In this issue of Blood, van den Biggelaar and colleagues demonstrate the cotargeting of FVIII and VWF to secretory granules in the absence of a high-affinity interactions between VWF and FVIII, using recombinant VWF type 2N variants. In addition to mediating the adhesion of platelets to subendothelial tissue at the site of vascular injury, von Willebrand factor (VWF) also serves as the carrier protein for coagulation factor VIII (FVIII) in plasma. The initial point of physical association between these 2 proteins remains unclear. When desmopressin (DDAVP) is administered to mild hemophilia or von Willebrand disease (VWD) pa-
tients, both FVIII and VWF are released into plasma. Presumably, VWF is released from endothelial cell Weibel-Palade bodies. While the liver is the major site of FVIII synthesis, the cell within the liver producing FVIII has not been definitively identified. Recent publications suggest that FVIII may be present in selected endothelial populations including human lung microvascular endothelial cells and murine sinusoidal endothelial cells.1,2

Several studies have demonstrated that expression of FVIII in VWF-producing cells, such as HUVEC, cultured megakaryocytes, and platelets, results in colocalized storage of VWF and FVIII in endothelial cell Weibel-Palade bodies or in α-granules of platelets and megakaryocytes.3,4 These studies have suggested that FVIII-regulated storage is secondary to VWF storage and results from a high-affinity VWF-FVIII association early in the secretory pathway. Seemingly in contrast to this conclusion is the observed comcomitant release of both VWF and FVIII in type 2N VWD patients after DDAVP administration.5 Type 2N VWD variants are characterized by a markedly decreased binding affinity for FVIII caused by homozygous or compound heterozygous mutations in VWF that impair FVIII binding. In these patients, the loss of high-affinity VWF-FVIII binding does not promote stabilization of FVIII in plasma and FVIII is degraded fairly rapidly. Given the lack of FVIII binding to 2N variants of VWF, the cellular source of the DDAVP-releasable pool of FVIII is an open question.

The current study by van den Biggelaar et al, which uses several recombinant type 2N VWF variants, provides valuable insight into the relationship between assembly of the VWF/FVIII complex and the cotrafficking of VWF and FVIII to the regulated secretory pathway.5 The authors employ complementary techniques, Surface Plasmon Resonance (SPR) and a pseudo-equilibrium binding assay to demonstrate a range of mildly to severely reduced FVIII-binding affinity of the 2N variants. Using an HEK293 cell expression system, the authors elegantly demonstrate that, despite the FVIII binding defects, all type 2N variants were able to target corexpressed FVIII and P-selectin to the VWF-containing pseudo–Weibel-Palade bod-
dies. This finally provides a mechanistic basis for the observed DDAVP-induced release of FVIII and VWF in type 2N VWD patients.

Comment on van den Biggelaar et al, page 3102

VWF and FVIII: the origins of a great friendship

Sandra L. Haberichter  MEDICAL COLLEGE OF WISCONSIN

In this issue of Blood, van den Biggelaar and colleagues demonstrate the cotargeting of FVIII and VWF to secretory granules in the absence of a high-affinity interactions between VWF and FVIII, using recombinant VWF type 2N variants. The current study by van den Biggelaar et al, which uses several recombinant type 2N VWF variants, provides valuable insight into the relationship between assembly of the VWF/FVIII complex and the cotrafficking of VWF and FVIII to the regulated secretory pathway.5 The authors employ complementary techniques, Surface Plasmon Resonance (SPR) and a pseudo-equilibrium binding assay to demonstrate a range of mildly to severely reduced FVIII-binding affinity of the 2N variants. Using an HEK293 cell expression system, the authors elegantly demonstrate that, despite the FVIII binding defects, all type 2N variants were able to target corexpressed FVIII and P-selectin to the VWF-containing pseudo–Weibel-Palade bod-
dies. This finally provides a mechanistic basis for the observed DDAVP-induced release of FVIII and VWF in type 2N VWD patients.
The data presented in this study indicate that assembly of a high-affinity VWF/FVIII complex is not required for cotrafficking of the 2 proteins, but rather FVIII storage may be secondary to the VWF-dependent biogenesis of the secretory granule itself. In a study by Yarovoi et al, expression of FVIII in VWF-deficient platelets resulted in some storage of FVIII in α-granules. This provided further evidence that interaction with VWF is not absolutely necessary for regulated storage of FVIII. Other yet unidentified cell-specific factors are likely to play a role in granule formation and protein targeting. An additional noteworthy observation from this study is the change in granule morphology from elongated, rodlike granules to round vesicles when FVIII is present in the granule. This type of morphologic change has been observed in previous studies examining other VWF variants and, in sum, these studies suggest that granule morphology may depend on the intragranule content. As pointed out by the authors, endothelial cells have organ-specific characteristics, and variations in expression and morphology may be dependent on the particular lineage of the vascular tree.

This study by van den Biggelaar et al provides a molecular explanation for the concomitant release of FVIII and VWF in type 2N VWD patients and demonstrates that a high-affinity VWF/FVIII complex is not necessary for targeting FVIII to the regulated secretory pathway. This study provides an answer to the long-standing question of FVIII release in type 2N patients and also creates new questions regarding FVIII biosynthesis and granule biogenesis.

Conflict-of-interest disclosure: The author declares no competing financial interests.

REFERENCES
VWF and FVIII: the origins of a great friendship

Sandra L. Haberichter