We now report that, contrary to previous expectations, 2′,3′-monophosphates can be used to drive the coupling of two oligoadenylates, if both oligomers contain only the natural 3′,5′ internuleotide linkage. The new bond formed in the coupling reaction is roughly 95 percent 2′,5′ linkage (*10*), as found previously for the coupling of two monomer units (*6*). This joining reaction is made possible by the large relative kinetic stability of the 3′,5′ bonds, when the oligomers are bound in a right-handed helix. Under these conditions, the rate of cleavage of the 2′,5′ bond is about equal to the rate of its formation; that of a 3′,5′ bond is several hundred times slower (*11*). If it were not for this stabilization, the equilibrium calculations of Renz *et al.* (*6*) would apply to both types of bond, and the rate of degradation of the hexamer to shorter oligomers would greatly exceed the rate of coupling. Thus hexaadenylic acid with a terminal 2′,3′-phosphate (Miles Laboratories) in the presence of a poly(U) template gave 24 percent of the dodecamer and 5 percent of the octadecamer in 5 days [1*M* aqueous ethylenediamine hydrochloride (*p*H 8, 2°C); the solution was 0.025*M* in adenosine units and 0.05*M* in uridine units]. The products were determined by chromatography on diethylaminoethyl-Sephadex with a triethylammonium bicarbonate buffer, and high-pressure liquid chromatography on RPC-5 (*12*) with a gradient of 0.2 to 0.6*M* KCl (0.01*M* tris-HCl, *p*H 8.1).

Mixing curves at 260 and 280 nm show that under our conditions the hexamer forms the expected triple helix (*13*) with two poly(U) strands (measured at 2°C in a cell having a 0.025-mm pathlength); the melting temperature of the triple helix is about 70°C. If the reactants are mixed in the ratio of one U to one A, dodecamer is still formed, but more slowly (16 percent after 5 days); if the poly(U) is omitted the yield of dodecamer is about 1 percent. When the cyclic phosphate ring was opened prior to mixing it with poly(U), no dodecamer was detected. The decrease in concentration of dodecamer at later times (Fig. 1) is due to the relatively rapid cleavage of the 2′,5′ bond to reform the hexamer cyclic phosphate, and gradual hydrolysis of the cyclic phosphate terminus to give "inactive" 2′- and 3′-phosphate monoesters. The cleavage reaction of the 2′,5′ and 3′,5′ linkages has been described (*11*).

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References and Notes

1. R. Naylor and P. T. Gilham, *Biochemistry* **5**, 2722 (1966).
2. J. Sulston, R. Lohrmann, L. E. Orgel, H. T. Miles, *Proc. Natl. Acad. Sci. U.S.A.* **59**, 726 (1968). For related work on template specificity see also J. Sulston, R. Lohrmann, L. E. Orgel, H. T. Miles, *ibid.* **60**, 409 (1968); and J. Sulston, R. Lohrmann, L. E. Orgel, H. Schneider-Bernloehr, B. J. Weimann, H. T. Miles, *J. Mol. Biol.* **40**, 227 (1969).
3. S. Uesugi and P. O. P. Ts'o, *Biochemistry* **13**, 3142 (1974).
4. L. E. Orgel and R. Lohrmann, *Accounts Chem. Res.* **7**, 368 (1974).
5. For references see: R. I. Zhdanov and S. M. Zhenodarova, *Synthesis* (1975), p. 222.
6. M. Renz, R. Lohrmann, L. E. Orgel, *Biochim. Biophys. Acta* **240**, 463 (1971).
7. J. A. Gerlt, F. H. Westheimer, J. M. Sturtevant, *J. Biol. Chem.* **250**, 5059 (1975).
8. F. H. Westheimer, *Accounts Chem. Res.* **1**, 70 (1968).
9. P. M. Pitha and J. Pitha, *Nature (London) New Biol.* **240**, 78 (1972).
10. The percentage of 2′,5′ bond was estimated from the amount of ApAp remaining after exhaustive digestion of the dodecamer with ribonuclease T_2. The standard error was large, however, and the figure of 95 percent was obtained from the kinetics of breakdown of the dodecamer.
11. D. A. Usher, *Nature (London) New Biol.* **235**, 207 (1972); _____ and A. H. McHale, *Proc. Natl. Acad. Sci. U.S.A.*, in press.
12. R. L. Pearson, J. F. Weiss, A. D. Kelmers, *Biochim. Biophys. Acta* **228**, 770 (1971).
13. C. L. Stevens and G. Felsenfeld, *Biopolymers* **2**, 293 (164).
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Human Glyceraldehyde-3-Phosphate Dehydrogenase in Man-Rodent Somatic Cell Hybrids

Abstract. *The human enzyme glyceraldehyde-3-phosphate dehydrogenase (GAPDH) forms heteropolymers with the rodent enzyme in man-rodent somatic cell hybrids. A gene specifying GAPDH is syntenic with the genes specifying the glycolytic enzymes triosephosphate isomerase (TPI) and lactate dehydrogenase B (LDH-B). The synteny of GAPDH, TPI, and LDH-B is the first evidence for the syntenic association of human genes that specify enzymes of the Embden-Meyerhof glycolytic pathway.*

The selective loss of human chromosomes by somatic cell hybrids formed between cultured aneuploid rodent cells and human fibroblasts or white blood cells (*1, 2*) affords a unique opportunity to determine the organization of loci in the human genome. In this report we describe the expression of human glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (E.C. 1.2.1.12) in man-mouse and man-hamster somatic cell hybrids and the syntenic association of a gene specifying this enzyme with the gene which specifies lactate dehydrogenase B (LDH-B), an enzyme catalytically and structurally similar to GAPDH (*3*), and with the gene which specifies triosephosphate isomerase (TPI), the enzyme responsible for the reversible isomerization of glyceraldehyde-3-phosphate and dihydroxyacetone phosphate. This is the first example of synteny of human genes which determine enzymes that catalyze sequential metabolic steps.

Rodent parental cells used for the preparation of hybrids included the mouse cell RAG and the hamster cell E36, both of which are deficient in hypoxanthine-guanine phosphoribosyl transferase (*4*). Human parental cells included white blood cells from a normal male and white blood cells from two unrelated females carrying different translocations involving chromosomes X and 19. Parental cells were treated in suspension with β-propiolactone inactivated Sendai virus (*2*), and hybrid colonies were selected in Dulbecco's modified Eagle's medium supplemented with 5 percent fetal calf serum, $1 \times 10^{-4}M$ hypoxanthine, $4 \times 10^{-7}M$ aminopterin, and $1.6 \times 10^{-5}M$ thymidine (HAT medium) (*5*). For electrophoretic analysis, cells from primary hybrid clones grown in HAT medium were harvested by trypsinization 9 to 12 weeks after hybridization.

Sonicates (4×10^7 cell/ml) in 0.1*M* tris-HCl, *p*H 7.0, containing $1 \times 10^{-3}M$ β-mercaptoethanol and $1 \times 10^{-4}M$ nicotinamide adenine dinucleotide (NAD) were analyzed for GAPDH on 9 percent starch gels. The hybrid clones were also analyzed by starch gel, agarose, acrylamide, or Cellogel electrophoresis for the presence of human and rodent forms of a number of additional enzymes which included: adenosine deaminase (E.C. 3.5.4.2), adenylate kinase-1 and -2 (AK-1, AK-2) (E.C. 2.7.4.3), aspartate aminotransferase-1 (E.C. 2.6.1.1), esterase D, α-galactosidase (E.C. 3.2.1.22), β-glucuronidase (β-GCR) (E.C. 3.2.1.31), glucose-6-phosphate dehydrogenase (G6PD) (E.C. 1.1.1.49), glucose phosphate isomerase (E.C. 5.3.1.9), glutathione reductase (GR) (E.C. 1.6.4.2), hexosaminidase A and B (E.C. 3.2.1.30), isocitrate dehydrogenase-1 (IDH-1) (E.C. 1.1.1.42), lactate dehydrogenase A and B (E.C. 1.1.1.27), lysosomal acid phosphatase ACP-2 (ACP-2) (E.C. 3.1.3.2), malate oxidoreductase-1

Table 1. Expression of GAPDH, TPI, LDH-B, and PEP-B in man-rodent primary hybrid clones.

Hybrid series pattern of expression*				Clones (No.)
GAPDH	TPI	LDH-B	PEP-B	
E36/WBC (X/19 translocation W)				
+	+	+	+	8
+	+	+	−	4
−	−	−	+	2
−	−	−	−	9
RAG/WBC (X/19 translocation B)				
+	+	ND†	+	7
+	+	ND	−	1
−	−	ND	+	1
−	−	ND	−	4
RAG/WBC (normal male)				
+	+	ND	+	11
−	−	ND	+	1
−	−	ND	−	3

*The other permutations of enzyme expression were not observed in this series of hybrid clones. †The expression of LDH-B was not determined (ND) in man-mouse hybrid clones because of the similar electrophoretic mobilities of the human and mouse isozymes.

(MDH-1) (E.C. 1.1.1.37), malate oxidoreductase (decarboxylating) (ME-1) (E.C. 1.1.1.40), mannosephosphate isomerase (E.C. 5.3.1.8), purine nucleoside phosphorylase (E.C. 2.4.2.1), peptidase A and B (PEP-A, PEP-B), phosphoglucomutase-1 and -2 (PGM-1, PGM-2) (E.C. 2.7.5.1), 6-phosphogluconate dehydrogenase (PGD) (E.C. 1.1.1.43), phosphoglycerate kinase (E.C. 2.7.2.3), phosphopyruvate hydratase (PPH) (E.C. 4.2.1.11), superoxide dismutase-1 and -2 (SOD-1, SOD-2) (E.C. 1.6.4.3), and triosephosphate isomerase (E.C. 5.3.1.1) (*6, 7*).

Extracts of the mouse cell RAG and the hamster cell E36 showed one major and one minor anodal GAPDH component on starch gels (Fig. 1). Extracts of human white blood cells or fibroblasts showed one major component, which remained at the origin, and a faint component, which migrated slowly toward the anode (not seen in Fig. 1). Extracts of certain man-mouse and man-hamster hybrid clones contained, in addition to the rodent components, one to three additional components whose electrophoretic mobilities were intermediate to those of the major human and rodent GAPDH isozymes (Fig. 1). As GADPH is a tetramer composed of identical subunits (*8*) the additional GAPDH components observed in certain of the hybrid cell extracts presumably represent the heteropolymers of rodent and human subunits expected for a tetrameric structure. The intensity of the putative heteropolymers expected to contain 3 and 2 rodent subunits was greater than the isozyme with only 1 rodent subunit in this series of man-rodent hybrid clones, and no hybrid clones were observed to express the human homopolymer. Some man-mouse and man-hamster hybrid clones expressed only the rodent isozymes (Fig. 1).

For resolution of the GAPDH isozymes on starch gels, addition of NAD to the sonication and gel buffers was essential. Enzymatic staining required the presence of glyceraldehyde-3-phosphate, NAD, and arsenate in the overlay. The GAPDH isozyme pattern was not observed when gels were stained for LDH, α-glycerolphosphate dehydrogenase, or TPI.

The expression of GAPDH was compared to the expression of 28 other human enzymes in the man-hamster hybrid clones and to the expression of 25 and 20 other human enzymes in two series of man-mouse hybrid clones. The expression of GAPDH, TPI, and LDH-B was concordant in all 23 primary man-hamster hybrid clones, and the expression of GAPDH and TPI was concordant in all 28 primary man-mouse hybrid clones (Table 1). The expression of LDH-B was not analyzed in the man-mouse clones because of the similar electrophoretic mobility of the mouse and human isozymes. In this series of man-mouse and man-hamster hybrid clones, the expression of GAPDH was independent of the expression of enzymes that have been assigned to chromosomes 1, 2, 5, 6, 10, 11, 13, 14, 15, 18, 19, 20, 21, and the X chromosome (*9*) and was also independent of the expression of PGM-2, GR, β-GCR, and AK-1.

Table 2. Human chromosome assignments of loci for enzymes of the Embden-Meyerhof pathway of glycolysis.

Enzyme	Chromosome assignment (*9*)
Hexokinase-1	10
Glucosephosphate isomerase	19
Phosphofructose kinase	?
Aldolase	?
Triosephosphate isomerase	12
Glyceraldehye-3-phosphate dehydrogenase	12
Phosphoglycerate kinase	X
2,3-Diphosphoglycerate mutase	?
Phosphopyruvate hydratase-1	1
Pyruvate kinase-3	15
Lactate dehydrogenase A	11
Lactate dehydrogenase B	12

The LDH-B and TPI loci have been assigned to chromosome 12 (*10, 11*), and preliminary data has suggested that both loci may be located in the 12pter to 12q14 region (*12*). The concordant expression of GAPDH, TPI, and LDH-B in the man-hamster clones and of GAPDH and TPI in the man-mouse clones suggests that a gene specifying GAPDH can be assigned to the TPI : LDH-B syntenic group and hence to chromosome 12. The lack of discordance in the expression of the three enzymes in the man-hamster hybrid clones and of GAPDH and TPI in

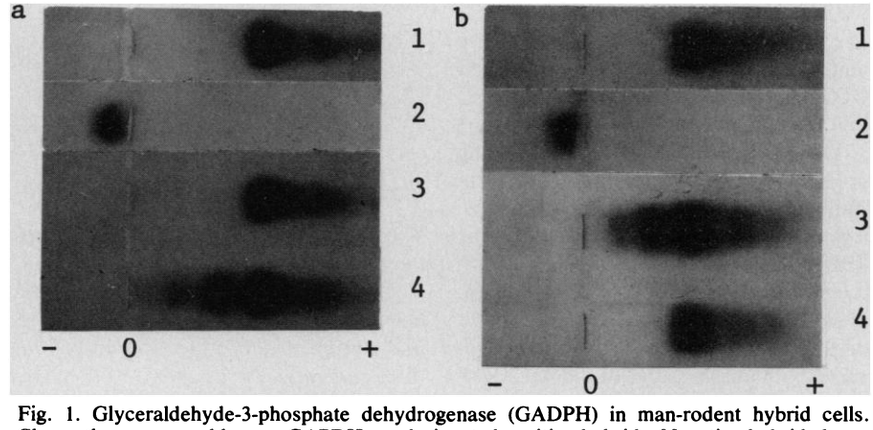

Fig. 1. Glyceraldehyde-3-phosphate dehydrogenase (GADPH) in man-rodent hybrid cells. Clones that expressed human GAPDH are designated positive hybrids. Negative hybrid clones did not express human GAPDH. (a) Man-mouse hybrids: (gel 1) RAG cells; (gel 2) human fibroblasts; (gel 3) negative clone from a hybridization of RAG with human white blood cells; (gel 4) positive clone from the same hybridization. (b) Man-hamster hybrid clones: (gel 1) E36 cells; (gel 2) human fibroblasts; (gel 3) positive clone from a hybridization of E36 with human white blood cells; (gel 4) negative clone from the same hybridization. The starch-gel buffer was 0.01*M* tris, sodium citrate, *p*H 6.5, containing $1 \times 10^{-4}M$ nicotinamide adenine dinucleotide (NAD), and the electrode buffer was 0.1*M* tris, sodium citrate, *p*H 6.5, containing $1 \times 10^{-4}M$ NAD. Electrophoresis was performed at 5 volt/cm for 20 hours at 4°C. Enzyme activity was detected in a 1 percent agar overlay containing 0.12*M* tris-HCl, *p*H 7.4, $1.5 \times 10^{-3}M$ DL-glyceraldehyde-3-phosphate, $3 \times 10^{-4}M$ NAD, $2.5 \times 10^{-2}M$ sodium pyruvate, $6 \times 10^{-3}M$ sodium arsenate, 7×10^{-4} MTT tetrazolium, and $2 \times 10^{-4}M$ phenazine methosulfate (*6*). Photographic composites were prepared from the same starch gel.

the man-mouse hybrid clones may indicate that the GAPDH, TPI, and LDH-B loci are close together.

The PEP-B locus has also been assigned to chromosome 12 (*13*) and may be located at 12q21 (*14*). The expression of PEP-B was independent of the expression of GAPDH, TPI, and LDH-B in 6 of 23 man-hamster hybrid clones (Table 1) and was independent of the expression of GAPDH and TPI in 3 of 28 man-mouse hybrid clones. The degree of discordancy in the expression of the PEP-B : LDH-B : TPI syntenic group in the man-hamster hybrid clones is three times greater than that observed in this series of hybrid clones for the PGD : PPH : AK-2 : PGM-1 syntenic group, the IDH-1 : MDH-1 pair, the ME-1 : SOD-2 pair, and the LDH-A : ACP-2 pair (*15*). If this is not due to chance, it may reflect the presence of a fragile site on a chromosome 12 from this particular white blood cell donor. The degree of discordancy in the expression of PEP-B and TPI in the man-mouse hybrids is comparable to that observed for the other known syntenic groups in these clones (*15*).

Nine loci specifying enzymes of the Embden-Meyerhof pathway of glycolysis have been assigned to particular human chromosomes (Table 2) and only three of the nine loci are syntenic (GAPDH, TPI, and LDH-B). If not due to chance, the synteny of these three loci may reflect their evolutionary relationship, which should also be evidenced by the structure of the enzymes.

Both GAPDH and LDH are tetramers whose molecular weight is 144,000 (*8, 16*). The four subunits of GAPDH are identical, as are those of the LDH-A and LDH-B homopolymers, and each subunit binds one molecule of the coenzyme NAD or NADH (*8, 16, 17*). The binding of NAD to GAPDH shows cooperativity, whereas in LDH the coenzyme binding sites do not interact (*17*). The three-dimensional structure of the coenzyme binding site of GAPDH is strikingly similar to that of LDH (*3*) and a lesser degree of structural homology is observed in the remainder of the two molecules (*3*). The structural and catalytic similarities of GAPDH and LDH may indicate that both dehydrogenases have evolved from a common ancestral gene (*18*).

TPI and GAPDH are sequential enzymes in the metabolism of glucose and 3-carbon intermediates. TPI is a dimer with a molecular weight of 52,000, is composed of identical subunits, and catalyzes the reversible isomerization of dihydroxyacetone phosphate and glyceraldehyde-3-phosphate (*19*). The TPI subunit is composed of alternating segments of α-helix and β-sheet structure (*20*), a structure which has also been observed in the dehydrogenases, in phosphoglycerate kinase, and in phosphoglycerate mutase (*3, 21*). These observations may indicate a distant evolutionary relationship of the glycolytic enzymes (*22*). The syntenic association of the genes specifying GAPDH and TPI may also be of significance for the regulation of these two glycolytic enzymes.

Further understanding of the significance of the syntenic association of the human genes specifying GAPDH, TPI, and LDH-B will require a determination of the distance between these loci and knowledge of the degree of conservation of this syntenic group in diverse species.

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References and Notes

1. M. C. Weiss and H. Green, *Proc. Natl. Acad. Sci. U.S.A.* **58**, 1140 (1967).
2. V. Miggiano, M. Nabholz, W. Bodmer, *Wistar Inst. Symp. Mongr.* **9**, 61 (1969).
3. M. G. Rossmann, M. J. Adams, M. Buehner, G. C. Ford, M. L. Hackert, P. J. Lentz, Jr., A. McPherson, Jr., R. W. Schevitz, I. E. Smiley, *Cold Spring Harbor Symp. Quant. Biol.* **36**, 179 (1971); M. Buehner, G. C. Ford, D. Moras, K. O. Olsen, M. G. Rossmann, *J. Mol. Biol.* **90**, 25 (1974).
4. R. J. Klebe, T. R. Chen, F. H. Ruddle, *J. Cell Biol.* **45**, 74 (1970); F. D. Gillin, O. J. Roufa, A. L. Beaudet, C. T. Caskey, *Genetics* **27**, 239 (1972).
5. J. W. Littlefield, *Exp. Cell Res.* **41**, 190 (1966).
6. G. S. Omenn and P. T. W. Cohen, *In Vitro* **7**, 132 (1971).
7. F. Ruddle and E. A. Nichols, *ibid.*, p. 120; P. Meera Khan, *Arch. Biochem. Biophys.* **145**, 470 (1971); C. W. Hall, M. Cantz, E. F. Neufeld, *ibid.* **155**, 32 (1973); D. A. Hopkinson, M. A. Mestriner, J. Cortner, H. Harris, *Ann. Hum. Genet.* **37**, 119 (1973); E. A. Nichols, V. M. Chapman, F. H. Ruddle, *Biochem. Genet.* **8**, 47 (1973); S. Okada and J. S. O'Brien, *Science* **165**, 698 (1969); C. B. Quick, R. A. Fisher, H. Harris, *Ann. Hum. Genet.* **35**, 445 (1972).
8. J. L. Harris and R. N. Perham, *Nature (London)* **219**, 1025 (1968).
9. J. L. Hamerton and P. J. L. Cook, in *Human Gene Mapping 2*, D. Bergsma, Ed. (National Foundation, New York, 1975), p. 3; F. H. Ruddle and E. R. Giblett, in *ibid.*, p. 13.
10. A. Jongsma, H. van Someren, A. Westerveld, A. Hagemeijer, P. Pearson, *Humangenetik* **20**, 195 (1973).
11. A. P. M. Jongsma, W. R. T. Los, A. Hagemeijer, in *Human Gene Mapping 1*, D. Bergsma, Ed. (National Foundation, New York, 1974), p. 106; R. S. Kucherlapati, R. P. Creagan, E. A. Nichols, D. S. Borgaonkar, F. H. Ruddle, in *Human Gene Mapping 2*, D. Bergsma, Ed. (National Foundation, New York, 1975), p. 194.
12. K. Mayeda, L. Weiss, R. Lindahl, M. Dully, *Am. J. Hum. Genet.* **26**, 59 (1974); A. P. M. Jongsma, A. Hagemeijer, P. Meera Khan, in *Human Gene Mapping 2*, D. Bergsma, Ed. (National Foundation, New York, 1975), p. 189.
13. T. R. Chen, F. A. McMorris, R. Creagan, F. Ricciuti, J. Tischfield, F. Ruddle, *Am. J. Hum. Genet.* **25**, 200 (1973).
14. J. L. Hamerton, T. Mohandas, P. J. McAlpine, G. R. Douglas, in *Human Gene Mapping 2*, D. Bergsma, Ed. (National Foundation, New York, 1975), p. 179.
15. G. A. P. Bruns and P. S. Gerald, *Biochem. Genet.* **14**, 1 (1976).
16. W. S. Allison, J. Admiraal, N. O. Kaplan, *J. Biol. Chem.* **244**, 4743 (1969).
17. A. Conway and D. E. Koshland, *Biochemistry* **7**, 4011 (1968); K. Kirschner, E. Gallego, I. Schuster, D. Goodall, *J. Mol. Biol.* **58**, 29 (1971); H. d'A. Heck, *J. Biol. Chem.* **244**, 4375 (1969); G. W. Schwert, B. R. Miller, R. J. Peanasky, *ibid.* **242**, 3245 (1967).
18. M. G. Rossmann, D. Moras, K. W. Olsen, *Nature (London)* **250**, 194 (1974).
19. P. H. Corran and S. G. Waley, *FEBS Lett.* **30**, 97 (1973).
20. D. W. Banner, A. C. Bloomer, G. A. Petsko, D. C. Phillips, C. I. Pogson, I. A. Wilson, P. H. Corran, A. J. Furth, J. D. Milman, R. E. Offord, J. D. Priddle, S. G. Waley, *Nature (London)* **255**, 609 (1975).
21. C. C. F. Blake and P. R. Evans, *J. Mol. Biol.* **84**, 585 (1974); T. N. Bryant, H. C. Watson, P. L. Wendell, *Nature (London)* **247**, 14 (1974); J. W. Campbell, H. C. Watson, G. I. Hodgson, *ibid.* **250**, 301 (1974).
22. G. E. Schulz and R. H. Schirmer, *Nature (London)* **250**, 142 (1974).
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Carbohydrate Deficiency of the Factor VIII/von Willebrand Factor Protein in von Willebrand's Disease Variants

Abstract. *Study of the normal human factor VIII/von Willebrand factor reveals a macromolecular glycoprotein composed of apparently identical subunits. This purified glycoprotein has procoagulant, antigen, and von Willebrand factor activities. In three patients with a variant of the von Willebrand's disease syndrome, their factor VIII/von Willebrand factor protein was present in normal amounts and had normal procoagulant and antigen activities; however, this protein was deficient in both carbohydrate and von Willebrand factor activity. The carbohydrate portion of the factor VIII/von Willebrand factor glycoprotein is of major importance in its interactions with platelets or the blood vessel wall, or both.*

Hemophilia A and von Willebrand's disease (vWd), two congenital hemorrhagic diseases, are similar in that they are both associated with reduced levels of procoagulant factor VIII (fVIII). Von Willebrand's disease can be distinguished from hemophilia by the findings of a long bleeding time, reduced ability of platelets to adhere to a column of glass beads (platelet retention), and autosomal dominant inheritance pattern (rather than X-linked recessive of hemophilia)