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STRUCTURE OF THE HUMAN BLOOD PLATELET MEMBRANE GLYCOPROTEIN Ibα GENE

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Summary: The gene for human platelet glycoprotein Ib α-chain has been cloned from a genomic cosmid library using a partial cDNA clone as probe. 3530 bp were sequenced including the entire transcribed part, as well as additional 5' and 3' regions. A single intron was found 6 bp upstream of the ATG initiation codon. An exceptionally long exon was identical to the recently published cDNA sequence (1). The 5' upstream promoter region is atypical for eukaryotic genes with only a weak homology to the characteristic promoter consensus sequences. The 3' region contains two repetitive Alu elements, belonging to distinct subfamilies, connected by an oligo(dA) linker.

Human blood platelet glycoprotein (GP) Ib is the receptor on unactivated platelets for von Willebrand factor (vWf), a large multimeric plasma component. As such it plays a critical role in adhesion of platelets to the subendothelium of damaged blood vessels in the primary stages of haemostasis (reviewed in refs. 2 and 3). GPIb consists of two subunits, α and β, with masses of 150 kDa and 25 kDa, respectively, joined by disulphide bonds (4). cDNA coding for both these subunits has been cloned and sequenced (1,5) and both contained leucine-rich repeats. In platelet membranes GPIb exists as a 1:1 complex with another glycoprotein, GPIX (6). The α subunit of GPIb contains a

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Abbreviations: GP: glycoprotein; vWf: von Willebrand factor; bp: base pair; kb: kilobase pair.

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thrombin binding site which is involved in the kinetics of platelet activation by thrombin (7). Several bleeding disorders are known in which there is an inherited deficiency or defect in GPIb. These include Bernard-Soulier syndrome (8,9), pseudo von Willebrand disease (10) and Bolin-Jamieson syndrome (11). In Bernard-Soulier syndrome both subunits of GPIb, GPIX and a further glycoprotein, GPV, are all missing or affected (12), suggesting an association between the genetic control or cellular expression of these components. As a step towards understanding the factors controlling expression of the GPIb complex in the megakaryocyte and in platelets we have isolated and sequenced the gene for GPIbα, which contains several unusual features.

MATERIALS AND METHODS

Preparation of a cDNA probe. Human platelet mRNA was isolated and used for the construction of a λgt11 expression library as described (13,14). Polyclonal antibodies against GPIbα were used for screening this library. A partial cDNA clone for GPIbα, identical to nucleotide sequence 661 to 1444 of the full length cDNA (1), was isolated and sequenced (13). The probe was labelled with [α^{32}P]dCTP by the random primed labelling method (Amersham).

Screening of genomic library. This cDNA probe was used for screening a pCV105 cosmid library (15,16). Colonies that hybridized positively were rescreened and the cosmid DNA isolated by the alkaline extraction procedure (17). DNA (25 μg) was digested with EcoRI (Boehringer Mannheim), separated by 0.8% agarose gel electrophoresis and blotted onto nitrocellulose membranes (BA 85, Schleicher & Schuell). The EcoRI fragment that hybridized with the cDNA probe on Southern blots, was isolated from agarose gels by electroelution in a membrane trap (Bio-Trap, Schleicher & Schuell), phenol extracted and precipitated with ethanol.

Nucleotide sequencing. This fragment was subcloned into M13 Bluescript (+) and (-) (Stratagene) by ligation to the linearized plasmid with T4 ligase (New England Bio-Labs) and transformation into E. coli strains JM 101, JM 107 and JM 109 by the $CaCl_2$ method (18). Single stranded DNA templates were prepared by superinfection with M13K07 helper phage. Alternatively, double stranded templates were used as described (19). The DNA sequence was determined by the dideoxy-chain-termination method (20) using [α^{35}S]dATP (New England Nuclear) and either the modified T7 DNA polymerase (Sequenase, United States Biochemicals) or the unmodified form (Pharmacia). The sequencing strategy involved synthetic primers and restriction mapping. Universal M13 and reversed M13 primer were purchased from Pharmacia, 18 to 24 bp oligodeoxynucleotides were prepared on a Applied Biosystems 381A DNA synthesizer and purified by gel filtration on NAP-10 columns (Pharmacia). Restriction enzymes were from Boehringer-Mannheim and New England Bio-Labs. Restriction fragments were isolated and subcloned for sequencing

as above. Sequences were assembled and analysed using the MicroGenie program (Beckmann).

RESULTS AND DISCUSSION

A pCV105 cosmid library (15,16) was screened (200,000 colonies) with a 783 bp partial cDNA probe (Fig. 1A) encoding human platelet GPIbα. Fifteen colonies gave a positive signal with this probe and secondary screenings identified 4 clones containing a 6.5 kb EcoRI fragment which hybridized to the same probe on Southern blots. Since genomic Southern blotting also revealed an EcoRI fragment of 6.5 kb (21), one of them (N10) was chosen for sequencing. 3530 bp were sequenced (Fig. 1B) including 261 bp of the 5' non-transcribed region, a single, 233 bp long intron 6 bp upstream of the ATG initiation codon and 646 bp downstream of the polyadenylation site at the 3' end (Fig. 2).

The 5' untranscribed region of the GPIbα gene has some unusual features. No typical TATA or CAAT consensus sequences, which play an important role in promotion of transcription in many eukaryotic genes (22), were found. However, there are two uncharacteristic sequences at relative positions where TATA and

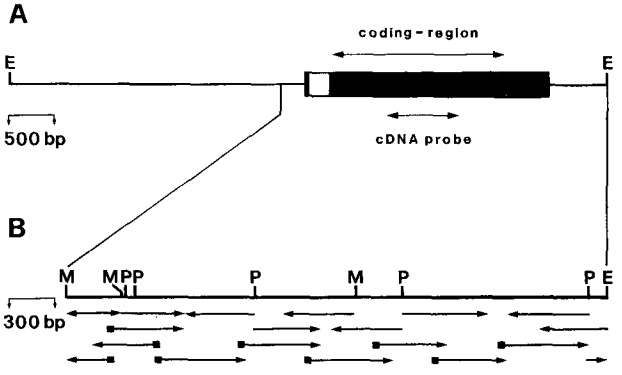

Figure 1: Cloning and sequencing strategy of the GPIbα gene.

Fig. 1A: The line represents the 6,5 kb EcoRI fragment of the GPIbα gene. 3530 bp were sequenced, including the transcribed part (filled bar) and a single intron (open bar). The lengths of the coding region and the cDNA probe used for screening the genomic library are indicated.

Fig. 1B: Restriction map and sequencing strategy. The extent and direction of sequencing are indicated by arrows. Solid boxes associated with the arrows indicate that the sequences were determined by using synthetic primers. The restriction sites are: E: EcoRI; M: MseI; P: PstI.

A

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5'- TTAAAAGATGGCAGAAGGCTGTTTGGAGGAGTCCACCCCCATCTCCCCTGTGTAAA 56
AGGAAAGCGGAAGAGAGAACCACAAAGAGGGCCTGGGGGAAAGCCGTGGAGTGAGGCGAT 116
AAGGGCTTGTGTCCAGGGGATTCCCGGTCACTGGAATCCCTATCAGGCCTGCATTTCCTC 176
CTCACCCCCATCCCCTTCCTTGCCACTGGCTTAGTCCTCCATGGGGCTAGAAGAGAGAAG 236
GACGGAGTCGAGTGGCACCCTAGAAGACGCTCTGTGCCTTCGGAGGTCTTTCTGCCTGCC 296
Tgtaagccggggttggtgctggggcaggagaggggtctgagggaggggaaagagccaagg 356
acctggagctagtagttttaagttctgcaggcaagggtgggagatgggagtagggaggac 416
aggaggtgtggatgctgtttctggaagcgaagctgcagggggaaggggctggggcctgg 476
ggggatgcttccaggggatgcaggggatccactcaaggctcccttgcccacagGTCCTC 536
 M  P  L  L  L  L  L  L  L  L  P  S  P  L  H  P  H  P  I  C  20
ATGCCTCTCCTCCTCTTGCTGCTCCTGCTGCCAAGCCCCTTACACCCCCACCCCATCTGT 596
 E  V  S  K  V  A  S  H  L  E  V  N  C  D  K  R  N  L  T  A  40
GAGGTCTCCAAAGTGGCCAGCCACCTAGAAGTGAACTGTGACAAGAGGAATCTGACAGCG 656
 L  P  P  D  L  P  K  D  T  T  I  L  H  L  S  E  N  L  L  Y  60
CTGCCTCCAGACCTGCCGAAAGACACAACCATCCTCCACCTGAGTGAGAACCTCCTGTAC 716
 T  F  S  L  A  T  L  M  P  Y  T  R  L  T  Q  L  N  L  D  R  80
ACCTTCTCCCTGGCAACCCTGATGCCTTACACTCGCCTCACTCAGCTGAACCTAGATAGG 776
 C  E  L  T  K  L  Q  V  D  G  T  L  P  V  L  G  T  L  D  L  100
TGCGAGCTCACCAAGCTCCAGGTCGATGGGACGCTGCCAGTGCTGGGGACCCTGGATCTA 836
 S  H  N  Q  L  Q  S  L  P  L  L  G  Q  T  L  P  A  L  T  V  120
TCCCACAATCAGCTGCAAAGCCTGCCCTTGCTAGGGCAGACACTGCCTGCTCTCACCGTC 896
 L  D  V  S  F  N  R  L  T  S  L  P  L  G  A  L  R  G  L  G  140
CTGGACGTCTCCTTCAACCGGCTGACCTCGCTGCCTCTTGGTGCCCTGCGTGGTCTTGGC 956
 E  L  Q  E  L  Y  L  K  G  N  E  L  K  T  L  P  P  G  L  L  160
GAACTCCAAGAGCTCTACCTGAAAGGCAATGAGCTGAAGACCCTGCCCCCAGGGCTCCTG 1016
 T  P  T  P  K  L  E  K  L  S  L  A  N  N  N  L  T  E  L  P  180
ACGCCCACACCCAAGCTGGAGAAGCTCAGTCTGGCTAACAACAACTTGACTGAGCTCCCC 1076
 A  G  L  L  N  G  L  E  N  L  D  T  L  L  L  Q  E  N  S  L  200
GCTGGGCTCCTGAATGGGCTGGAGAATCTCGACACCCTTCTCCTCCAAGAGAACTCGCTG 1136
 Y  T  I  P  K  G  F  F  G  S  H  L  L  P  F  A  F  L  H  G  220
TATACAATACCAAAGGGCTTTTTTGGGTCCCACCTCCTGCCTTTTGCTTTTCTCCACGGG 1196
 N  P  W  L  C  N  C  E  I  L  Y  F  R  R  W  L  Q  D  N  A  240
AACCCCTGGTTATGCAACTGTGAGATCCTCTATTTTCGTCGCTGGCTGCAGGACAATGCT 1256
 E  N  V  Y  V  W  K  Q  G  V  D  V  K  A  M  T  S  N  V  A  260
GAAAATGTCTACGTATGGAAGCAAGGTGTGGACGTCAAGGCCATGACCTCTAACGTGGCC 1316
 S  V  Q  C  D  N  S  D  K  F  P  V  Y  K  Y  P  G  K  G  C  280
AGTGTGCAGTGTGACAATTCAGACAAGTTTCCCGTCTACAAATACCCAGGAAAGGGGTGC 1376
 P  T  L  G  D  E  G  D  T  D  L  Y  D  Y  Y  P  E  E  D  T  300
CCCACCCTTGGTGATGAAGGTGACACAGACCTATATGATTACTACCCAGAAGAGGACACT 1436
 E  G  D  K  V  R  A  T  R  T  V  V  K  F  P  T  K  A  H  T  320
GAGGGCGATAAGGTGCGTGCCACAAGGACTGTGGTCAAGTTCCCCACCAAAGCCCATACA 1496
 T  P  W  G  L  F  Y  S  W  S  T  A  S  L  D  S  Q  M  P  S  340
ACCCCCTGGGGTCTATTCTACTCATGGTCCACTGCTTCTCTAGACAGCCAAATGCCCTCC 1556
 S  L  H  P  T  Q  E  S  T  K  E  Q  T  T  F  P  P  R  W  T  360
TCCTTGCATCCAACACAAGAATCCACTAAGGAGCAGACCACATTCCCACCTAGATGGACC 1616
 P  N  F  T  L  H  M  E  S  I  T  F  S  K  T  P  K  S  T  T  380
CCAAATTTCACACTTCACATGGAATCCATCACATTCTCCAAAACTCCAAAATCCACTACT 1676
 E  P  T  P  S  P  T  T  S  E  P  V  P  E  P  A  P  N  M  T  400
GAACCAACCCCAAGCCCGACCACCTCAGAGCCCGTCCCGGAGCCCGCCCCAAACATGACC 1736
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Figure 2: Sequence of the GPIbα gene.

5' region: The intron is indicated by lower case letters. The stretch with a certain homology to the CAAT consensus is double underlined and the direct repeated stretches with weak homology to the TATA consensus are single underlined. The beginning of the cloned full length cDNA (1) is represented by an arrow.

3' region: The polyadenylation signal is double underlined and the polyadenylation site is marked with a triangle. The two Alu repetitive elements are underlined.

CAAT boxes have usually been found. These are the GGCCTGCAT and the repeated CTAGAAGA stretch starting at position -100, -39 and -6 respectively, relative to the estimated beginning of the cDNA

B

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 T  L  E  P  T  P  S  P  T  T  P  E  P  T  S  E  P  A  P  S  420
ACCCTGGAGCCCACTCCAAGCCCGACCACCCCAGAGCCCACCTCAGAGCCCGCCCCCAGC 1796
 P  T  T  P  E  P  T  P  I  P  T  I  A  T  S  P  T  I  L  V  440
CCGACCACCCCGGAGCCCACCCCAATCCCGACCATCGCCACAAGCCCGACCATCCTGGTG 1856
 S  A  T  S  L  I  T  P  K  S  T  F  L  T  T  T  K  P  V  S  460
TCTGCCACAAGCCTGATCACTCCAAAAAGCACATTTTTAACTACCACAAAACCCGTATCA 1916
 L  L  E  S  T  K  K  T  I  P  E  L  D  Q  P  P  K  L  R  G  480
CTCTTAGAATCCACCAAAAAAACCATCCCTGAACTTGATCAGCCACCAAAGCTCCGTGGG 1976
 V  L  Q  G  H  L  E  S  S  R  N  D  P  F  L  H  P  D  F  C  500
GTGCTCCAAGGGCATTTGGAGAGCTCCAGAAATGACCCTTTTCTCCACCCCGACTTTTGC 2036
 C  L  L  P  L  G  F  Y  V  L  G  L  F  W  L  L  F  A  S  V  520
TGCCTCCTCCCCCTGGGCTTCTATGTCTTGGGTCTCTTCTGGCTGCTCTTTGCCTCTGTG 2096
 V  L  I  L  L  L  S  W  V  G  H  V  K  P  Q  A  L  D  S  G  540
GTCCTCATCCTGCTGCTGAGCTGGGTTGGGCATGTGAAACCACAGGCCCTGGACTCTGGC 2156
 Q  G  A  A  L  T  T  A  T  Q  T  T  H  L  E  L  Q  R  G  R  560
CAAGGTGCTGCTCTGACCACAGCCACACAAACCACACACCTGGAGCTGCAGAGGGGACGG 2216
 Q  V  T  V  P  R  A  W  L  L  F  L  R  G  S  L  P  T  F  R  580
CAAGTGACAGTGCCCCGGGCCTGGCTGCTCTTCCTTCGAGGTTCGCTTCCCACTTTCCGC 2276
 S  S  L  F  L  W  V  R  P  N  G  R  V  G  P  L  V  A  G  R  600
TCCAGCCTCTTCCTGTGGGTACGGCCTAATGGCCGTGTGGGGCCTCTAGTGGCAGGAAGG 2336
 R  P  S  A  L  S  Q  G  R  G  Q  D  L  L  S  T  V  S  I  R  620
AGGCCCTCAGCTCTGAGTCAGGGTCGTGGTCAGGACCTGCTGAGCACAGTGAGCATTAGG 2396
 Y  S  G  H  S  L TER                                          626
TACTCTGGCCACAGCCTCTGAGGGTGGGAGGTTTGGGGACCTTGAGAGAAGAGCCTGTGG 2456
GCTCTCCTATTGGAATCTAGTTGGGGGTTGGAGGGGTAAGGAACACAGGGTGATAGGGGA 2516
GGGGTCTTAGTTCCTTTTTCTGTATCAGAAGCCCTGTCTTCACAACACAGGCACACAATT 2576
TCAGTCCCAGCCAAAGCAGAAGGGGTAATGACATGGACTTGGCGGGGGGACAAGACAAAG 2636
CTCCCGATGCTGCATGGGGCGCTGCCAGATCTCACGGTGAACCATTTTGGCAGAATACAG 2696
CATGGTTCCCACATGCATCTATGCACAGAAGAAAATCTGGAAAGTGATTTATCAGGATGT 2756
GAGCACTCGTTGTGTCTGGATGTTACAAATATGGGTGGTTTTATTTTCTTTTTCCCTGTT 2816
TAGCATTTTCTAGTTTTCCACTATTATTGTATATTATCTGTATAATAAAAAATAATTTTA 2876
GGGTTGGGAGTGATGGCTCATGCCTGTAATCCTAGCACTTTGGGAGGCCGAGGCGGGTGG 2936
AATCACCAGAGGTAGGGAGTTCAAGACCAGCCTGGCAAACATGGTGAAACCCTGGTCTCT 2996
ACTAAAAATACAAAAATTAGGCCAGGCGTGGTGGTGCACACCTATAACCCCAGCTACTCG 3056
GGAGGGTGGGGCAGGAGAATCGCTTGAACCTGGGAGGCGGAAGTTGCCGTGAGCCAAGAT 3116
CGTACCACTGAACTCCAGCCTGGGTAACAGAGTGAGACTCCGTCTAAAAAAAAAAAAAAA 3176
AAAAAAAAAAACTTCTGGCCGGGTGCAGGGGCTCATGCCTGTAATTCCAGCACTCTGGAA 3236
GGCTGAGGCGGGTGGGTTGCTTGAACCCAGGAGTTTGGCCCAGGCTTGGCAACATGGCAA 3296
AACCCGACCTCTACAAAAAATACAAAACATTAGCCAGGTGTGGTGGCATGCACCTGTGGT 3356
CCCAGGTACCCGGGTGGCTGAGGAGGGAGGATCACCTGAGCCTGGGAGATGGAGGCTGCA 3416
GTGAGCCCTGAAGGTGCCACTGTACTCCAGCCTGGGTGACAGAGTGAGAGCCTGTCTCAA 3476
AACAACTTGGCTTCTTTTGGTGAAGAGTGGCTGGGGCACCTGTCATGAGAATTC - 3' 3530
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Figure 2 - Continued.

(1) (Fig. 2). Further investigations will show whether the homology to the $TATA^{A}/_{T}A^{A}/_{T}$ and $GG^{T}/_{C}CAATCA$ consensus sequences (22) is mere chance or if there exists a new family of promoter sequences controlling the expression of platelet GPIb complex proteins.

The exon-intron structure of the GPIbα gene is also rather uncommon for eukaryotic genes. The whole precursor of GPIbα (626 amino acids) is expressed by a single, exceptionally large exon of 2,4 kb. No difference in the nucleotide sequences was found between our genomic clone and the published cDNA (1). The gene

contains a single intron of 233 bp, 6 bp upstream from the ATG translation initiation codon in the 5' non-translated region. This intron has an unusually high content of guanosine (46% of total). A computer-assisted search of the GeneBank (DNAstar, release 55) revealed no significant homology to any other known sequence. The exon-intron splice junctions are in good agreement with the consensus sequences determined for correct splicing of the precursor mRNA (23). Interestingly, von Willebrand factor, for which GPIbα is the receptor on platelets, also contains an intron just before the initiation site (24,25). Whether this is important for cell specific expression of GPIbα, as has been suggested for vWf, remains to be shown.

The 3' region of the GPIbα gene contains two Alu repeats (Fig. 2). An alignement of the first of these (293 bp) with the published Alu-S consensus sequence (26,27) revealed 88% homology. The second (291 bp) is 83% homologous to the Alu-J subfamily, matching 11 of 15 diagnostic identification points determined for this subfamily, which is more similar to the 7SL DNA (28) in these positions (27). In contrast the Alu-S element did not match in any of these positions. These two repetitive Alu elements are connected by a 26 bp oligo(dA) linker, an element that is characteristic for Alu repeats. It should be noted that the first Alu consensus already starts 6 bp upstream from the polyadenylation site of the determined cDNA (Fig. 2), excluding the possibility of further mRNA processing signals downstream of the first polyadenylation signal.

While the origin of the genetic defect in Bernard-Soulier syndrome, which affects not only GPIbα, but also GPIbβ, GPV and GPIX, is not yet established the information supplied here on the structure of the GPIbα gene is a necessary step on the way to understanding how the biosynthesis of the GPIb complex is regulated. The sequences of the genes for the other members of the complex will be required to determine whether there are common regulatory elements or whether concerted expression is a post transcriptional event as has been postulated for the GP IIb/IIIa complex (29).

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