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Physical linkage of the genes for platelet membrane glycoproteins IIb and IIIa
(subchromosomal localization/pulsed-field electrophoresis/fibrinogen receptor/integrins)

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ABSTRACT The fibrinogen receptor on human platelets is a prototypic member of the integrin family and is composed of subunit glycoproteins IIb (gpIIb) and IIIa (gpIIIa) in a 1:1 stoichiometric ratio. We have isolated cDNA clones for gpIIb and gpIIIa and localized both genes to chromosome 17. In the current study, several approaches were used to localize and map the genes for gpIIb and gpIIIa. A preliminary evaluation of subchromosomal localization was performed by using a panel of mouse–human somatic cell hybrids that contain different amounts of the long arm of human chromosome 17. Southern hybridization to the DNA of these hybrids shows that both genes map near the thymidine kinase gene. In situ hybridization to intact human chromosomes localized both genes to the 17q21-22 region. To better define the physical distance between the two genes, we examined the genomic hybridization pattern of each cDNA probe to high molecular weight restriction fragments separated by pulsed-field gel electrophoresis. Serial hybridizations of the same filter have allowed construction of long-range Mlu I and Sfi I restriction maps spanning more than 500 kilobases. Finally, nonoverlapping portions of the cDNAs for both gpIIb and gpIIIa were used to probe Sfi I digests of genomic DNA separated by field-inversion gels. This confirmed that the genes are physically linked within the same 260-kilobase Sfi I fragment and suggests that the gene for gpIIb is located on the 3' side of the gene for gpIIIa. These results suggest that coordinate expression of gpIIb and gpIIIa may depend on physical proximity.

Regulated expression of heteropolymeric proteins requires a mechanism for coordinating the synthesis of individual subunits. Subunit genes that have evolved from duplication of a common ancestor may contain conserved cis-acting control elements that respond to alterations in a single diffusible factor (1–4). Coordinate expression of nonhomologous subunits must be achieved by a different mechanism.

The platelet fibrinogen receptor is a heterodimer of glycoproteins IIb (gpIIb) and IIIa (gpIIIa) (5, 6) that correspond to the α and β subunits in the integrin superfamily of membrane-adhesion receptors (7). In vitro translation of RNA from human erythroblasts (HEL) cells (8) and isolation of individual cDNA clones for gpIIb and gpIIIa (9, 10) have shown that gpIIb and gpIIIa are transcribed from separate mRNAs. Analysis of these cDNA clones illustrates a general rule for integrins—although there is a high degree of sequence conservation between different α subunits and different β subunits, there is little or no similarity between the α and β groups. Nonetheless, the 1:1 stoichiometry of gpIIb and gpIIIa in normal platelets (5, 6) and the similar reduction in concentration of both subunits in the hereditary bleeding disorder Glanzmann thrombasthenia (11–15) imply that there are cellular mechanisms for coordinate expression of gpIIb and gpIIIa. Northern analysis of RNA gel blots reveals similar levels of gpIIb and gpIIIa mRNA in HEL cells (16), suggesting possible coordinate expression at the level of transcription.

Few of the genes for integrin subunits have been mapped, and none have been found to be linked (18, 31). During previous studies of gpIIb and gpIIIa gene structure, we discovered that both genes were located on human chromosome 17 (16, 19). By using a combination of physical and somatic cell genetic techniques, we now report the subchromosomal localization and orientation of the gpIIb and gpIIIa genes. Both are contained on a 260-kilobase segment that maps to the 17q21-22 region. This close physical linkage is unlikely to be coincidental and suggests that regulated expression may be based on physical proximity.

MATERIALS AND METHODS
cDNA Probes. A partial-length gpIIb cDNA clone (16) was used to obtain a 3.3-kb full-length clone for gpIIb. This was sequenced and is identical to that described by Poncz et al. (9). The gpIIIa cDNA is 3.3 kb long and contains the entire coding region except for the 5' most 30 base pairs (19). Both gpIIb and gpIIIa cDNA clones were obtained from HEL-cell cDNA libraries and were subcloned into pBS (Strategene Cloning Systems) and pBl13 (International Biotechnologies, respectively). Purified cDNA inserts were prepared by standard techniques (20). Probes used include the 1.3-kb gpIIb fragment on the 5' side of the internal Acc I site, the 2.0-kb gpIIb fragment on the 3' side of the internal Acc I site, the 2.2-kb gpIIIa fragment on the 5' side of the internal EcoRI site, and the 1.5-kb gpIIIa fragment on the 3' side of the internal EcoRI site.

Human–Mouse Hybrid DNA. The parent hybrid line WL contains the long arm of human chromosome 17 fused to a mouse chromosome. This line and the daughter lines 12B, 12A, 12D, and 12G have been characterized (21).

Southern Blotting and Hybridization. Genomic DNA was digested with restriction endonucleases, separated by electrophoresis through 0.8% agarose gels, and transferred to a nylon membrane by standard methods (16). Hybridizations were performed in a solution of 2× SSC (300 mM NaCl/30 mM sodium citrate, pH 7.0), 1% NaDodSO4, salmon sperm DNA at 100 µg/ml, and 10% (wt/vol) dextran sulfate with 32P-labeled probe at 2 × 106 cpm/ml at 65°C. Filters were washed in 2× SSC/1% NaDodSO4 at 65°C for 60 min with

Abbreviations: gpIIb, glycoprotein IIb; gpIIIa, glycoprotein IIIa; HEL, human erythroblasts.
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two changes. Filters to be rehybridized were "stripped" with 0.1 x SSC/1% NaDodSO4/50% (vol/vol) formamide at 65°C for 30 min and exposed to film for 4 days to ensure complete removal of the first probe.

**In Situ Hybridization.** Plasmids containing 2.2 kb of gpIIla cDNA and 3.3 kb of gpIIb cDNA were radiolabeled by nick-translation (22) with 3[H]dCTP plus 3[H]dTTTP to specific activities of $\approx 3.7 \times 10^8$ and $\approx 3.0 \times 10^8$ dpm/µg of DNA, respectively. In situ hybridization to human metaphase chromosomes was performed as described (22, 23).

**Pulsed-Field Gel Electrophoresis.** DNA from HEL cells was prepared in agarose plugs at a concentration of 10³ cells per ml (24). A 50-µl plug was incubated with 20 units of the indicated restriction enzyme in a total volume of 100 µl and the reaction was terminated after 8 hr by the addition of 1 ml of 50 mM Tris-HCl, pH 8.0/50 mM EDTA. Electrophoresis was performed at 13°C at 330 V (10 V/cm) in 0.5 x TBE buffer (45 mM Tris base/45 mM boric acid/1.25 mM EDTA, pH 8.3) by using a commercial apparatus (LKB Pulsaphor). Total electrophoresis time was 40 hr with a switching interval of 100 sec.

**Field-Inversion Gel Electrophoresis.** DNA from HEL cells was prepared as above and digested with Sfi I. Electrophoresis was as described (25) with the following modifications. Field strength was 240 V (7 V/cm) in 0.5 x TBE buffer containing ethidium bromide at 0.5 µg/ml, with recirculation of buffer through a heat exchanger to maintain the gel temperature at 13°C. The electric field was periodically inverted by computer-controlled relays according to a program that increased the forward interval exponentially from 10 to 60 sec during a 4-hr cycle, such that 40% through the cycle, half of the increase in forward switching time had been achieved. The reverse interval was maintained at one-third of the forward interval, and the field was interrupted between each inversion by a period equal to 2% of the forward interval. The total electrophoresis time was 16 hr.

**RESULTS**

**Somatic Cell Hybrid Analysis.** A preliminary evaluation of subchromosomal localization was performed by using a panel of mouse–human somatic cell hybrids that contain different amounts of the long arm of human chromosome 17 (21). WL, the parent hybrid line, contains all of human chromosome 17q; lines 12B and 12A carry the human thymidine kinase, galactokinase, and procollagen αI(1) genes; 12D carries the thymidine kinase and galactokinase genes; and 12G carries only the thymidine kinase gene (Fig. 1A). All these genes have been mapped centromere-proximal to 17q22 (21).

DNA from each cell line was digested with EcoRI, fractionated on a 0.7% agarose gel, and transferred to a nylon filter. Hybridization with a gpIIla cDNA probe revealed 11.0-kb, 7.8-kb, and 3.8-kb fragments in all the cell lines (Fig. 1B, lanes 1-5). Comparison with the pattern generated by total mouse and human genomic DNA (Fig. 1B, lanes 6 and 7) showed that the 11.0-kb and 7.8-kb fragments were of mouse origin and the 3.8-kb fragment was of human origin. The same blot, washed and then hybridized with a gpIIb cDNA probe, detected a prominent 4.5-kb fragment in all cell lines as well as a less intense 6.4-kb fragment; comparison with the control lanes showed the 6.4-kb fragment originated from the mouse and the 4.5-kb fragment was from the human gene (Fig. 1C). These results demonstrate that the gpIIla and gpIIb genes are located in the same region of chromosome 17q21-22, centromere-proximal to the galactokinase and procollagen αI(1) genes.

**In Situ Hybridization of Human Chromosomes.** To exclude the possibility of cosegregation of the gpIIla and gpIIb genes in the hybrid cell lines might be due to retention of a small, unrecognized fragment of human DNA, we performed direct in situ hybridization to intact human chromosomes. By using a gpIIla cDNA probe, 203 metaphase cells were counted and scored. Seventeen percent had silver grains on the long arm of chromosome 17 (Fig. 2A). Although the subchromosomal distribution was more diffuse than with the gpIIb probe, the majority of grains were found over 17q21-3q22. The results of a similar experiment performed with a cDNA probe for gpIIb are shown in Fig. 2B. Of 114 metaphase cells counted and scored, 29% had grains on chromosome 17q with the highest density over 17q21.3. These results confirm the somatic cell hybrid mapping data and identify locations for the gpIIla and gpIIb genes that are virtually indistinguishable.

**Pulsed-Field Gel Electrophoresis.** The limits of resolution by cytogenetic mapping are one to two orders of magnitude greater than the distances usually considered as significant in terms of physical linkage. To directly address this issue, we examined the genomic hybridization pattern of each cDNA probe to high molecular weight restriction fragments separated by orthogonal-field gel electrophoresis (Fig. 3). Careful alignment of autoradiograms produced from serial hybridizations of the same filter revealed that the gpIIla (Fig. 3A) and gpIIb (Fig. 3B) probes detected several identical fragments produced by Mlu I (470 kb and 530 kb), Nar 1 (280 kb), and Sfi I (260 kb). The Sfi I digestion contained the smallest overlapping fragment, placing a maximum distance of 260 kb between coding sequences for the gpIIla and gpIIb genes.

**Field-Inversion Gel Electrophoresis and Construction of a Long-Range Restriction Map.** Apparent identity of the fragments detected by each probe was confirmed by performing the experiment several times under different fractionation conditions and with DNA from different sources (foreskin fibroblasts, HeLa cells, and HEL cells). Both probes hybridized to multiple fragments in the 1000- to 5000-kb range, detecting apparently identical high molecular weight fragments produced by the enzymes BstHII, Mlu I, and Sfi I (data not shown). Field-inversion gel electrophoresis allowed the best resolution of the HEL cell DNA fragments resulting
from Sfi I digestion. By using nonoverlapping portions of both gpIIa and gpIIb cDNAs as probes, Southern analysis revealed a common 260-kb fragment (Fig. 4A, lanes 1-4). However, only the 5' gpIIa probe hybridized to an additional 125-kb fragment (Fig. 4A, lane 1). These results confirm the overlapping Sfi I fragment seen in the previous experiment, suggest that the gene for gpIIb is oriented on the 3' side of the gene for gpIIa, and permit construction of a long-range Sfi I map (Fig. 4C).

The largest fragments seen in Fig. 3 are likely to be partial cleavage products, perhaps due to a variable degree of secondary DNA modification (27), because additional experiments with portions of each cDNA as hybridization probes detect the same fragments as are detected by the full-length cDNAs (data not shown). Had complete digestion occurred, the shorter cDNA probes would have only detected subsets of these fragments. Comparison of the Mlu I pattern produced by each probe (Fig. 4B) allowed construction of a long-range restriction map based on these partial digests (Fig. 4C). Probes for both cDNAs identified overlapping 470-kb and 530-kb Mlu I fragments. The difference in length of these fragments, 60 kb, corresponds to the difference in length between the two smaller fragments identified by gpIIa (180 kb and 240 kb). The small fragments detected only by the gpIIb probe (290 kb, 320 kb, and 390 kb) must extend in the opposite direction.

**DISCUSSION**

Pulsed-field gel electrophoresis has been used to establish linkage relationships for several mammalian genes (28-30). Because such conclusions are based upon coincidental detection of seemingly identical restriction fragments with unrelated probes, the possibility that each probe produces similar hybridization patterns by chance must always be considered. Factors that suggest physical linkage of two
unrelated probes are the previous localization of the two probes to a small subchromosomal region, the presence of overlapping bands produced by more than one enzyme or enzyme combination, and similar banding patterns seen with partial enzyme digests. In cases where the data allow construction of a long-range restriction map, there should be no inconsistencies between maps generated from each probe. All of these criteria are met by the present study. In particular, our somatic cell mapping and in situ hybridization data narrow the total target size for gpIIa and gpIIb genes to less than 1% of the haploid genome or approximately 30,000 kb, in which it is exceedingly unlikely that two probes would share several comigrating restriction fragments by chance. Although final confirmation of the long range Sfi I and Mlu I restriction map will depend on isolation of genomic DNA, the data presented here conclusively demonstrate physical linkage of gpIIa and gpIIb coding sequences.

The analysis of the Sfi I digests with nonoverlapping portions of each cDNA suggests that the gene for gpIIb is on the 3' side of the gene for gpIIa. An alternative interpretation of this data is that the additional 125-kb fragment identified by the 5' gpIIa probe (Fig. 4A, lane 1) represents a homologous region of the genome not adjacent to the 260-kb Sfi I fragment. Since under these experimental conditions, the gpIIa probe has not hybridized to other homologous integrin β subunits (19), such an alternative explanation would require a highly conserved sequence in the 17q21-22 region.

Sosnoski et al. (31) colocallized the genes for gpIIb and gpIIa to 17q21-23 region by using somatic cell hybrids and in situ hybridization. Our data confirm their results and narrow the distance between the two genes to a maximum of 260 kb.

Other genes that have been previously mapped to this region of chromosome 17 include the HOX2 homeobox gene cluster, the procollagen α1(I) gene, the galactokinase gene, and the ERBA oncogene (32-35). A region of conserved synteny for all of these genes exists on mouse chromosome 11 (36), baboon chromosome 16 (37), and African green monkey chromosome 19 (37); and it would not be surprising if subunit genes for the fibrinogen receptor in these other species are also physically linked.

In the hereditary bleeding disorder Glanzmann thrombasthenia, both gpIIb and gpIIa are absent or markedly reduced and platelets from affected patients fail to bind fibrinogen and aggregate (11-15). The Glanzmann phenotype is likely to be caused by a heterogeneous group of genetic defects (38). For example, a defect in the expression of one gene may result in the inability of the other subunit to be expressed in the platelet membrane. Close physical linkage between the genes

![Fig. 3](image-url) Pulsed-field gel electrophoretic analysis of gpIIa and gpIIb genes. DNA was digested with the restriction endonucleases indicated. (A) Hybridization with two pooled gpIIa cDNA probes: a 2.2-kb probe on the 5' side of the internal EcoRI site plus a 1.5-kb probe on the 3' side of the internal EcoRI site (19). (B) Hybridization with the 3.3-kb gpIIb cDNA. Prior to the experiment, the filter used in A was stripped. Arrowheads indicate overlapping fragments. Size markers were estimated with chromosomes from the yeast strain HY-1 (26). Ethidium bromide stains of the agarose gels revealed inadequate DNA in the Not I and Pvu I digests (data not shown).

![Fig. 4](image-url) Southern hybridization analysis of large fragment DNA and long-range restriction map. (A) Field-inversion gel analysis of gpIIa and gpIIb genes. cDNA probes were as follows. Lanes: 1, a 2.2-kb gpIIa cDNA on the 5' side of the internal EcoRI site; 2, a 1.5-kb gpIIa cDNA on the 3' side of the internal EcoRI site; 3, a 1.3-kb gpIIb cDNA on the 5' side of the internal Acc I site; 4, a 2.0-kb gpIIb cDNA on the 3' side of the internal Acc I site. The same filter was hybridized with all four probes. Size markers are as described in Fig. 3. (B) The same Mlu I digests as in Fig. 3 A and B. (C) Long-range restriction map based on the Sfi I and Mlu I digests. The horizontal arrows indicate that the 5' and 3' extents of gpIIa and gpIIb are not certain. The Sfi I map demonstrates orientation of gpIIa with respect to gpIIb since only the 5' gpIIa probe identified the 125-kb band. The Mlu I map is constructed from the partial Mlu I digests seen in B and, like the Sfi I map, is aligned below the indicated extents of gpIIa and gpIIb.
for gpIIb and gpIIIa could be coincidental; but considering the coordinate expression of gpIIb and gpIIIa in both normal and Glanzmann platelets, it seems other explanations must be entertained. Any regulatory mechanism for expression that depends on physical proximity could serve as a selective pressure to establish and maintain this linkage. A cis-acting signal that mediates coordinate expression is an obvious candidate for such a selective pressure, perhaps in the form of a tissue-specific enhancer (4) or derepression of a large chromatin domain (39). However, although 260 kb is the maximum distance between the genes for gpIIIa and gpIIb, we do not know precisely how close they are and they may be too far apart to be controlled by a single cis element.

gpIIb and gpIIIa correspond to α and β subunits in the integrin superfamily (17) that includes the vitronectin and fibronectin receptors, the chicken integrin complex, the group of leukocyte-adhesion proteins lymphocyte function-associated antigen 1 (LFA-1), Mac-1, and p150,95, the very late antigens (VLA) group of proteins on lymphocytes, and the position-specific antigens found in Drosophila imaginal disks (for reviews, see refs 7 and 38). Most integrin subfamilies combine different α subunits with a common β subunit (7), implying that cellular mechanisms exist for independent regulation of each subunit. Few of the integrin genes have been mapped (17, 18), and only gpIIIa and gpIIb have been found to be linked. It will be interesting to see whether the linkage of gpIIIa and gpIIb genes has been conserved in other vertebrates and whether other integrin subunit genes are physically linked.

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