prising is the fact that even for very simple compounds like methyl and ethyl nitrate, basic questions of their chemistry in the environment are still open.

References

1. A. L. Chuck, S. M. Turner, P. S. Liss, *Science* **297**, 1151 (2002).
2. F. Flocke, E. Atlas, S. Madronich, S. M. Schauffler, *Geophys. Res. Lett.* **25**, 1891 (1998).
3. E. Atlas, *Nature* **331**, 426 (1988).
4. M. Schneider, O. Luxenhofer, A. Deissler, K. Ballschmiter, *Environ. Sci. Technol.* **32**, 3055 (1998).
5. R. G. Fischer, J. Kastler, K. Ballschmiter, *J. Geophys. Res.* **105**, 14473 (2000).
6. G. Werner, J. Kastler, R. Looser, K. Ballschmiter, *Angew. Chem. Int. Ed.* **38**, 1634 (1999).
7. F. Flocke, A. Volz-Thomas, H. J. Buers, H. J. Garthe, D. Kley, *J. Geophys. Res.* **103**, 5729 (1998).
8. J. J. Orlando, G. S. Tyndall, J. G. Calvert, *Atmos. Environ.* **26**, 3111 (1992).
9. S. M. Fan *et al.*, *J. Geophys. Res.* **99**, 16867 (1994).
10. E. Atlas *et al.*, *Eos* **78** (fall meeting suppl.), 46 (1997).
11. N. J. Blake *et al.*, *J. Geophys. Res.* **104**, 21803 (1999).
12. A. E. Jones *et al.*, *J. Geophys. Res.* **104**, 21355 (1999).
13. R. G. Fischer, R. Weller, H.-W. Jacobi, K. Ballschmiter, *Chemosphere* **48**, 981 (2002).
14. T. Urhahn, K. Ballschmiter, *Chemosphere* **37**, 1017 (1998).

PERSPECTIVES: BIOMEDICINE

Contact—How Platelets Touch von Willebrand Factor

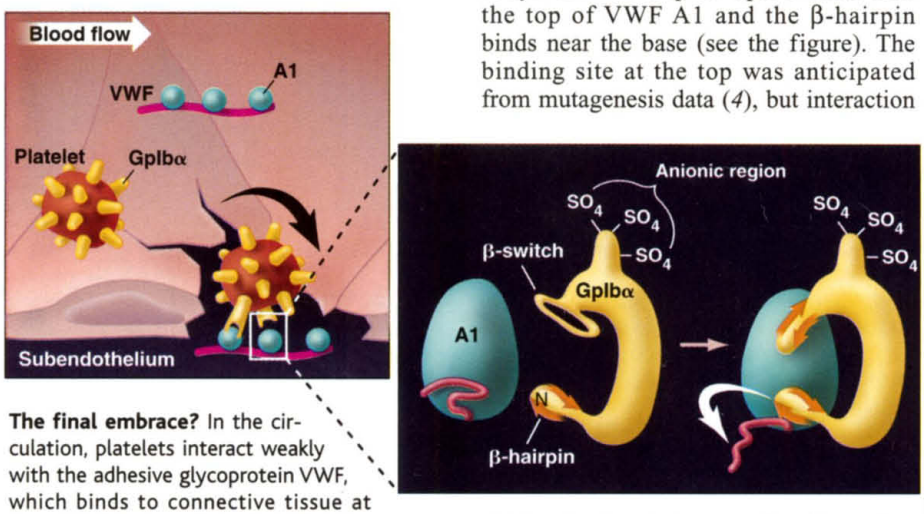
J. Evan Sadler

We cannot live without platelets, the small anucleate blood cells that aggregate to seal leaks at sites of vascular injury. Plasma von Willebrand factor (VWF) acts as an extracellular adapter in this process, binding to collagen in the wall of damaged blood vessels and then to membrane glycoprotein Ibα (GpIbα) on the platelet surface (*1*, *2*). Bleeding ensues when this interaction cannot occur, and fatal thrombosis (thrombotic thrombocytopenic purpura) follows when it cannot be terminated by feedback proteolysis of VWF. The kinetic properties of the binding of VWF to GpIbα have evolved to satisfy some rather special requirements that make platelet adhesion possible. On page 1176 of this issue, Huizinga *et al.* (*3*) give us our first look at the structural features that underlie this life-saving interaction.

VWF is a multimeric protein composed of identical 250-kD subunits, with the multimer typically exceeding 10,000 kD. Each subunit has a single 24-kD A1 domain that binds to the amino-terminal 45-kD segment of platelet GpIbα (see the figure). Platelet GpIbα is the largest component of a cell surface complex that contains at least three other membrane proteins. The affinity of binding appears to depend on the assay conditions. Soluble VWF and platelets do not readily interact in the blood, but when platelets flow across a VWF-coated surface, they adhere rapidly and begin to roll along the surface. Reversible rolling provides enough interaction time for other kinetically slow receptors to engage their ligands and initiate stable platelet adhesion and activation. The VWF-dependent capture and transient tethering of platelets is most efficient at the relatively high fluid shear rates found in small arterioles. Platelet adhesion therefore behaves as though it is regulated by the surface adsorption of VWF and by fluid shear stress (*1*).

These observations often are interpreted as evidence that immobilization or shear stress induces conformational changes in VWF that increase its affinity for GpIbα. This model is consistent with a surprising phenotype caused by certain mutations in either protein. von Willebrand disease (VWD) type 2B is a bleeding disorder characterized by increased binding of mutant VWF to GpIbα. The type 2B mutations are clustered in a small patch on the VWF A1 domain, which is remote from the GpIbα binding site that has been localized by mutagenesis (*4*). The clinically similar "platelet-type pseudo-VWD" is caused by mutations in the amino-terminal region of GpIbα that increase its affinity for VWF (*5*, *6*). Thus, low-affinity conformations of both the VWF A1 domain and GpIbα appear to be maintained by inhibitory mechanisms that are relieved by constitutive gain-of-function mutations. Whether shear stress or surface binding causes similar conformational changes remains controversial, and the behavior of adhering platelets can be modeled satisfactorily without invoking shear-dependent changes in affinity (*7*).

Against this background, the structure of the VWF A1-GpIbα complex (*3*) has remarkable explanatory power. To facilitate crystallization, Huizinga and colleagues engineered high-affinity variants: VWF A1 carried the VWD type 2B mutation R543Q, and the GpIbα fragment carried the platelet-type pseudo-VWD mutation M239V. The GpIbα fragment consists of eight leucine-rich repeats (LRRs) that form an elongated curve. The LRR framework is flanked by an amino-terminal β-hairpin motif and by a more complex carboxyl-terminal region with a protruding loop. The concave face of GpIbα grabs VWF A1 in a pincer-like grip: The carboxyl-terminal loop of GpIbα binds near the top of VWF A1 and the β-hairpin binds near the base (see the figure). The binding site at the top was anticipated from mutagenesis data (*4*), but interaction



The final embrace? In the circulation, platelets interact weakly with the adhesive glycoprotein VWF, which binds to connective tissue at sites of injury. At sufficiently high fluid shear rates, platelets bind to VWF and exhibit rolling adhesion. Each VWF subunit of the multimer has a single A1 domain (blue) that binds to platelet GpIbα (yellow). In the free A1 domain, an amino-terminal extension (pink) appears to block a binding site for the amino-terminal β-hairpin (orange arrows) of GpIbα. Binding requires the amino-terminal extension of A1 to move, and also induces the β-switch (yellow loop) of GpIbα to form a β-strand motif (orange arrows). The anionic sulfated region of GpIbα (closer to the carboxyl-terminal than the β-switch) does not directly contact the VWF A1 domain.

CREDIT: KATHARINE SUTLIFF/*SCIENCE*

The author is in the Department of Medicine, Department of Biochemistry and Molecular Biophysics, and Howard Hughes Medical Institute, Washington University School of Medicine, St. Louis, MO 63110, USA. E-mail: esadler@im.wustl.edu

of the β-hairpin with the base of the A1 domain is unexpected and may account for the ability of VWD type 2B mutations to increase the binding affinity.

The A1 domain contains a compact α/β fold delimited by a single disulfide loop. In the free A1 domain, the sequence extending on the amino-terminal side of the disulfide bond lies against the α/β surface (*8*), and this amino-terminal extension is displaced when GpIbα binds (*3*). The amino-terminal extension is inhibitory: Recombinant A1 domains lacking this extension bind 5- to 10-fold more tightly to GpIbα (*9*). The structure of another high-affinity A1 domain mutant with the substitution I546V suggests that conformational changes are propagated from the mutation through the A1 domain to the GpIbα binding site at the top (*10*). Many VWD type 2B mutations, however, are within the amino-terminal extension or affect residues that it contacts, suggesting that the mutations mainly promote the displacement of the amino-terminal extension and facilitate binding of the GpIbα β-hairpin (*3*). The relative importance of these two mechanisms remains to be determined.

The mutations in GpIbα that cause platelet-type pseudo-VWD also stabilize the A1-GpIbα complex, but by a different mechanism. As determined by Huizinga *et al.* (*3*) and independently by Uff *et al.* (*11*), the β-switch loop is flexible and lacks defined secondary structure in uncomplexed GpIbα. Upon binding to the VWF A1 domain, the β-switch region forms a two-stranded antiparallel β sheet that aligns with the central β sheet of A1. Mutations in GpIbα that increase the affinity of this platelet glycoprotein for VWF A1 are located in the β-switch region and are predicted to stabilize the β sheet (*3*). Because VWD type 2B mutations and platelet-type pseudo-VWD mutations affect widely separated binding sites, one might expect them to be additive. Indeed, binding affinity was increased two- to threefold by either type of mutation singly, and fivefold when the mutations were combined (*3*).

The structure of the VWF A1-GpIbα complex challenges several widely held notions about how the proteins might interact. For example, the properties of chimeric GpIbα proteins suggest that LRRs 1 to 4 may be important for binding (*12*). Instead, the structure reveals that there is one contact with LRR 1 and multiple contacts with LRRs 5 to 8 at the opposite end of the GpIbα fragment. An anionic region closer to the carboxyl-terminal than the β-switch region contains three sulfated tyrosine residues (see the figure). Mutations that affect these tyrosine residues reduce binding of GpIbα to VWF (*13*) and impair cell adhesion (*14*), suggesting that they might bind to VWF A1. However, this anionic region does not contact A1 in the complex, and Huizinga *et al.* could not show that deleting the anionic region reduced the binding affinity (*3*). Additional studies are needed to reconcile these observations.

The large structural changes associated with binding of GpIbα to VWF A1 are certain to breathe new life into studies of how protein conformation modulates platelet adhesion. The bidentate character of the complex suggests that binding (and dissociation) might develop in stages, so that a weak interaction at one site could evolve into a tight interaction involving both sites. If so, the present structure presumably represents the final embrace rather than the first molecular touch, which raises the question of whether certain conditions in vivo might help to tighten an initial low-affinity interaction between platelets and VWF. Perhaps required conformational changes are induced by surface binding of VWF or by fluid shear stress, or perhaps they simply develop in the course of binding. Many studies measuring the binding affinity of native VWF for GpIbα have obtained values for the dissociation constant (K_d) of >1 μM, much higher than the 30 nM calculated by Huizinga *et al.* (*3*). This suggests that there are uncharacterized inhibitory interactions involving sites outside of the A1 and GpIbα domains represented in the crystal structure. The answers to such questions may be clinically relevant. For example, they may determine whether one or both of the identified binding interfaces between A1 and GpIbα could be useful targets for the prevention or treatment of thrombosis.

References

1. B. Savage, E. Saldivar, Z. M. Ruggeri, *Cell* **84**, 289 (1996).
2. J. E. Sadler, *Annu. Rev. Biochem.* **67**, 395 (1998).
3. E. G. Huizinga *et al.*, *Science* **297**, 1176 (2002).
4. T. Matsushita, D. Meyer, J. E. Sadler, *J. Biol. Chem.* **275**, 11044 (2000).
5. J. L. Miller, D. Cunningham, V. A. Lyle, C. N. Finch, *Proc. Natl. Acad. Sci. U.S.A.* **88**, 4761 (1991).
6. S. D. Russell, G. J. Roth, *Blood* **81**, 1787 (1993).
7. T. A. Doggett *et al.*, *Biophys. J.* **83**, 194 (2002).
8. J. Emsley, M. Cruz, R. Handin, R. Liddington, *J. Biol. Chem.* **273**, 10396 (1998).
9. M. Sugimoto, J. Dent, R. McClintock, J. Ware, Z. M. Ruggeri, *J. Biol. Chem.* **268**, 12185 (1993).
10. R. Celikel, Z. M. Ruggeri, K. I. Varughese, *Nature Struct. Biol.* **7**, 881 (2000).
11. S. Uff, J. M. Clemetson, T. Harrison, K. J. Clemetson, J. Emsley, *J. Biol. Chem.*, 26 June 2002 (10.1074/jbc.M205271200).
12. Y. Shen *et al.*, *Blood* **95**, 903 (2000).
13. P. Marchese *et al.*, *J. Biol. Chem.* **270**, 9571 (1995).
14. J. Dong *et al.*, *J. Biol. Chem.* **276**, 16690 (2001).

PERSPECTIVES: GENETIC NETWORKS

Small Numbers of Big Molecules

Nina Fedoroff and Walter Fontana

Biologists tend to think deterministically. A case in point is their prolonged search for the "founder cells" of the slime mould *Dictyostelium*. These amoebae emit pulsatile cAMP signals under starvation conditions, mobilizing neighboring cells to surround them and form a motile multicellular slug that wanders off to form spores. Despite their efforts, biologists never could find the founder cells. The reason is that all *Dictyostelium* amoebae have the capacity to produce cAMP signals, and becoming a founder cell is a matter of chance—it's a stochastic process (*1*).

The issue of stochasticity—randomness or "noise," if you prefer—in living systems has been addressed theoretically (*2–4*). Two recent papers (*5*, *6*), including one by Elowitz and colleagues on page 1183 of this issue (*6*), provide direct and elegant experimental evidence that functional interactions among cellular macromolecules involved in gene expression are "noisy." Stochasticity arises because of the very small number of macromolecules involved in certain biological processes. Small numbers mean that both the randomness of molecular encounters and the fluctuations in the transitions between the conformational states of a macromolecule become noticeable.

The two new studies both measure the stochasticity of gene expression in bacteria using green fluorescent protein (GFP) reporter genes under the control of promoters regulated by the Lac repressor. Elowitz and colleagues (*6*) distinguish between different sources of noise, which they term "intrinsic" (inherent in the biochemical process of gene expression) and "extrinsic" (due to fluctuations in other cellular components required for gene expression).

N. Fedoroff is at Penn State University, University Park, PA 16803, USA. E-mail: nvf1@psu.edu W. Fontana is at Santa Fe Institute, Santa Fe, NM 87501, USA. E-mail: walter@santafe.edu