receptor VEGFR-2, which has been shown to cooperate with VEGFR-3 in lymphatic endothelial cell migration and proliferation. Whereas VEGF-C is essential in mouse lymphatic vascular development, it remains to be resolved to what extent the lack of VEGFR-2 binding accounts for the dispensable role of VEGF-D in this process.[5,6] Elucidation of the receptor-binding pattern of *Xenopus* VEGF-D and finding whether it explains the functional difference between VEGF-C and VEGF-D in this organism might bring us a step forward in solving this question. A hint to the lymphangiogenic modifier role of VEGF-D is provided by the fact that exogenous VEGF-D protein administration is able to partially rescue the impaired sprouting and migration of lymphatic endothelial cells in VEGF-C–deficient mouse embryos,[6] although loss of endogenous VEGF-D does not aggravate the VEGF-C null phenotype.[7]

Death of VEGFR-3–deficient mice due to blood vascular defects prior to lymphatic vessel development has precluded studies of the role of VEGFR-3 in mouse embryonic lymphangiogenesis.[8] The importance of VEGFR-3 signaling in embryonic lymphatic vascular development, postnatal lymphatic vessel survival, and stimulation of adult lymphangiogenesis has been directly or indirectly demonstrated in various experimental settings in several species. By using genetic down-regulation and chemical inhibition, Ny et al document the critical role of VEGFR-3 in developmental lymphangiogenesis in the *Xenopus* tadpole model and thus the functional conservation of VEGFR-3 in this species.

A profound understanding of the molecular regulation of lymphatic vascular development and the entire range of functions of its major players and modifiers should enable the development of controlled antilymphangiogenic and prolymphangiogenic therapies. Characterization of the lymphatic system in small model organisms has provided new tools to study lymphangiogenic processes and to complement the methods available in mammals. Ny et al highlight the potential of *Xenopus* tadpoles in lymphatic vascular research by demonstrating the subtle modifier role of VEGF-D in lymphatic vascular development.

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● ● ● HEMOSTASIS

Comment on Gao et al, page 1713

Shear tango: dance of the ADAMTS13/VWF complex

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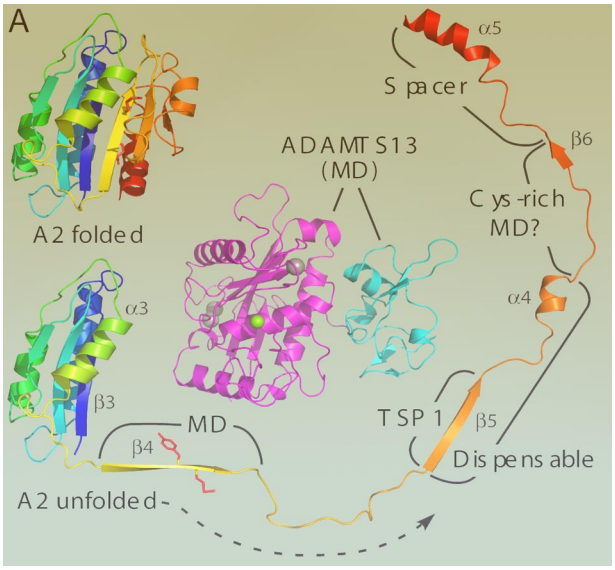
ADAMTS13, the metalloprotease that regulates the size of von Willebrand factor (VWF),[1] is critical in preventing microvascular platelet clumping, such as occurs in thrombotic thrombocytopenic purpura. The article in this issue of *Blood* by Gao et al addresses how these two proteins use multiple binding sites to facilitate proteolytic regulation of VWF.

ADAMTS13 cleaves only a single bond, Tyr^{1605}-Met^{1606}, buried within the VWF A2 domain. Shear forces unravel the normally tightly packed globular VWF, exposing this cleavage site. How does ADAMTS13 position itself on this flexible and dynamic multimeric glycoprotein, in such a way that it remains in place throughout the shear-induced folding and unfolding that occurs during transit through microcirculatory beds?

Binding is known to take place directly on the A2 domain of VWF spatially adjacent to the cleavage site,[2] but also on a more remote site of the A2 domain.[3,4] There might be additional domain interaction sites on VWF. Complementary binding sites have been identified on ADAMTS13, certainly within its Spacer[5] and Cys-rich regions,[6] but also within the TSR/CUB domains,[7] depending on shear conditions. Binding between each distinct remote site (exosite) ramps up the specificity constant of cleavage. According to Gao et al, collective exosite-driven proteolysis of the Tyr^{1605}-Met^{1606} bond accounts for approximately 2 orders of magnitude increase in cleavage of a small VWF substrate.

Remote exosite interactions are common in the hemostatic process and are exemplified by thrombin,[8] which uses exosites to direct and enhance cleavage of multiple protein and cellular substrates. In order for ADAMTS13 to utilize a similar mechanism of exosite recognition to partner its conformationally-responsive substrate during its conformational dance to shear, this enhanced proteolysis also requires that the protease can retain its binding under changing shear conditions during its transit through vessels of different sizes. Gao et al introduce an important concept of portability of exosites. This can be seen in the figure. The A2 domain model is shown folded as a compact globular molecule with the cleavage site hidden within the structure. When unfolded, it presents several distinct interaction sites near the cleavage site and extending to its C-terminus. Flexibility in spatial relationships (portability) between interacting sites is provided and/or enhanced by a redundant (dispensable) sequence. If there is no fixed distance between exosite and protease cleavage site, then the 2 partner macromolecules can be tightly bound but move together, elongating when shear stress is high in the microcirculation, and contracting in length to globular form in larger vessels when shear stress is low. Such a rhythmic dance depends on mutual and multiple exosite engagements on dynamical structures that nevertheless precisely position the active site



A homology model of the ADAMTS13 metalloprotease (magenta) and disintegrin-like domains (cyan) is shown (MD) to provide a sense of scale, with active site Zn2+ ion (green) and 3 structural Ca2+ ions (gray) as spheres. The VWF A2 domain is predicted to consist of a 6-stranded β-sheet surrounded by 5 α-helices. Residues Tyr[1605]-Met[1606] (side chains in red) are buried in strand β4. Exposure of this bond to ADAMTS13 requires substantial unfolding of domain A2; more distal segments that interact with specific domains of ADAMTS13 are labeled. The locations of these ADAMTS13 domains relative to the MD moiety are not known. Deletion of strand β5 through helix α4 (dispensable) has a minimal effect on the rate of substrate cleavage. Molecular graphics prepared with PyMOL (DeLano Scientific, Palo Alto, CA). See the complete figure in the article beginning on page 1713.

within the protease domain. This makes it poised to strike when the cleavage site is revealed by the shear-induced unfolding of the A2 domain.

Ultimately, then, such flexible proximity enables the catalytic center of ADAMTS13 to proteolyse the Tyr[1605]-Met[1606] bond as it becomes exposed and thereby reduces the size and reactivity of the VWF polymer. The unique preference of ADAMTS13 for a single bond contrasts with that of thrombin, the latter being an example of exosite-enhanced (almost) promiscuous proteolysis. Numerous crystal structures have given insight into the mechanisms of functional specificity of thrombin. What precisely determines the unique cleavage specificity of ADAMTS13 remains to be determined.

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HEMOSTASIS

Comment on van Schooten et al, page 1704

Where does von Willebrand factor go?

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VWF is removed from the blood by macrophages rather than scavenger receptors, suggesting a possible strategy for improving treatment of VWD and hemophilia.

In clinical hematology, von Willebrand factor (VWF) is a bit like the actor Jake Gyllenhaal: we care about him because of who clings to his arm rather than for any inherent quality. VWF is the consort for factor VIII, and many hematologists care more that VWF brings factor VIII to the party than getting to chat with VWF. This is unfair to VWF, of course. VWF is also essential for normal hemostasis and mild von Willebrand disease(VWD), due to partial deficiency of VWF, is more common than hemophilia A. However, we all know how bad bleeding can be when factor VIII is absent, so we care whether VWF is able to protect factor VIII. Thus, we also care whether factor VIII stays with VWF, even when it is removed from the blood by a clearance pathway.

VWF is an unusually large protein. It differs from most plasma proteins in that it is synthesized and secreted primarily by endothelial cells rather than hepatocytes. Rolled up like a spool of yarn, the secreted VWF travels in the blood until it unravels under vascular sheer stress or after engagement of the vascular wall. The unrolled VWF is a sticky, very long molecule. A single VWF multimer, attached to a vessel wall, can be a good deal longer than the width of an endothelial cell. As blood rushes by, the unrolled VWF thread is soon decorated by platelets that cling tightly enough to resist sheer force and the bumping of red cells. Thus, deficiency of VWF leads to bleeding problems caused by failure of platelet deposition. In a pathologic situation, excessive function of VWF is a dramatic feature of thrombotic thrombocytopenic purpura. In that disease, abnormally large VWF multimers in blood causes deposition of platelet-rich thrombi that fragment red blood cells and occlude blood flow to essential organs.

Each of the 20 to 50 subunits that make up a VWF multimer has a high affinity niche for a factor VIII molecule. When bound to VWF, factor VIII is prevented from binding to platelets and participating in the coagulation cascade. Factor VIII is also protected from spontaneous dissociation, from proteolytic degradation, and from clearance by scavenger receptors that line blood vessels. Thus, VWF substantially prolongs the plasma half-life of factor VIII. VWF has a longer plasma half-life than factor VIII and, until now, it hasn't been clear how VWF molecules are removed from the circulation or whether they take bound factor VIII molecules when they go.

In this issue of *Blood*, van Schooten and colleagues identify a cellular clearance pathway for VWF and, apparently, for VWF-bound factor VIII. The surprise is that VWF is cleared via a macrophage pathway rather than via the usual scavenger receptors that are localized primarily on hepatocytes, renal cells, and endothelial cells. It is fitting that VWF, the giant molecule, is cleared by a cellular pathway