgtgtacag <u>GTGAGCTCTGACTAGGGGGAGGGAC</u> V Y R	14 (84 bp)	AGCGAGCCTCTCCAATCCCATCAG <u>agctcagcc</u> A Q P
agtcagctg <u>GTGAGGAGGTGGAGGTCACGGACTT</u> V S C	15 (84 bp)	CACAAACCTGCCCTCATCTTGCAG <u>cttcaacat</u> F N I
agaagctgc <u>GTGAGTGCCATGGAGTGAGGGGTTG</u> Q K L	16 (89 bp)	TGGATCCTTCCTGCTGCCATGCAG <u>atctaaagg</u> H L K
ttccttcgg <u>GTATGCTCAGGCTAGGATGGGAGGG</u> F L R	17 (107 bp)	CAGACTCTTGCCGTGCGCACGCTAG <u>gatgagggc</u> D E A
caggagcag <u>GTAGGAATAGTGGGACGAGCAAGAT</u> Q E Q	18 (310 bp)	ACACGCATCCCATTGTGTCGCCCTAG <u>gacaaggatc</u> T R I
agctactgc <u>GTGAGAGAGTCTTCACTCTACCCA</u> A T A	19 (117 bp)	CCTAAACCAAGCACTCCCCATACAG <u>gggggactc</u> G D S
aacattgag <u>GTGAGGTACCACCATGGGGCATAGC</u> N I E	20 (1,438 bp)	GCCCCACCTGTCTGTGCATCTCCAG <u>ggctttgag</u> G F E
gacaccggg <u>GTAAGGGCTCTGTGATGTAACCTTA</u> D T R	21 (149 bp)	TGACGTTGGATTTTCCCTCTTCCAG <u>atagggaatc</u> I G I
ggtcaggag <u>GTAAGTACTGAGCTGGGCAGCATGGCTGA</u> V R S	22 (260 bp)	CTTCTTTTACCTTCTCCCTCCAG <u>caagaacag</u> K N S
gcttcgagg <u>GTGAGAGACCAAGCATGGGACGGGG</u> L R G	23 (212 bp)	TCTCTGGGACTTGGACACTTGCAG <u>gaattcctt</u> N S F
acctatgag <u>GTAGGGAGGAGCCTCTGGGTAAGAT</u> T Y E	24 (141 bp)	GCCACCCCGCTATCTCCACCCCA <u>gctccacaac</u> L H N
tctcccaag <u>GTAAGTTCTGGGAGAGAGAAGGAG</u> S P K	25 (100 bp)	TTGATTGTGCTCTGTCTCCCAAG <u>gtggaactgg</u> V D W
gttctggtg <u>GTGAGAAGGCTCAGCGGGCTCGAGC</u> V L V	26 (630 bp)	CTTACCACACATCCATCCCCCTCAG <u>agctgagac</u> S C D
ctccgcccag <u>GTGGGGCTAGACCCGGATGGGCGGG</u> L R Q	27 (229 bp)	CGCCTGTACACCCCGCCCATACAG <u>agggccgacg</u> R P Q
caagctcgg <u>GTAAGAGACCCTGGTTCTCTGCTG</u> Q A R	28 (218 bp)	GAAGTGACATCTAGTTTCCCTCAG <u>gtgagacaca</u> V Q T
atgtggaag <u>GTGAGGCTGAAAGGGAGACACAAAC</u> M W K	29 (2,050 bp)	AGCTCCTGTGCCCTTCCCACTCCAG <u>gctggcttc</u> A G F
Gaagag <u>tga</u> CAGCAGAGGGGGCGGGTTCCTGGT E E ter	(((103 bp)))	ACC <u>TAATAAA</u> GAGCTTGACAGTGAT poly A signal

Genomic organization at the exon/intron borders are shown beginning with exon 1's splice donor region. 9 bp of the exons and 25 bp of the introns are shown at the splicing junctions. Total intron size is indicated. Splice donors and acceptors are underlined. The "GC" instead of "GT" splice donors for exons 5 and 8 are double-underlined. The termination codon and polyadenylation signal in exon 30 are boxed, and the left out sequence between the sequences shown is indicated.

but the region surrounding the start of the mature protein is well conserved (see arrow in Fig 1). The least-conserved region is near the cleavage site into the light and heavy chain. Although the cleavage site itself is conserved (underlined "RR" in Fig 1), homology with α IIb and α IIb is only 50% and 60%, respectively. In contrast, the transmembrane and cytoplasmic domains are highly conserved.

Studies of natural-occurring and directed mutations have suggested that the 3 upper surface loops of the N-terminal β -propeller of α IIb may be involved in ligand binding. Our cross-species comparison shows that there is 100% conservation at the middle of these 3 loops (grayed area for the "W3 loop" in Fig 1). However, there is poor conservation in the other 2 loops ("W2/3 loop" and "W3/4 loop" in Fig 1). We would have expected a loop involved in ligand binding to be highly preserved. One possible explanation for this divergence could be that these differences in receptor loop structure help to accommodate for species differences in the primary structure of the fibrinogen ligand itself. Supporting this, it has been shown that the α IIb/ β 3 receptor of different species have different sensitivities to RGD peptide inhibition of fibrinogen binding so that rodent and murine platelets are much more resistant to RGD peptide inhibition than human platelets.¹² Whether this difference in species RGD sensitivity is caused by the rapid evolution of sequences in the W2/3 and W3/4 loops remains to be investigated.

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