association and clot retraction. Using raft-specific affinity labels, they demonstrated that fibrin associates with a subset of lipid rafts that are rich in sphingomyelin (SM) (as opposed to the cholesterol-rich raft population). Platelets from mice lacking either of the enzymes that synthesize sphingomyelin retracted clots poorly.

Previous studies have implicated both pFXIII and cFXIII in fibrin association with the platelet surface on thrombin receptor activation.[5] Here, Kasahara et al demonstrate that FXIII is required for translocation of fibrin to SM-rich rafts and that this fibrin is a substrate for FXIII. The translocation required the γ-chain of fibrin: a recombinant γ-chain fusion protein bound to rafts in a FXIII-dependent manner in thrombin-activated platelets. Covalent crosslinking was required for this association because mutating the FXIII crosslinking sites prevented association of the γ-chain fusion protein with rafts, as did the transglutaminase inhibitor cystamine.

These results point to a novel 2-step mechanism whereby fibrin(ogen) binds to integrin $\alpha_{IIb}\beta_3$, translocates to SM-rich lipid rafts in a FXIII-dependent manner, and covalently associates with an as yet unidentified receptor. Considered in light of the data indicating that cFXIII translocates to the cytoskeleton of activated platelets[6] (which in activated platelets attaches to lipid rafts[7]), a mechanism emerges whereby, within SM-rich rafts, FXIII crosslinks cytoskeletal and motor elements to promote force transduction from the platelet interior (see figure). The raft-associated fibrin is crosslinked to an unknown receptor (one candidate being $\alpha_v\beta_3$), which itself might be crosslinked to the cytoskeleton on the cytoplasmic side to allow the strongest possible linkage between the cytosolic motors and the extracellular cables they tug on to retract the clot. Lack of FXIII would not only be expected to increase fibrin clot lysis, it could also impair that ability of the fibrin to attach to the underlying platelets. These findings also suggest that changes in lipid raft structure or organization could alter clot retraction and impair wound healing.

As with many thought-provoking papers, the current paper raises many new questions and opens new areas of research. These include the following. What is the receptor for fibrin within the SM-rich rafts? Is this mechanism specific for thrombin-activated platelets or is it applicable to platelet activation by other agonists? Is there a role for cFXIII-mediated crosslinking of intracellular proteins within lipid rafts? What effect does this crosslinking have on the platelets' ability to generate contractile forces? Answers to these and other questions will provide further insight into the clinical signs and symptoms of FXIII deficiency.

Conflict-of-interest disclosure: The authors declare no competing financial interests. ■

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Comment on Kahr et al, page 3349

α-Granules at the BEACH

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In this issue of *Blood*, Kahr et al report the description of a mouse model lacking the Nbeal2 protein and demonstrate its utility in the study of α-granule biogenesis and megakaryocyte development in gray platelet syndrome (GPS).[1]

GPS (Mendelian Inheritance in Man [MIM] 139090) was first identified by Raccuglia in 1971.[2] The first patient, an 11-year-old boy, presented with thrombocytopenia and platelets that had a "peculiar gray color" on Wright-stained blood smears. Analysis showed that these platelets lacked a class of granules that are now known as *α-granules*. GPS is a rare autosomal recessive disorder associated with macrothrombocytopenia, splenomegaly, myelofibrosis, increased serum B_{12}, and mild-to-moderate bleeding tendencies.[3] Although fatal in some cases, in other cases, patients have lived into their 7th decade. However, where data are available, it seems that GPS is progressive, because the thrombocytopenia and myelofibrosis worsen with age. Additionally, there is evidence that genetic modifiers affect disease outcomes. Individuals with identical mutations, but from different families, have discordant disease severities.[4] The mouse strain reported by Kahr et al[1] and also by Deppermann et al[5] represents the first animal model for GPS.

The causative gene for GPS was identified on chromosome 3p by 3 groups in 2011.[4,6,7] These studies showed that mutations in the coding region of the neurobeachin-like 2 (*NBEAL2*) gene correlated with GPS in humans. Nbeal2 is a member of a family of proteins known as *BEACH–domain–containing proteins* (BDCP).[8] The BEACH domain gained its name because the charter family member—LYST/CHS1 (lysosomal trafficking regulatory/Chediak-Higashi syndrome 1 protein), defective in the beige mouse and in human Chediak-Higashi syndrome (MIM 214500)—contained this unusual ~300 amino acid domain.[9] This domain has a unique peptide-backbone fold, in that its core does not assume regular secondary structures.[10] The highly conserved BEACH domain has now been identified in 9 human proteins and in numerous species.[8] Most members of this family of generally high molecular weight proteins contain

membrane-binding domains (eg, Pleckstrin homology and FYVE [Fab1, YOTB, Vac1, and EEA1] zinc finger) and domains that form multiprotein complexes (tryptophan-aspartic acid repeat of ~40 amino acids). Family members also contain additional domains; for example, Nbeal2 contains a Concanavilin A-like, lectin domain. BDCPs have been linked to several membrane-related processes such as lysosome size regulation, synaptosome formation, and autophagy. They are thought to be scaffolds that assemble protein complexes at specific membranes and direct either membrane fusion or fission.[8] Two BDCPs, Nbeal2 and lysosomal trafficking regulatory protein, differentially affect granule biogenesis in megakaryocytes. The processes controlled by BDCPs are of central importance, because autosomal mutations in 4 of the 9 genes encoding family members are associated with human diseases. However, their mechanisms of action are largely unknown.

To study the role of Nbeal2, Kahr et al[1] and Deppermann et al[5] generated and characterized a Nbeal2 knockout mouse strain that, in part, mimics human GPS. The mice are macrothrombocytopenic and have splenomegaly, as in humans, but lack the obvious reticulin staining associated with myelofibrosis. Platelets from these animals lack identifiable α-granules and show significant reduction in several α-granule cargo proteins (ie, thrombospondin, von Willebrand factor, fibrinogen, platelet factor 4). Interestingly, the levels of von Willebrand factor in endothelial cells and in plasma are not affected.[5] The α-granule membrane protein P-selectin is present, but reduced, indicating that Nbeal2 is required for granule cargo sorting or packing but is not essential for creating α-granule membranes. Consistently, Nbeal2$^{-/-}$ platelets exhibit increased numbers of membrane-delineated vacuoles that appear devoid of content. Ex vivo platelet assays showed that Nbeal2 deficiency causes defects in platelet aggregation and adhesion but has only a limited effect on α_{IIb}/β_3 activation and dense granule release. Remarkably, Nbeal2 deficiency decreases procoagulant activity and annexin binding, suggesting that α-granules contribute to activation-induced phosphatidylserine exposure.[5] Consistent with these results, in vivo studies showed that Nbeal2 is required for effective hemostasis, implying that properly formed α-granules are important for thrombus formation and stability.

Further analysis by Kahr et al[1] suggests that Nbeal2 has a role in megakaryocyte development. Microscopy analysis of Nbeal2$^{-/-}$ bone marrow megakaryocytes showed abnormalities in platelet territories and increased emperipolesis. Upon culture in the presence of thrombopoietin, the Nbeal2$^{-/-}$ megakaryoctyes showed reduced survival and ploidy, suggesting that Nbeal2 plays a role in megakaryocyte growth and differentiation. Morphologically, the Nbeal2$^{-/-}$ cells appear to be arrested in an early state of differentiation. A role for Nbeal2 in megakaryocyte development is consistent with earlier studies in zebrafish. Silencing the Nbeal2 ortholog with morpholino oligonucleotides led to an almost complete abrogation of thrombocytes in injected embryos with no effect on erythropoiesis.[6] Further studies will be required to fully understand Nbeal2's role in megakaryocyte differentiation.

Mouse models have been invaluable tools in understanding human disease. This mouse strain will be no different. It can be used to dissect the function of Nbeal2 at the molecular and cellular level to determine which trafficking steps are affected in GPS and perhaps shed light on the functions of other BDCPs. It can be used to identify genetic modifiers that affect the severity of GPS and perhaps allow for a better understanding of how the disease progresses with age. Finally, these animals will be instrumental in dissecting the biological role of α-granules and the release of their myriad cargo. As an example, Deppermann et al[5] show that the Nbeal2$^{-/-}$ mice were protected from thrombo-inflammatory brain injury following focal cerebral ischemia. Given the many α-granule cargo proteins and their multiple biological targets in both normal and damaged vasculature, the Nbeal2$^{-/-}$ mice generated and described by Kahr et al[1] and Deppermann et al[5] will be of significant value to a number of future studies.

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

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