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### Commentary

Details of the pathophysiologic mechanisms that underlie complex disorders, such as the thrombo-occlusive events associated with myocardial infarction, stroke, and venous thromboembolism, are challenging to address. Recent advances have been made through the application of genome-wide association studies (GWAS) to identify genetic loci associated with plasma levels of procoagulant proteins and risk of thrombotic disease. GWAS have consistently identified the gene encoding syntaxin-binding protein 5 (STXBP5) in this context. STXBP5 is expressed in both endothelium and platelets, and SNPs within the *STXBP5* locus have been associated with plasma levels of vWF and increased venous thrombosis risk. In this issue of the *JCI*, two complementary reports from the laboratories of Charles Lowenstein and Sidney Whiteheart describe studies that highlight the complexity of the function of STXBP5 in control of storage granule development and exocytosis in platelets and endothelium. Together, these studies demonstrate that STXBP5 differentially regulates exocytosis in these two cell types. While STXBP5 facilitates granule release from platelets, it inhibits secretion from the Weibel-Palade bodies (WPBs) of endothelial cells.

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# Syntaxin-binding protein 5 exocytosis regulation: differential role in endothelial cells and platelets

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Details of the pathophysiologic mechanisms that underlie complex disorders, such as the thrombo-occlusive events associated with myocardial infarction, stroke, and venous thromboembolism, are challenging to address. Recent advances have been made through the application of genome-wide association studies (GWAS) to identify genetic loci associated with plasma levels of procoagulant proteins and risk of thrombotic disease. GWAS have consistently identified the gene encoding syntaxin-binding protein 5 (STXBP5) in this context. STXBP5 is expressed in both endothelium and platelets, and SNPs within the *STXBP5* locus have been associated with plasma levels of vWF and increased venous thrombosis risk. In this issue of the *JCI*, two complementary reports from the laboratories of Charles Lowenstein and Sidney Whiteheart describe studies that highlight the complexity of the function of STXBP5 in control of storage granule development and exocytosis in platelets and endothelium. Together, these studies demonstrate that STXBP5 differentially regulates exocytosis in these two cell types. While STXBP5 facilitates granule release from platelets, it inhibits secretion from the Weibel-Palade bodies (WPBs) of endothelial cells.

## Background

Thrombo-occlusive cardiovascular disease remains a major cause of death globally. It is well documented that the mechanisms that coordinate these pathologies are multifactorial and involve both genetic and acquired risk factors (1). Key players in thrombo-occlusive events are the vascular endothelium and platelets, both of which are involved in maintaining vascular integrity and normal hemostatic responses (2). However, under pathophysiologic circumstances, activation of the endothelium and platelets can trigger the release of procoagulant and cellular-adhesive molecules that can interact to promote the development of thrombotic events (3, 4).

## STXBP5 as a regulator of cardiovascular homeostasis

It is not completely understood how the secretory status of platelets and endotheli-

um is regulated. Various agonists are known to promote the secretion of granular contents from platelets (including thrombin, ADP, and collagen; ref. 5) and endothelial cells (such as histamine and shear flow; ref. 6); however, it is not clear how these inducers mediate exocytosis. Two studies in this issue directly address the role of syntaxin-binding protein 5 (STXBP5; also known as Tomosyn-1) in this process. The studies by Ye et al. (7) and Zhu et al. (8) build upon the consistent identification of STXBP5 as a contributor to the regulation of plasma vWF levels (9–11) and as a risk factor for venous thrombosis (12), which suggests that STXBP5 function is important for maintaining normal hemostatic balance (Figure 1). In further support of this notion, a nonsynonymous STXBP5 SNP, N436S, is associated with a decreased incidence of venous thromboembolism (9) and an increased bleeding phenotype in females (13).

## STXBP5 expression in endothelium and platelets

STXBP5 is expressed in both endothelial cells and platelets as an approximately 130-kDa protein. Multiple isoforms have been identified that derive from differential splicing of the *STXBP5* transcript. Ye et al. and Zhu et al. both report that the pattern of isoform expression appears similar in these two cell types. Ye and colleagues isolated STXBP5 as a t-SNARE-binding protein from platelet extracts (7), whereas Zhu and colleagues identified STXBP5 in endothelial cells through its interaction with syntaxin-4, a component of SNARE complexes, and synaptotagmin, the sensor for mediating calcium-induced exocytosis (8). These findings confirmed prior observations in neurons (14) and pancreatic  $\beta$  cells (15) that STXBP5 can form complexes with SNARE proteins and is thus well situated to regulate the exocytic process. In endothelial cells, STXBP5 was located in cytoplasmic granules, and colocalization with vWF in Weibel-Palade bodies (WPBs) was minimal. Additionally, STXBP5 did not appear to influence WPB number, size, or morphology.

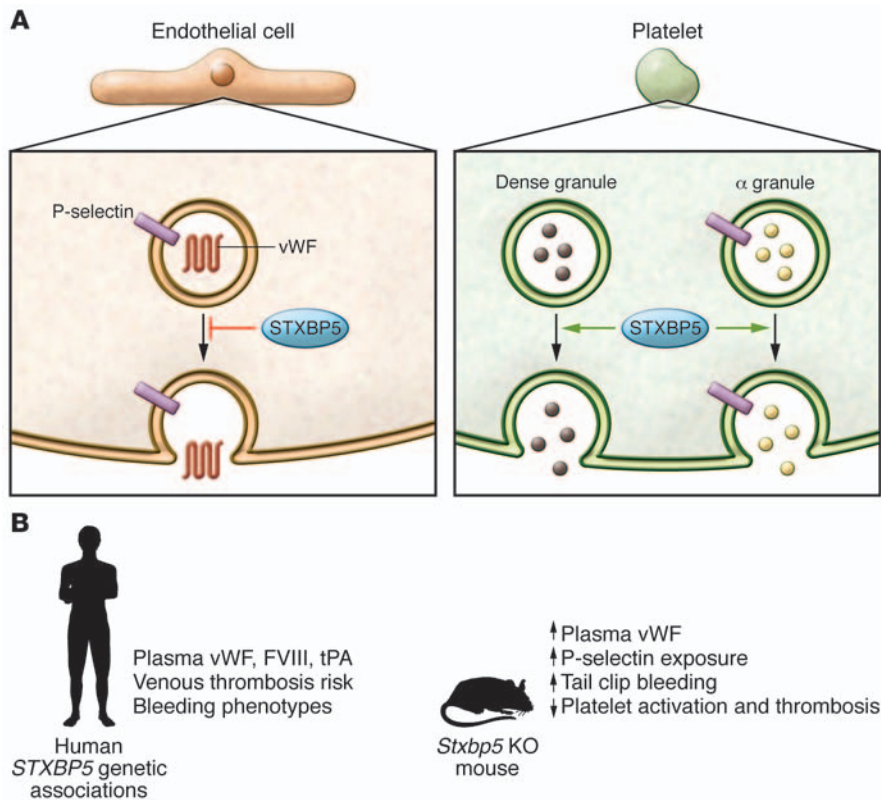
## STXBP5 inhibits exocytosis in endothelial cells

In neurons and pancreatic  $\beta$  cells, STXBP5 inhibits neurotransmitter release and insulin secretion, respectively (14, 15). Zhu and colleagues demonstrated that STXBP5 also inhibits exocytosis in endothelial cells (8). STXBP5 knockdown in endothelial cells resulted in increased histamine- and calcium ionophore-mediated secretion of vWF and increased externalization of the adhesion molecule P-selectin. In contrast, STXBP5 knockdown did not affect constitutive vWF release. Interestingly, the N436S STXBP5 variant, which is associated with reduced plasma vWF levels and increased bleeding tendency in females (13), inhibited endothelial vWF secretion more efficiently than did WT STXBP5.

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**Figure 1. STXBP5 differentially influences exocytosis in platelets and endothelial cells.** (A) STXBP5 inhibits regulated exocytosis from endothelial cells, but promotes granule secretion from platelets. In both cell types, STXBP5 interacts with components of the SNARE machinery, but the details of these interactions appear distinct. In endothelial cells, there is no colocalization with WPBs, and the number, size, and morphology of these organelles is not influenced by STXBP5. In platelets, there is no colocalization of STXBP5 with  $\alpha$ , dense, and lysosomal granules, but the granule cargo composition is altered. (B) Genetic associations and effects of STXBP5 deficiency. In humans, *STXBP5* has consistently been identified in GWAS to be associated with the regulation the plasma levels of several coagulation factors, with bleeding, and with the risk of venous thrombosis. *Stxbp5* KO mice exhibit a spectrum of hemostatic phenotypes that are consistent with the role of STXBP5 function in endothelial cells and platelets. FVIII, factor VIII; tPA, tissue plasminogen activator.

In *Stxbp5* KO mice, plasma levels of vWF were increased, consistent with the demonstrated STXBP5-dependent inhibition of endothelial vWF exocytosis. In this *Stxbp5* KO mouse model, calcium ionophore-mediated WPB release also resulted in increased platelet adhesion to the walls of venules and increased exposure of P-selectin. Despite these apparent signs of hypercoagulability resulting from uninhibited endothelial exocytosis, tail bleeding was increased in *Stxbp5* KO mice. Moreover, *Stxbp5* KO mice also exhibited delayed thrombus development after mesenteric and carotid artery injury. These unexpected results suggested that STXBP5 deficiency might induce a platelet function defect.

**STXBP5 facilitates exocytosis in platelets**

Contrary to the findings in endothelial cells, both groups showed that in platelets, STXBP5 promotes exocytosis (platelet secretion; refs. 7, 8). Both demonstrated reduced release of  $\alpha$  and dense granule contents and impaired surface exposure of P-selectin in the absence of STXBP5 (Figure 1). In addition, platelets from *Stxbp5* KO mice exhibited a complex granule cargo defect that included reduced levels of

some constituents, including platelet factor 4 and factor V, but only modest changes in other proteins, such as fibrinogen and vWF. Numbers of  $\alpha$  and dense granules in *Stxbp5* KO mice were normal, as were platelet counts.

Both Ye et al. and Zhu et al. observed an increase in tail bleeding and defective thrombus generation in *Stxbp5* KO mice (7, 8). To test whether this defect was the result of a platelet-specific influence, bone marrow transplant experiments were performed. Transfer of *Stxbp5* KO hematopoietic cells into WT animals resulted in defective hemostasis, whereas transfer of WT hematopoietic cells corrected the hemostatic defect in *Stxbp5* KO mice. Together, these results highlight the importance of platelet-specific STXBP5 in mediating granule secretion and generating a normal hemostatic response.

**Conclusions**

The reports by Ye et al. and Zhu et al. (7, 8) extend previous observations of a genetic association among STXBP5, plasma levels of vWF, and venous thrombosis risk. These two studies revealed that STXBP5 regulates exocytosis from endothelial cells and platelets, but the influence is dis-

tinct in these two cell types, with STXBP5 inhibiting endothelial cell exocytosis, but promoting secretion from platelets. Why the difference in effect? STXBP5 transcript variants are similarly expressed in the two cell types; however, the STXBP5 binding partners within SNARE complexes appear to be cell type-specific and may provide clues as to the differential functional outcomes. Overall, these studies demonstrate the power of GWAS to identify previously unrecognized contributors to key cardiovascular functions. Furthermore, these reports highlight the need for additional dissection of the cell-specific functions of STXBP5 to unravel the subtle but critical differences among different cell types.

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