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REVIEW ARTICLE

A new look at an old body: molecular determinants of Weibel-Palade body composition and von Willebrand factor exocytosis

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Abstract

Endothelial cells, forming a monolayer along blood vessels, intricately regulate vascular hemostasis, inflammatory responses, and angiogenesis. A key determinant of these functions is the controlled secretion of Weibel-Palade bodies (WPBs), which are specialized endothelial storage organelles housing a presynthesized pool of the hemostatic protein von Willebrand factor and various other hemostatic, inflammatory, angiogenic, and vasoactive mediators. This review delves into recent mechanistic insights into WPB biology, including the biogenesis that results in their unique morphology, the acquisition of intraluminal vesicles and other cargo, and the contribution of proton pumps to organelle acidification. Additionally, in light of a number of proteomic approaches to unravel the regulatory networks that control WPB formation and secretion, we provide a comprehensive overview of the WPB exocytotic machinery, including their molecular and cellular mechanisms.

KEYWORDS
endothelial cells, exocytosis, organelle biogenesis, von Willebrand factor, Weibel-Palade bodies

1 | INTRODUCTION

Weibel-Palade bodies (WPBs) are rod-shaped storage organelles found only in the cytoplasm of vascular endothelial cells [1]. The principal soluble component of WPB is the mosaic glycoprotein von Willebrand factor (VWF) [2]. VWF is a secreted protein that functions physiologically in primary and secondary hemostasis. VWF first appeared in chordates [3] at around the same time that important evolutionary steps were occurring in the early blood coagulation system in response to selective pressures posed by more complex, high-pressure circulatory systems [4,5]. WPBs are classified as lysosome-related organelles [6], a class of subcellular organelles that arose in metazoans as cell-specific functional adaptations of the endolysosomal pathway. The adaptable size and unique shape of WPBs facilitate the optimal release of VWF. As secretory organelles, WPBs are also involved in the controlled release of additional cargo, thereby contributing to hemostasis, angiogenesis, and inflammation (Figure 1A). Ongoing research aims to unravel the complexities of their cargo composition, including the contribution of organelle acidification to organelle rigidity, exploring how they respond to various signals and investigating the potential existence of subclasses tailored for specific functions. Recent advances, including insights from techniques like proteomic interactome screening, have significantly expanded our understanding since the last review from our laboratory [7]. In this updated overview, we delve into these recent discoveries, providing novel molecular and mechanistic insights into WPB biology.

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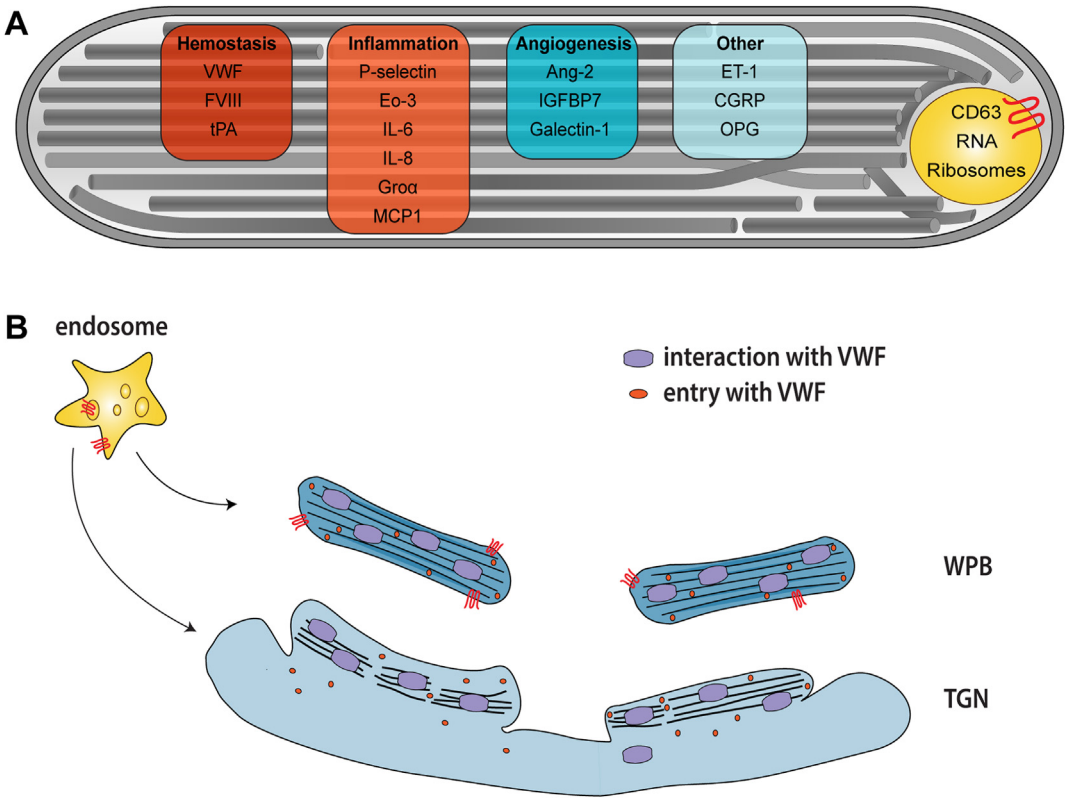

FIGURE 1 Contents of a Weibel-Palade body (WPB). (A) Cargo sorted by function. (B) Mechanisms of cargo inclusion in WPB. The model depicts molecules binding to von Willebrand factor (VWF), independent cargo entry, and the endosome recycling pathway. Ang-2, angiopoietin-2; CGRP, calcitonin gene-related peptide; Eo-3, eotaxin-3; ET-1, endothelin 1; FVIII, factor VIII; Groα, growth related oncogene α; IGFBP7, insulin-like growth factor-binding protein 7; IL-6, interleukin-6; IL-8, interleukin-8; MCP1, monocyte chemoattractant protein-1; OPG, osteoprotegerin; TGN, *trans*-Golgi network; tPA, tissue-type plasminogen activator.

2 | NOVEL MECHANISTIC INSIGHTS INTO WPB BIOGENESIS: DEPENDENCE ON GOLGI STRUCTURE AND THE ROLE OF SOLUBLE NSF ATTACHMENT RECEPTORS AND ADP-RIBOSYLATION FACTORS IN ENDOPLASMIC RETICULUM-GOLGI TRANSPORT

VWF was the first protein identified as a cargo molecule within WPB, confirming its function in hemostasis [2]. The formation of the organelles is entirely driven by the expression of VWF [8,9]; therefore, genetic defects in *VWF*, such as in von Willebrand disease, can impair WPB biogenesis and morphology [10,11]. The biosynthesis of VWF is extensively reviewed and will not be covered here [12,13]. A number of studies have established that there is a correlation between the length of the WPB and the hemostatic potential of its main cargo VWF, which includes the length of VWF strings formed upon release, the number of platelets that can adhere to these strings, and the self-association with VWF from plasma [14,15]. How the size of WPBs, which can range from 0.5 μm up to 5 μm in length, is regulated is now beginning to be understood. Large-scale morphometric analysis revealed that WPB lengths seemed to occur at a rhythm of approximately 0.5 μm intervals [16], which is best explained by a scenario that involves an amalgamation of equally sized building blocks. In the model that was put forward, VWF arrives at the *trans*-Golgi network (TGN) in discrete "quanta" of protein cargo that is condensed within TGN cisterna [16]. Simultaneous arrival of such quanta in adjacent regions within the TGN would lead to their joint incorporation in a newly budding WPB, its length being determined by the number of quanta that are organized alongside each other. Interestingly, the dimensions of these VWF quanta correspond to those of so-called Golgi mini-stacks, the minimal units from which Golgi cisterna self-organize in endothelial cells [17]. This is supported by the observation that unlinking the Golgi into smaller-sized units is accompanied by a similar step-wise reduction in WPB size. In 2-dimensional and 3-dimensional reconstructions of WPBs imaged by cryo-electron microscopy (EM), densely packed and parallel-arranged VWF tubules that extend from one end of the WPB to the other can be found [18]. VWF tubules are essentially helically condensed ultralong VWF multimers, and to reconcile the existence of such long tubules with the joining of quanta, multimerization needs to take place after they arrive in the TGN with sufficient room and time for rearrangements into the VWF paracrystal. From this model, it also follows that Golgi integrity, ie, an extended Golgi ribbon, and a continuous flux of VWF through the early secretory pathway are essential for the formation of elongated WPBs (Figure 2A). Recently, 2 protein families crucial for VWF

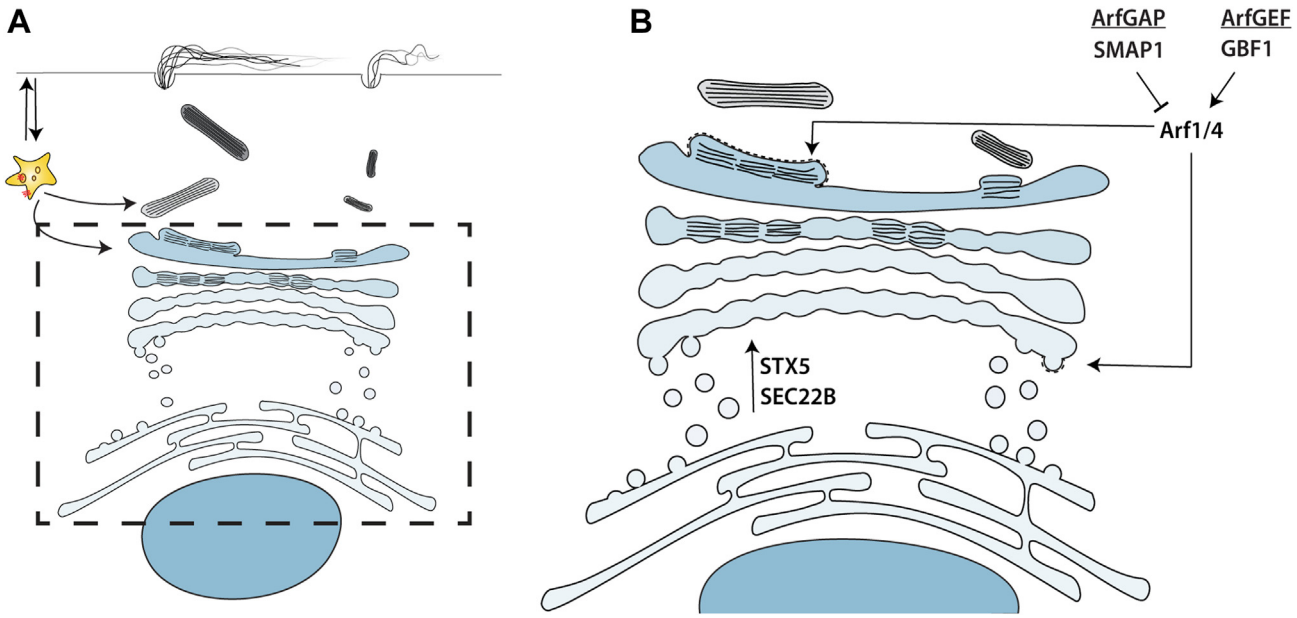

FIGURE 2 Weibel-Palade body (WPB) size is a regulated process. (A) Illustration depicting the regulated biogenesis of WPBs where von Willebrand factor quanta arrive at the *trans*-Golgi network (TGN) and are copackaged into newly forming WPBs. (B) Close-up view of the selected area highlighting the regulatory role of soluble NSF attachment receptor proteins SEC22B and syntaxin-5 (STX5), along with ADP-ribosylation factor (Arf) proteins, in the regulation of endoplasmic reticulum-Golgi trafficking. GAP, GTPase-activating protein; GEF, guanine nucleotide exchange factor.

trafficking from the endoplasmic reticulum (ER) to the TGN have been identified: soluble NSF attachment receptors (SNAREs) and ADP-ribosylation factor (Arf) proteins. Anterograde transport of VWF from the ER requires the longin-SNARE SEC22B and its cognate SNARE syntaxin-5 (STX5), located on the *cis*-Golgi membrane (Figure 2B). Depletion of either SNARE results in impaired ER-Golgi trafficking and Golgi fragmentation and leads to loss of elongated WPBs [19,20]. In SEC22B-depleted cells, dilations of the ER cisterna containing aggregates of VWF were observed by EM, indicative of defective progression of VWF to the TGN [19]. Arf proteins and the guanine nucleotide exchange factors (GEFs) that regulate them are now known to be crucial in VWF trafficking (Figure 2B). The Golgi-specific GEF, GBF1, plays a key role in VWF trafficking and links WPB biogenesis to cellular metabolism. When glucose levels drop, AMP-activated protein kinase activates GBF1, which in turn regulates Arf1 and Arf4 to control anterograde VWF trafficking, leading to smaller WPBs and reduced VWF secretion [21]. The Arf GTPase-activating protein SMAP1 is also reported to regulate WPB size [22]; although the mechanism appears not to involve Arf6, the Arf GTPase is associated with SMAP1 [21,22]. The mechanism by which SMAP1 regulates WPB size remains to be established but could be through clathrin recruitment during organelle formation. Importantly, loss of function of SNAREs or Arf proteins associated with WPB formation can affect their morphology in ways that are similar to what is observed in endothelial cells from patients with von Willebrand disease that cause mutations in VWF, emphasizing the relevance of the trafficking machinery in WPB biology and VWF function.

3 | NEW INSIGHTS INTO THE COMPOSITION OF WPBs

Although VWF is the primary cargo within WPBs, it has been known for many years that the luminal composition of these storage organelles can be highly complex and plastic, changing in response to fluid shear and pro- or anti-inflammatory signals [2,23–36] (Figure 1A). The diversity of cargo found within WPBs suggests that these organelles can take part in various physiological processes beyond hemostasis, notably leukocyte recruitment and angiogenesis [9,37,38]. How all these different soluble cargo molecules enter into and are subsequently retained within WPBs remains of considerable interest (Figure 1B). In several cases, the low pH and high calcium (Ca^{2+}) environment of the TGN appear to be important. Using *in vitro* binding assays with purified proteins, 2 studies have recently shown that the Tie-2 ligand angiopoietin-2 (Ang2) binds with high affinity to VWF under conditions of low pH and high Ca^{2+} similar to that of the TGN [39,40], indicating a mechanism similar to that of another soluble WPB cargo, osteoprotegerin [25,41]. However, while both studies confirm the binding of Ang2 to VWF, they diverge in their conclusions regarding the specific domains involved. Mobayen et al. [39] show binding to the A1 domain, whereas Texier et al. [40] suggest a broader interaction encompassing multiple domains in VWF. To what extent such *in vitro* protein-protein interactions can be extrapolated to what happens in the complex mix of proteins within the confines of the Golgi is uncertain. pH-dependent binding of chemokines to VWF may also mediate inclusion in WPBs [42,43], but mobility analysis of enhanced green fluorescent protein-tagged chemokines inside mature

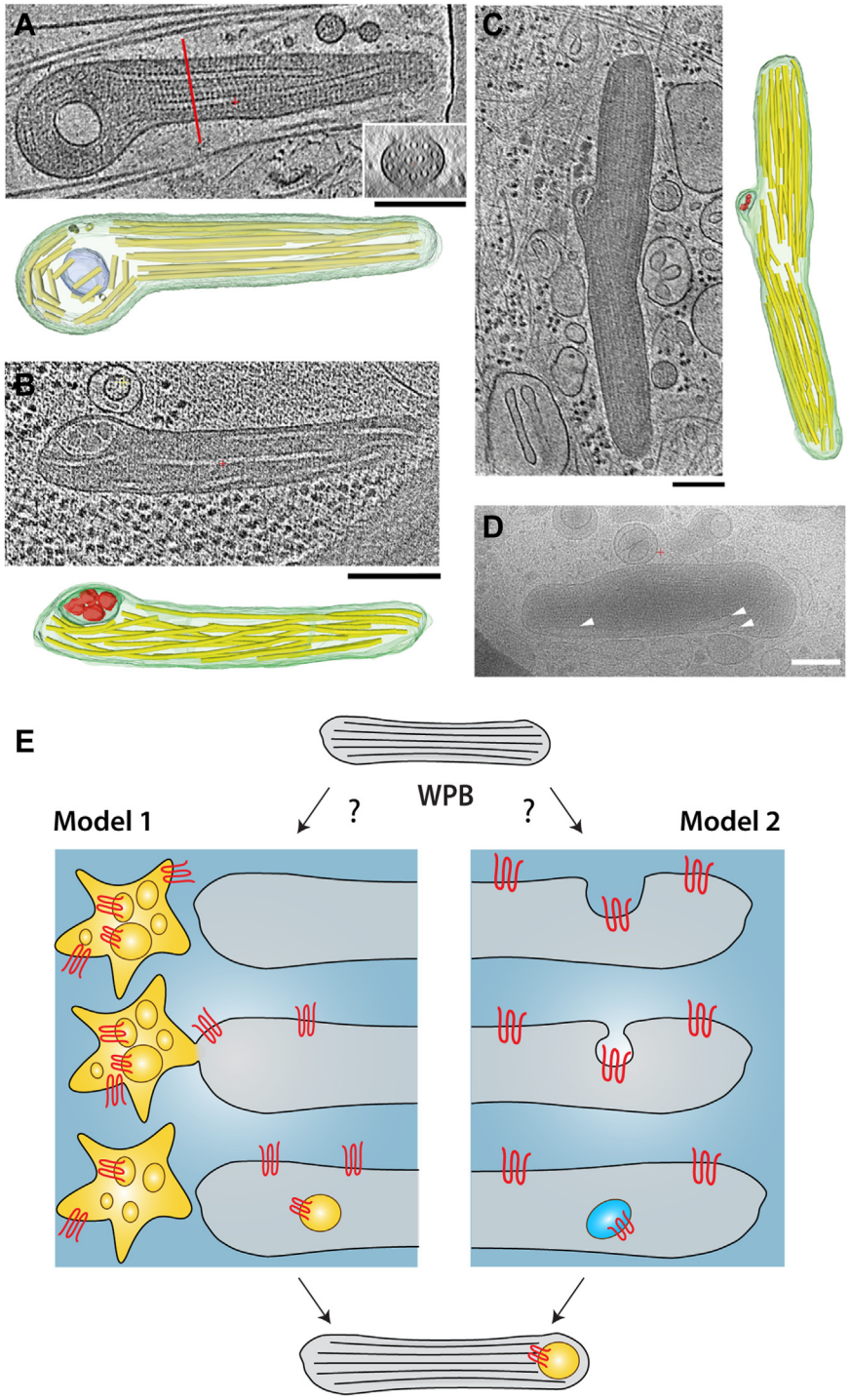

FIGURE 3 How may Weibel-Palade bodies (WPBs) acquire intraluminal vesicles? (A–D) Examples of WPBs containing intraluminal vesicles (ILVs). (B–C) ILVs often contain cytoplasmic components. (D) ILVs can be seen compressed between the WPB limiting membrane and the von Willebrand factor para-crystal. Scale bars 200 nm. (E) The 2 possible models by which WPBs may acquire ILVs. (A–D) Reproduced with permission of the rights holder, Elsevier, license numbers 5471370060842 and (A–C), 5481321142311 (D).

WPBs shows that such interactions are at most weak, enabling selectively rapid chemokine secretion during WPB exocytosis [44,45]. A route into the forming granule that is independent of interaction with VWF is also possible since a variant of factor VIII that lacks the high-affinity binding site for VWF and even enhanced green fluorescent protein attached to a signal peptide are able to enter the WPB when ectopically expressed in endothelial cells [26,46].

A key new discovery has been the identification of intraluminal vesicles (ILVs) inside WPBs (Figure 3A–D) and the demonstration of their release as exosomes during regulated exocytosis [47]. The discovery of a hitherto unrecognized pathway for exosome-mediated signaling by the endothelium raises many new questions, including when and how WPBs acquire ILVs, what they contain, and what they do once secreted. WPB-ILVs can be identified in individual organelles as discrete regions of enrichment of the endosomal/exosomal protein CD63. The absence of CD63 in newly forming WPBs connected to the TGN [48], coupled with the absence of ILVs detected in this compartment by ultrastructural studies [49], suggest that

CD63-positive ILVs are acquired at a later step in the life cycle of the organelle. Mature WPBs studied by cryo-electron tomography of vitrified cells show a very smooth limiting membrane shrink-wrapping a tightly packed para-crystalline assembly of tubules [18,47]. The absence of membrane ruffles suggests little or no excess membrane for ILV formation, and the rigid para-crystalline matrix is a physical obstacle to inward budding, which makes it unlikely that ILVs can still be incorporated into WPBs at this stage. In 2-dimensional and 3-dimensional cryo-EM images, ILVs were often seen to be highly distorted/squeezed between the smooth, tight-wrapped membrane and the para-crystalline tubule matrix (Figure 3D), suggesting compression during organelle dehydration. VWF tubules are inherently flexible but pack to a ridge para-crystal in the mature organelle [18]. Tubule disruption around ILVs indicates they were most likely present before dehydration and tubule packing. Thus, it seems likely that ILVs appear during the post-Golgi maturation phase when WPBs are actively acquiring endosomal components and sorting away unwanted materials [48–52] prior to final dehydration. The presence of cytoplasmic components inside WPB-ILVs (Figure 3B, C) [47] indicates they form by a process of inward membrane budding, but in which membrane compartment does this process occur? The WPB limiting membrane acquires CD63 and other integral membrane proteins (eg, VAMP8) from a late endosomal compartment as part of the post-Golgi maturation process [48,50,51,53]. CD63 delivery to post-Golgi-WPBs involves the adapter complex AP-3 [48,50], annexin-8 (CD63) [54], and potentially BLOC-2 [52]. While direct fusion and content transfer between endosomes and WPB has yet to be directly visualized, the nature of the proteins trafficked (integral membrane components), along with the intimate associations seen between WPBs and multivesicular bodies [18] or small carrier vesicles, make it the likely scenario. Tracking the fate of cell surface labeled CD63 in live cells showed that CD63 in WPB-ILVs (like that in the WPB limiting membrane) is also derived from endosomal trafficking [47]. This key observation gives rise to 2 possible scenarios for how WPBs acquire ILVs: first, preformed CD63-positive ILVs are transferred from late endosomes/multivesicular bodies to WPBs during fusion as part of the organelle's maturation process (Figure 3E model 1). Second, inward budding of the WPB membrane itself captures endosomal-derived CD63 after its delivery to the WPB limiting membrane (Figure 3E model 2). Future ultrastructural examination of WPBs in endothelial cells either depleted of CD63 or where endosomal-WPB trafficking is blocked (eg, in Hermansky-Pudlak syndrome type 2 or 6 [50,52]) will help clarify a role for endosomal-derived factors in WPB-ILV formation. Identification of a pore connecting an ILV to the WPB membrane would provide strong evidence for ILV biogenesis within the WPB.

The composition of WPB-ILVs is likely to be complex. Each ILV contains a small biopsy of the cell's cytoplasm with components such as ribosomes and glycogen granules clearly visible [47]. Proteomic and RNA sequence analysis of endothelial cell (EC)-derived exosomes has shown a rich diversity of proteins and RNA types, and functional studies implicate EC exosomes in regulating a wide range of processes, including cellular protection [55], atheroprotection [56], immune tolerance [57], antitumor response [58], and angiogenesis [59,60]. Interestingly, the angiogenic function of EC exosomes overlaps with those of other soluble WPB cargo proteins such as Ang2, VWF, and IGFBP7 [24,27,37,61], and it is possible that exosome release via the WPB-ILV pathway complements or augments the function of soluble angiogenic mediators in WPBs. Whether the exact composition of WPB-ILVs differs from other EC-derived exosomes and if the quantities of cargo released via this novel pathway are of sufficient magnitude to drive biological processes requires further study. Establishing how WPB-ILVs are formed will be an important step toward elucidating their physiological role. Such insights may also provide new approaches to selectively promote or interfere with the formation of WPB-ILVs, allowing for systematic investigations of their composition using multiomic profiling and functional studies of (purified) WPB-ILVs.

4 | MOLECULAR BASIS FOR WPB ACIDIFICATION

Recent studies have shed new light on the molecular basis for the regulation of intra-WPB pH during organelle formation and maturation [62–64]. During WPB formation, the acidic pH within the TGN (pH 6.5-6.2) facilitates the noncovalent association of VWF dimers through the N-terminal D1-D3 domains, leading to the formation of tubule-like structures [65,66] that position the D3 domains in such a way that allows intermolecular disulfide bond formation and VWF multimerization [67]. As VWF tubules grow, they drive the formation of the WPB, and the order packing of these long tubule structures is thought to be important for the way in which VWF multimers unfurl to form extracellular strings following secretion [68–70]. Since the acidification of TGN is maintained by the activity of vacuolar-type H^+ ATPases (v-ATPase) [71], it seems logical that such a pump operates within the WPB membrane since following scission from the TGN, the intra-WPB pH continues to decrease rapidly during maturation to close to the isoelectric point for VWF [69]. v-ATPases consist of 2 distinct domains that operate in tandem. The V1 domain, composed of 8 subunits, hydrolyses ATP, while the V0 domain, comprising 6 subunits, translocates the resulting H^+ ions through the host membrane [71] (Figure 4A). Recent proteomic analysis of WPB content identified components of this pump [27,72,73]. Blocking the v-ATPase complex has no effect on exocytosis but reduces VWF unfurling and, therefore, platelet recruitment, producing results similar to those observed with overall forced neutralization of the WPB interior through the use of ionophores or weak bases [63,70]. So far, the subunits V1G1, V1A, V0c, V0d1, V0a1, and V0a2 have been shown by immunofluorescence or proteomic analysis to be associated with WPBs [27,62,63]. Intriguingly, V0a1 and V0a2, 2 isoforms of the V0a subunit, appear to localize to WPBs at different maturation stages. V0a1 colocalizes with the maturation marker Rab27a on mature WPBs, while V0a2 is predominantly found in nascent WPBs in the perinuclear region [62] (Figure 4B). The presence of these isoforms, and thus 2 (or more) v-ATPase complexes with different functions, is confirmed by the distinct effects of depleting either of them. V0a1, primarily found on

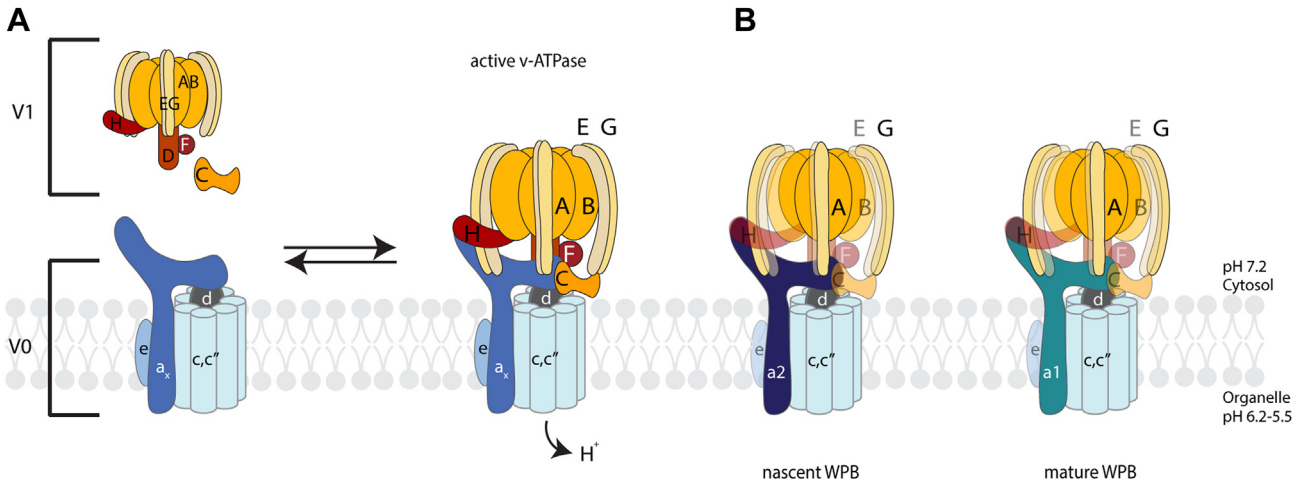

FIGURE 4 Vacuolar-type H^+ ATPases (v-ATPase) pump is present in the Weibel-Palade body (WPB) membrane. (A) The v-ATPase pump comprises 2 essential subunits: the soluble V1 domain, responsible for ATP hydrolysis, and the membrane-spanning V0 domain, which transfers resulting H^+ ions into the organelle. The pump functions effectively only when these subunits are together. (B) Distinct v-ATPase complexes, characterized by different isoforms of the V0a domain, are localized to different stages of WPB. V0a2 localizes to nascent WPB at the *trans*-Golgi network (TGN), while V0a1 is only found on mature WPB. The complete pump is shown for reference, with only the subunits identified through immunofluorescence or proteomic studies rendered opaque. Other subunits are depicted as transparent.

mature WPBs, appears to be crucial for separating WPB buds from the TGN. Whether this is a direct function of V0a1 or mediated by the recruitment of other proteins is still unclear. On the contrary, depletion of the V0a2 subunit results in WPB that are able to separate from the TGN but appear to have an altered tubule organization [62]. Their presence at different stages could indicate a regulatory mechanism where the transition from nascent to mature WPBs involves a switch in v-ATPase isoform composition, possibly influenced by signals from the cellular environment or specific cues within the TGN. Overall, it seems like V0a2 is a resident TGN subunit, and then V0a1 is recruited for membrane fission. Importantly, the depletion of these subunits not only affects WPBs but also leads to the neutralization of the luminal pH in the Golgi. This perturbation in Golgi pH balance could potentially have additional downstream effects, such as aberrant glycosylation of secretory proteins. Interestingly, in some patients with V0a2 mutations, bleeding diathesis and wound healing defects have been reported, as well as a low VWF activity to antigen ratio [74,75].

How might v-ATPase subunits be trafficked to mature WPBs? Rab27a depletion does not disrupt WPB acidification, suggesting that HPS6, a component of the BLOC-2 complex responsible for cargo trafficking from endosomes to lysosome-related organelles, may be involved in V0d1 delivery to WPBs, influencing their acidification and size [63,64]. More work is needed to establish the specific routes by which different v-ATPase subunits are delivered to WPBs. Many questions remain to be addressed: Do v-ATPases play a role in initiating acidification, or is their sole purpose to maintain the acidic luminal environment within mature WPBs? What is the mechanism and timing of complex assembly? Are there distinct subsets of organelles with varying v-ATPase compositions, and if so, how does this diversity affect WPB integrity and exocytosis?

5 | PROTEOMIC INSIGHTS INTO WPB MATURATION AND SECRETION

Newly formed WPBs are secretion-incompetent and acquire their secretion competence as they mature by the recruitment of soluble factors to their membrane [76]. Among these factors are Rab27a and Rab3d, 2 members of the Ras superfamily of small G proteins [77,78]. Recent research has revealed that the GEF, MAP kinase activating death domain (MADD) [79], activates both GTPases, resulting in their localization to WPB and the subsequent assembly of effector complexes on the WPB membrane [80–82]. Given its role in WPB maturation, Rab27a has been employed as bait in numerous proteomic studies to identify novel effector proteins that localize to WPB (Figure 5A). We anticipate that these proteomic approaches will continue to unveil both known and previously unexplored proteins relevant to WPB biology. Four studies on the WPB interactome have so far been published that use 2 different approaches. The first approach involved "classical" pulldown experiments with the Rab27a effector synaptotagmin-like protein 4a (Slp4-a) or the SNARE proteins STX3 and syntaxin binding protein (STXBP) 5 (STXBP5, formerly known as tomosyn) [72,83]. The second approach, applied by 2 different groups, is the use of Rab27a-APEX2 proximity biotinylation [73,84] (Figure 5A). The strength of proteomic studies lies in the combination of results from multiple research teams, thereby enriching the pool of biologically significant discoveries. To pinpoint frequently detected protein interactors, we have combined the results of the 4 published studies with WPB effector proteins in Figure 5B. While there is not a single common hit across all studies, there are 85 proteins found in multiple of them. Twelve out of these 85 proteins have been published in the context of WPB biogenesis (**bold** in Figure 5B [21,63,64,72,76,85–93]). Other hits

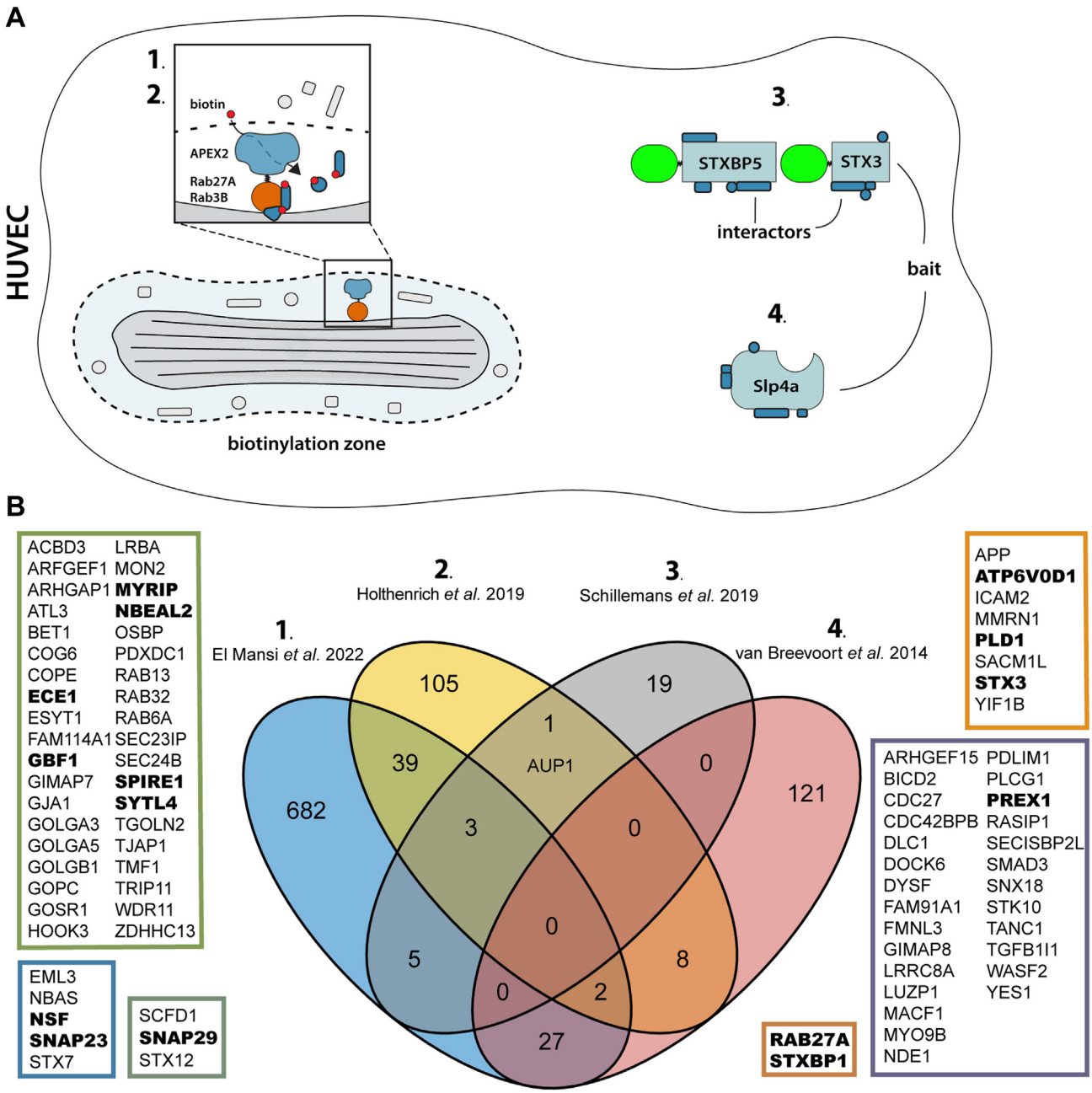

FIGURE 5 Combined results of 4 published proteomic screens for regulators of Weibel-Palade body exocytosis. (A) Simplified visualization of methodologies used in distinct studies: 1) proximity biotinylation using APEX2-EGFP-Rab27a by El-Mansi et al. [84], 2) proximity biotinylation with APEX2-Rab27a by Holthenrich et al. [73], 3) pulldown assays with STXBP5-EGFP and STX3-EGFP by Schillemans et al [83], and 4) GST pulldown with synaptotagmin-like protein 4a (Slp4-a) by van Breevoort et al. [72] (B) Venn diagram displaying gene names of identified hits across all studies. No common hit was observed in all 4 studies. Genes found in 2 or 3 studies are displayed in colored boxes matching the respective Venn diagram segment colors. Proteins previously associated with Weibel-Palade body biology are denoted in **bold font**. EGFP, enhanced green fluorescent protein; GST, glutathione S-transferase; STX3, syntaxin-3; STXBP5, syntaxin binding protein 5.

consist of proteins involved in various cellular processes, including vesicle transport (such as SNARE, COPII, and NRZ), Golgi proteins (some of them previously associated with Golgi fragmentation upon knockdown), Rab and Rho GTPases along with their GEFs and GTPase activating proteins and the actin cytoskeleton. These findings provide further support for the involvement of these protein groups in the regulation, formation, and function of WPB, highlighting the complexity of WPB trafficking and fusion mechanisms. Notably, biallelic mutations in some of the identified proteins, such as ARFGEF1 (BIG1), ATL3, BET1, and WDR11, have been associated with rare and severe diseases with predominantly neurologic phenotypes [94–97]. The rarity of these diseases shows the critical importance of intracellular trafficking. However, it has been shown that knocking down ARFGEF1 has no apparent impact on WPB formation [21]. This underscores the need for

validation of the specific contribution of these individual proteins to WPB biology and VWF secretion and that such a role cannot be assumed.

6 | CALCIUM-SENSITIVE PROTEINS AND REGULATORS IN WPB EXOCYTOSIS

In endothelial cells, WPB exocytosis is primarily driven by sustained high levels of intracellular Ca^{2+} ions, which occur during stimulation by physiological agonists or cellular injury. However, our understanding of how these Ca^{2+} increases are sensed and translated into WPB exocytosis remains incomplete, prompting research to focus on this aspect of the process. Prior to stimulated exocytosis, WPBs located in the cell periphery are anchored to the actin cytoskeleton via the Rab27a effectors MyRIP and Myosin Va [90,98,99]. This serves to strategically position the organelles close to the plasma membrane (PM) while preventing premature exocytosis. As calcium levels increase within the cell in response to hormones or injury, a series of calcium-sensitive proteins come into play, contributing to PM docking and fusion of WPBs. WPBs bind to specific PM microdomains enriched for phosphatidylinositol 4,5-bisphosphate (PIP_2) [100]. The formation of these microdomains is orchestrated by calcium-dependent regulation of RalA, which, in turn, promotes Arf6-dependent phospholipase D1 (PLD1) activity [85]. Two Ca^{2+} binding effector proteins of Rab27a, Slp4-a and Munc13-4, guide WPB localization to the PM [76,82,101]. Munc13-4 associates with S100A10 and operates in conjunction with annexin A2 (AnxA2), which is recruited in a Ca^{2+}-dependent fashion to newly formed PIP2 microdomains. Together, these proteins form the adapter complex that links WPB to the PM [82]. Additionally, WPBs dock to the SNARE machinery on the PM via a Rab27a/Slp4-a/STXBP1 or STXBP3 complex [72,76]. The resulting SNARE complexes that are required for WPB-PM fusion consist of the R-SNAREs VAMP3/VAMP8 (on the organelle) [50,88] and the Q-SNAREs STX3/STX4/SNAP23 (on the PM) [86,87,102]. While STX2 is frequently identified in genetic association studies with levels of WPB proteins [103,104], its role in this fusion process remains unconfirmed by experimental evidence. A more recent discovery introduces Slp2-a as a Ca^{2+}-dependent effector of Rab27a. This protein mediates tip-end fusion of the organelle, a noteworthy revelation given their unconventional shape [81]. This mediation is achieved through the Ca^{2+}-dependent condensation of Slp2-a, which can bind to PIP2 at the WPB tip. Although this is beneficial for the release of highly multimeric VWF, it does not significantly impact WPB-PM fusion. This explains the relatively mild inhibitory effect of Slp2a knockdown on total VWF secretion [81].

When WPBs have reached the PM, the stage is set for fusion to occur, a process intricately linked to the regulation of calcium levels in conjunction with the SNARE fusion machinery. Notably, SNAREs themselves lack inherent sensitivity for Ca^{2+}. Instead, the calcium dependence of exocytosis is widely attributed to synaptotagmins, a family of Ca^{2+}-sensing proteins renowned for their interaction with SNAREs. Among these synaptotagmin proteins, synaptotagmin 5 (SYT5) emerged as a key player in the orchestration of WPB exocytosis, functioning as a vesicle-associated calcium sensor [105]. SYT5 exhibits a lower affinity for Ca^{2+} than other synaptotagmins, a trait that seemingly serves as a regulatory mechanism. It is believed that SYT5 predominantly recruits effector molecules when intracellular Ca^{2+} levels increase to higher thresholds, a feature that functions as a safeguard [105]. This selective recruitment strategy effectively reduces the risk of untimely release of high molecular mass forms of VWF and inflammatory mediators when their presence is unwarranted. The link of SYT5 to the SNARE complexes involved in WPB exocytosis has not been found, and it is unknown whether other synaptotagmins play a role in this process. At the final stages of WPB exocytosis, Rab46, a calcium-sensing Rab GTPase, might play a role by redirecting a subpopulation of WPBs away from the PM to the microtubule organizing center in response to histamine [106].

In summary, the stimulated release of WPBs is a highly orchestrated process involving preparation of the PM by altering the lipid composition, moving the organelle to the PM, granule polarization, and SNARE formation that finally results in fusion and release (Figure 6). Novel key participants have been identified, but the underlying molecular mechanisms governing their functions require further investigation.

7 | DIVERSE MECHANISMS OF VWF SECRETION AND ACTOMYOSIN DYNAMICS IN WPB EXOCYTOSIS

There are 3 ways VWF can be secreted. Constitutive secretion consists of constant, unimpeded release of newly synthesized VWF that is directly released from the TGN via constitutive vesicles without the need for cellular stimulation. Cargo from this route is characterized by short, low molecular weight forms of VWF that are primarily released at the endothelial cell's basolateral side into the subendothelial matrix [107]. This pool of VWF can bind platelets [108] but consists of short multimers and does not form long strings [14]. Basal release, which involves nonstimulated release of regulated cargo, consists of continuous low-rate secretion of higher molecular weight VWF multimers that originate from WPBs and are preferentially released at the apical face of the endothelium [107,109]. This route has been postulated to account for the vast majority of circulating VWF. The regulated secretion pathway, triggered by agonists, releases a rapid bolus of high-molecular-weight VWF in the vessel lumen by simultaneous exocytosis of a large number of WPBs. Ca^{2+} or cAMP serve as second messengers in response to these signals. Regulated secretion of WPB involves 2 distinct mechanisms. In the majority of WPBs, VWF is rapidly expelled from the organelle upon fusion with the PM. The explosive release of tubularly packed VWF is driven by the entry of water molecules and an increase in WPB luminal pH that occurs immediately upon fusion [69,110]. In a smaller fraction of WPBs, approximately 20% to 30%, expulsion of VWF is not immediate but occurs several seconds after fusion and is associated with the formation of an actomyosin ring around the postfusion organelle [81,83,111,112]

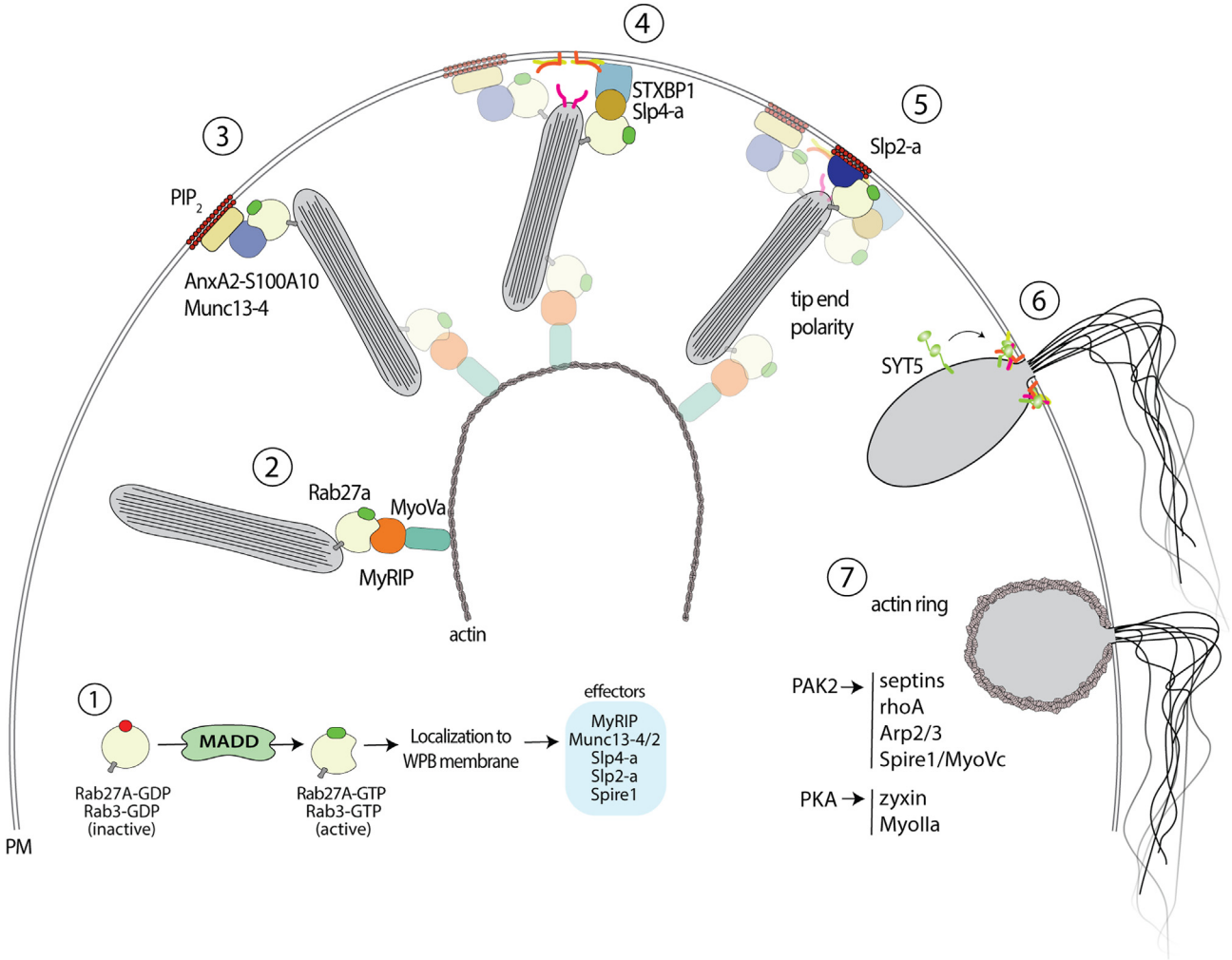

FIGURE 6 Exocytosis of Weibel-Palade body (WPB). This diagram depicts the key steps in WPB exocytosis, which may occur simultaneously. 1) The activation of soluble factors (Rab GTPases) that localize to the WPB membrane and recruit several effector complexes. 2) Anchoring of WPBs to the actin skeleton via MyRIP and Myosin Va (MyoVa). 3) Tethering to the PM facilitated by the formation of a complex involving Munc13-4, which interacts with AnxA2-S100A10 bound to 4,5-bisphosphate (PIP_2) microdomains, bringing the vesicle in close proximity to the target membrane. 4) WPBs dock to the plasma membrane as synaptotagmin-like protein 4a (Slp4-a) binds to syntaxin binding proteins (STXBP), linking the organelle to the soluble NSF attachment receptors fusion machinery. 5) Polarity of the WPB is established by the condensation of Slp2-a at the tip-end of the vesicle, ensuring a specific orientation during the fusion process. 6) Soluble NSF attachment receptor interactions, facilitated by synaptotagmin 5 (SYT5) upon a surge in Ca^{2+} levels, lead to membrane fusion between the WPB and the plasma membrane (PM) and expulsion of content. 7) In a subset of postfusion events, actin ring formation occurs. PAK2, p21-activated kinase 2; PKA, protein kinase A.

(Figure 6). Two functions for actomyosin structures are proposed: firstly, to stabilize the fusion pore and contribute to sustained exocytosis; secondly, to facilitate the expulsion of high-molecular-weight VWF through a mechanical "squeezing" process. The actomyosin ring refers to a complex structure formed by the coordinated interaction between actin filaments and myosin motor proteins. It is not a single protein or entity but rather a composite structure made up of multiple proteins working together. It typically consists of actin filaments that provide the structural framework, myosin motor proteins that generate contractile forces, and other regulatory proteins that modulate its structure and activity. It is worth noting that the depletion of Slp2-a also disrupts actin ring assembly [81]. This suggests that a specific organelle orientation might be a requirement for actin ring formation. Furthermore, ongoing investigations continue to explore the composition and functional nuances of actin rings in the context of WPB exocytosis, revealing variability based on the specific stimulus employed [69,110–114]. During cAMP-mediated stimulation, the focal adhesion protein zyxin mediates the formation of actin frameworks around the WPB in a myosin II-a-dependent manner [113,114]. The knockdown of zyxin does not affect Ca^{2+}-mediated VWF secretion, suggesting that different mechanisms of actin nucleation are employed after different stimuli [114]. Other studies revealed that histamine stimulation or wounding, both Ca^{2+} mediated, can induce actin ring

formation in a subset of postfusion WPBs, again confirming that this phenomenon occurs at a later stage than the initial release [111,115]. This process is dependent on RhoA, which potentially activates Arp2/3 or Spire1, effectors of the Rho GTPase that facilitate actin nucleation at WPB fusion sites [115,116]. Additionally, Spire1 interacts with MyoVc and can bind to Rab27a or Rab3 isoforms, thereby facilitating its recruitment to WPB-PM fusion sites. Of note, even with a mutated Rab3 binding site in MyoVc, its recruitment to WPBs remains observable, hinting at additional recruitment modes, potentially involving interactions with Spire1. Depletion of Spire1 or MyoVc results in diminished surface VWF and reduced actin ring formation following histamine stimulation [115]. This underscores their roles in initiating or stabilizing these actin structures. The mechanisms by which Spire1 and MyoVc are activated remain unknown. Septin rings are recruited to fused WPBs before actin rings form. Their primary role is likely to assist in the coordination of myosin proteins (eg, NMII or Myo1C) or their activators to facilitate actomyosin ring function. The recruitment of septin hetero-oligodimers to WPBs is initiated by p21-activated kinase 2 (PAK2). In the absence of septins, the actomyosin ring exhibits slower contraction kinetics and eventually disassembles [84].

The complexity of actomyosin ring formation is exemplified by the diverse array of processes described, including signaling pathways (involving PAK2 and Rho GTPases), actin nucleation (influenced by Spire1 and Arp2/3, as well as zyxin for reorganization), linking, and actomyosin contraction (involving septins and myosins). However, several fundamental questions remain unanswered: Firstly, the mechanism by which actomyosin attaches to the organelle membrane remains a puzzle. Secondly, the molecular physical route that drives the "squeezing" process itself remains unknown. Lastly, there is the question surrounding the biological significance of these actomyosin rings, given that the majority of VWF is secreted rapidly in a subsecond expulsion manner that is independent of their function.

8 | CONCLUSIONS AND FUTURE DIRECTIONS

In conclusion, WPBs intricately regulate the storage and release of VWF and an array of additional cargo molecules, responding to environmental cues. From the formation and maturation of WPBs to the precise cargo release mechanisms, there remains much to explore and comprehend. By deciphering the complex pathways that result in WPB formation and expulsion, we can potentially intervene in pathologic conditions, such as vascular complications seen in COVID-19, and advance treatments for disorders related to aberrant WPB function. Moreover, selective tuning of the endothelial response, including aspects like WPB size selection, cargo inclusion, and the strength of expulsion, remain interesting questions in WPB biology that will be explored further in the next few years. The journey through WPB complexity offers great potential for both scientific discovery and clinical application, promising innovative strategies to manipulate these cellular structures for therapeutic benefit.

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AUTHOR CONTRIBUTIONS

S.H. created the figures, and all authors contributed to the writing of the paper.

DECLARATION OF COMPETING INTERESTS

The authors state that they have no conflict of interest.

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REFERENCES

[1] Weibel ER, Palade GE. New cytoplasmic components in arterial endothelia. *J Cell Biol*. 1964;23:101–12.
[2] Wagner DD, Olmsted JB, Marder VJ. Immunolocalization of von Willebrand protein in Weibel-Palade bodies of human endothelial cells. *J Cell Biol*. 1982;95:355–60.
[3] Grant MA, Beeler DL, Spokes KC, Chen J, Dharaneeswaran H, Sciuto TE, Dvorak AM, Interlandi G, Lopez JA, Aird WC. Identification of extant vertebrate Myxine glutinosa VWF: evolutionary conservation of primary hemostasis. *Blood*. 2017;130:2548–58.
[4] Doolittle RF. Step-by-step evolution of vertebrate blood coagulation. *Cold Spring Harb Symp Quant Biol*. 2009;74:35–40.
[5] Zakas PM, Coyle CW, Brehm A, Bayer M, Solecka-Witulska B, Radford CE, Brown C, Nesbitt K, Dwyer C, Kannicht C, Trent Spencer H, Gaucher EA, Doering CB, Lillicrap D. Molecular coevolution of coagulation factor VIII and von Willebrand factor. *Blood Adv*. 2021;5:812–22.
[6] Karampini E, Bierings R, Voorberg J. Orchestration of primary hemostasis by platelet and endothelial lysosome-related organelles. *Arterioscler Thromb Vasc Biol*. 2020;40:1441–53.
[7] Schillemans M, Karampini E, Kat M, Bierings R. Exocytosis of Weibel–Palade bodies: how to unpack a vascular emergency kit. *J Thromb Haemost*. 2019;17:6–18.
[8] Schillemans M, Kat M, Westeneng J, Gangaev A, Hofman M, Nota B, van Alphen FPJ, de Boer M, van den Biggelaar M, Margadant C, Voorberg J, Bierings R. Alternative trafficking of Weibel-Palade body proteins in CRISPR/Cas9-engineered von Willebrand factor–deficient blood outgrowth endothelial cells. *Res Pract Thromb Haemost*. 2019;3:718–32.
[9] Denis CV, André P, Saffaripour S, Wagner DD. Defect in regulated secretion of P-selectin affects leukocyte recruitment in von Willebrand factor-deficient mice. *Proc Natl Acad Sci U S A*. 2001;98:4072–7.
[10] Wang JW, Bouwens EAM, Pintao MC, Voorberg J, Safdar H, Valentijn KM, De Boer HC, Mertens K, Reitsma PH, Eikenboom J. Analysis of the storage and secretion of von willebrand factor in blood outgrowth endothelial cells derived from patients with von Willebrand disease. *Blood*. 2013;121:2762–72.
[11] Valentijn KM, Eikenboom J. Weibel-Palade bodies: a window to von Willebrand disease. *J Thromb Haemost*. 2013;11:581–92.
[12] Springer TA. von Willebrand factor, Jedi knight of the bloodstream. *Blood*. 2014;124:1412–25.

[13] Valentijn KM, Sadler JE, Valentijn JA, Voorberg J, Eikenboom J. Functional architecture of Weibel-Palade bodies. *Blood*. 2011;117:5033–43.

[14] Ferraro F, Patella F, Costa JR, Ketteler R, Kriston-Vizi J, Cutler DF. Modulation of endothelial organelle size as an antithrombotic strategy. *J Thromb Haemost*. 2020;18:3296–308.

[15] Kat M, Margadant C, Voorberg J, Bierings R. Dispatch and delivery at the ER–Golgi interface: how endothelial cells tune their hemostatic response. *FEBS J*. 2022;289:6863–70.

[16] Ferraro F, Kriston-Vizi J, Metcalf DJ, Martin-Martin B, Freeman J, Burden JJ, Westmoreland D, Dyer CE, Knight AE, Ketteler R, Cutler DF. A two-tier golgi-based control of organelle size underpins the functional plasticity of endothelial cells. *Dev Cell*. 2014;29:292–304.

[17] Page KM, McCormack JJ, Lopes-da-Silva M, Patella F, Harrison-Lavoie K, Burden JJ, Quah YYB, Scaglioni D, Ferraro F, Cutler DF. Structure modeling hints at a granular organization of the Golgi ribbon. *BMC Biol*. 2022;20:111.

[18] Berriman JA, Li S, Hewlett LJ, Wasilewski S, Kiskin FN, Carter T, Hannah MJ, Rosenthal PB. Structural organization of Weibel-Palade bodies revealed by cryo-EM of vitrified endothelial cells. *Proc Natl Acad Sci U S A*. 2009;106:17407–12.

[19] Karampini E, Bürgisser PE, Olins J, Mulder AA, Jost CR, Geerts D, Voorberg J, Bierings R. Sec22b determines Weibel-Palade body length by controlling anterograde endoplasmic reticulum-Golgi transport. *Haematologica*. 2021;106:1138–47.

[20] Kat M, Karampini E, Hoogendijk AJ, Bürgisser PE, Mulder AA, van Alphen FPJ. Syntaxin 5 determines Weibel-Palade body size and von Willebrand factor secretion by controlling Golgi architecture. *Haematologica*. 2022;107:1827–39.

[21] Lopes-da-Silva M, McCormack JJ, Burden JJ, Harrison-Lavoie KJ, Ferraro F, Cutler DF. A GBF1-dependent mechanism for environmentally responsive regulation of ER-Golgi transport. *Dev Cell*. 2019;49:786–801.e6.

[22] Watanabe A, Hataida H, Inoue N, Kamon K, Baba K, Sasaki K, Kimura R, Sasaki H, Eura Y, Ni W-F, Shibasaki Y, Waguri S, Kokame K, Shiba Y. Arf GTPase-activating proteins SMAP1 and AGFG2 regulate the size of Weibel-Palade bodies and exocytosis of von Willebrand factor. *Biol Open*. 2021;10:bio058789.

[23] Bonfanti R, Furie BC, Furie BC, Wagner DD. PADGEM (GMP140) is a component of Weibel-Palade bodies of human endothelial cells. *Blood*. 1989;73:1109–12.

[24] Fiedler U, Scharpfenecker M, Koidl S, Hegen A, Grunow V, Schmidt JM, Kriz W, Thurston G, Augustin HG. The Tie-2 ligand Angiopoietin-2 is stored in and rapidly released upon stimulation from endothelial cell Weibel-Palade bodies. *Blood*. 2004;103:4150–6.

[25] Zannettino ACW, Holding CA, Diamond P, Atkins GJ, Kostakis P, Farrugia A, Gamble J, To LB, Findlay DM, Haynes DR. Osteoprotegerin (OPG) is localized to the Weibel-Palade bodies of human vascular endothelial cells and is physically associated with von Willebrand factor. *J Cell Physiol*. 2005;204:714–23.

[26] Knipe L, Meli A, Hewlett L, Bierings R, Dempster J, Skehel P, Hannah MJ, Carter T. A revised model for the secretion of tPA and cytokines from cultured endothelial cells. *Blood*. 2010;116:2183–91.

[27] Van Breevoort D, Van Agtmaal EL, Dragt BS, Gebbinck JK, Dienava-Verdoold I, Kragt A, Bierings R, Horrevoets AJG, Valentijn KM, Eikenboom JC, Fernandez-Borja M, Meijer AB, Voorberg J. Proteomic screen identifies IGFBP7 as a novel component of endothelial cell-specific weibel-palade bodies. *J Proteome Res*. 2012;11:2925–36.

[28] Rondaij MG, Bierings R, Kragt A, Van Mourik JA, Voorberg J. Dynamics and plasticity of Weibel-Palade bodies in endothelial cells. *Arterioscler Thromb Vasc Biol*. 2006;26:1002–7.

[29] Rosenberg JB, Foster PA, Kaufman RJ, Vokac EA, Moussalli M, Kroner PA, Montgomery RR. Intracellular trafficking of factor VIII to von Willebrand factor storage granules. *J Clin Invest*. 1998;101:613–24.

[30] Huber D, Cramer EM, Kaufmann JE, Meda P, Massé JM, Kruithof EKO, Vischer UM. Tissue-type plasminogen activator (t-PA) is stored in Weibel-Palade bodies in human endothelial cells both in vitro and in vivo. *Blood*. 2002;99:3637–45.

[31] Ozaka T, Doi Y, Kayashima K, Fujimoto S. Weibel-Palade bodies as a storage site of calcitonin gene-related peptide and endothelin-1 in blood vessels of the rat carotid body. *Anat Rec*. 1997;247:388–94.

[32] Saint-Lu N, Oortwijn BD, Pegon JN, Odouard S, Christophe OD, de Groot PG, Denis CV, Lenting PJ. Identification of galectin-1 and galectin-3 as novel partners for von Willebrand factor. *Arterioscler Thromb Vasc Biol*. 2012;32:894–901.

[33] McEver RP, Beckstead JH, Moore KL, Marshall-Carlson L, Bainton DF. GMP-140, a platelet α-granule membrane protein, is also synthesized by vascular endothelial cells and is localized in Weibel-Palade bodies. *J Clin Invest*. 1989;84:92–9.

[34] Utgaard JO, Jahnsen FL, Bakka A, Brandtzaeg P, Haraldsen G. Rapid secretion of prestored interleukin 8 from Weibel-Palade bodies of microvascular endothelial cells. *J Exp Med*. 1998;188:1751–6.

[35] Øynebråten I, Bakke O, Brandtzaeg P, Johansen FE, Haraldsen G. Rapid chemokine secretion from endothelial cells originates from 2 distinct compartments. *Blood*. 2004;104:314–20.

[36] Vischer UM, Wagner DD. CD63 is a component of Weibel-Palade bodies of human endothelial cells. *Blood*. 1993;82:1184–91.

[37] Cossutta M, Darche M, Carpentier G, Houppe C, Ponzo M, Raineri F, Vallée B, Gilles ME, Villain D, Picard E, Casari C, Denis C, Paques M, Courty J, Cascone I. Weibel-Palade bodies orchestrate pericytes during angiogenesis. *Arterioscler Thromb Vasc Biol*. 2019;39:1843–58.

[38] Doyle EL, Ridger V, Ferraro F, Turmaine M, Saftig P, Cutler DF. CD63 is an essential cofactor to leukocyte recruitment by endothelial P-selectin. *Blood*. 2011;118:4265–73.

[39] Mobayen G, Smith K, Ediriwickrema K, Starke RD, Solomonidis EG, Laffan MA, Randi AM, McKinnon TAJ. von Willebrand factor binds to angiopoietin-2 within endothelial cells and after release from Weibel-Palade bodies. *J Thromb Haemost*. 2023;21:1802–12.

[40] Texier A, Lenting PJ, Denis CV, Roullet S, Christophe OD. Angiopoietin-2 binds to multiple interactive sites within von Willebrand factor. *Res Pract Thromb Haemost*. 2023;7:102204.

[41] Shahbazi S, Lenting PJ, Fribourg C, Terraube V, Denis CV, Christophe OD. Characterization of the interaction between von Willebrand factor and osteoprotegerin. *J Thromb Haemost*. 2007;5:1956–62.

[42] Bierings R, van den Biggelaar M, Kragt A, Mertens K, Voorberg J, van Mourik JA. Efficiency of von Willebrand factor-mediated targeting of interleukin-8 into Weibel-Palade bodies. *J Thromb Haemost*. 2007;5:2512–9.

[43] Hol J, Küchler AM, Johansen FE, Dalhus B, Haraldsen G, Øynebråten I. Molecular requirements for sorting of the chemokine interleukin-8/CXCL8 to endothelial Weibel-Palade bodies. *J Biol Chem*. 2009;284:23532–9.

[44] Kiskin NI, Babich V, Knipe L, Hannah MJ, Carter T. Differential cargo mobilisation within weibel-palade bodies after transient fusion with the plasma membrane. *PLoS One*. 2014;9:e108093.

[45] Kiskin NI, Hellen N, Babich V, Hewlett L, Knipe L, Hannah MJ, Carter T. Protein mobilities and P-selectin storage in Weibel-Palade bodies. *J Cell Sci*. 2010;123:2964–75.

[46] Van Den Biggelaar M, Bierings R, Storm G, Voorberg J, Mertens K. Requirements for cellular co-trafficking of factor VIII and von Willebrand factor to Weibel-Palade bodies. *J Thromb Haemost*. 2007;5:2235–43.

[47] Streetley J, Fonseca AV, Turner J, Kiskin NI, Knipe L, Rosenthal PB, Carter T. Stimulated release of intraluminal vesicles from Weibel-Palade bodies. *Blood*. 2019;133:2707–17.
[48] Harrison-Lavoie KJ, Michaux G, Hewlett L, Kaur J, Hannah MJ, Lui-Roberts WW, et al. P-selectin and CD63 use different mechanisms for delivery to Weibel-Palade bodies. *Traffic*. 2006;7:647–62.
[49] Zenner HL, Collinson LM, Michaux G, Cutler DF. High-pressure freezing provides insights into Weibel-Palade body biogenesis. *J Cell Sci*. 2007;120:2117–25.
[50] Karampini E, Schillemans M, Hofman M, Van Alphen F, De Boer M, Kuijpers TW, Van Den Biggelaar M, Voorberg J, Bierings R. Defective AP-3-dependent VAMP8 trafficking impairs Weibel-Palade body exocytosis in Hermansky-Pudlak syndrome type 2 blood outgrowth endothelial cells. *Haematologica*. 2019;104:2091–9.
[51] Kobayashi T, Vischer UM, Rosnoblet C, Lebrand C, Lindsay M, Parton RG, Kruithof EKO, Gruenberg J. The tetraspanin CD63/lamp3 cycles between endocytic and secretory compartments in human endothelial cells. *Mol Biol Cell*. 2000;11:1829–43.
[52] Sharda AV, Barr AM, Harrison JA, Wilkie AR, Fang C, Mendez LM, Ghiran IC, Italiano JE, Flaumenhaft R. VWF maturation and release are controlled by 2 regulators of Weibel-Palade body biogenesis: exocyst and BLOC-2. *Blood*. 2020;136:2824–37.
[53] Vischer UM, Wagner DD. von Willebrand factor proteolytic processing and multimerization precede the formation of Weibel-Palade bodies. *Blood*. 1994;83:3536–44.
[54] Poeter M, Brandherm I, Rossaint J, Rosso G, Shahin V, Skryabin BV, Zarbock A, Gerke V, Rescher U. Annexin A8 controls leukocyte recruitment to activated endothelial cells via cell surface delivery of CD63. *Nat Commun*. 2014;5:1–13.
[55] Abid Hussein MN, Nieuwland R, Hau CM, Evers LM, Meesters EW, Sturk A. Cell-derived microparticles contain caspase 3 in vitro and in vivo. *J Thromb Haemost*. 2005;3:888–96.
[56] Hergenreider E, Heydt S, Tréguer K, Boettger T, Horrevoets AJG, Zeiher AM, Scheffer MP, Frangakis AS, Yin X, Mayr M, Braun T, Urbich C, Boon RA, Dimmeler S. Atheroprotective communication between endothelial cells and smooth muscle cells through miRNAs. *Nat Cell Biol*. 2012;14:249–56.
[57] Song J, Chen X, Wang M, Xing Y, Zheng Z, Hu S. Cardiac endothelial cell-derived exosomes induce specific regulatory B cells. *Sci Rep*. 2014;4:7583.
[58] Bovy N, Blomme B, Frères P, Dederen S, Nivelles O, Lion M, Carnet O, Martial JA, Noël A, Thiry M, Jérusalem G, Josse C, Bours V, Tabruyn SP, Struman I. Endothelial exosomes contribute to the antitumor response during breast cancer neoadjuvant chemotherapy via microRNA transfer. *Oncotarget*. 2015;6:10253–66.
[59] van Balkom BW, de Jong OG, Smits M, Brummelman J, den Ouden K, de Bree PM, van Eijndhoven MA, Pegtel DM, Stoorvogel W, Würdinger T, Verhaar MC. Endothelial cells require miR-214 to secrete exosomes that suppress senescence and induce angiogenesis in human and mouse endothelial cells. *Blood*. 2013;121:3997–4006.
[60] Lombardo G, Dentelli P, Togliatto G, Rosso A, Gili M, Gallo S, Deregibus MC, Camussi G, Brizzi MF. Activated Stat5 trafficking via endothelial cell-derived extracellular vesicles controls IL-3 pro-angiogenic paracrine action. *Sci Rep*. 2016;6:25689.
[61] Starke RD, Ferraro F, Paschalaki KE, Dryden NH, McKinnon TAJ, Sutton RE, Payne EM, Haskard DO, Hughes AD, Cutler DF, Laffan MA, Randi AM. Endothelial von Willebrand factor regulates angiogenesis. *Blood*. 2011;117:1071–80.
[62] Yamazaki Y, Eura Y, Kokame K. V-ATPase V0a1 promotes Weibel-Palade body biogenesis through the regulation of membrane fission. *Elife*. 2021;10:e71526.
[63] Terglane J, Menche D, Gerke V. Acidification of endothelial Weibel-Palade bodies is mediated by the vacuolar-type H+-ATPase. *PLoS One*. 2022;17:e0270299.
[64] Lu J, Ma J, Hao Z, Li W. HPS6 regulates the biogenesis of Weibel-Palade body in endothelial cells through trafficking v-ATPase to its limiting membrane. *Front Cell Dev Biol*. 2021;9:743124.
[65] Zeng J, Shu Z, Liang Q, Zhang J, Wu W, Wang X, Zhou A. Structural basis of von Willebrand factor multimerization and tubular storage. *Blood*. 2022;139:3314–24.
[66] Huang RH, Wang Y, Roth R, Yu X, Purvis AR, Heuser JE, Egelman EH, Sadler JE. Assembly of Weibel-Palade body-like tubules from N-terminal domains of von Willebrand factor. *Proc Natl Acad Sci U S A*. 2008;105:482–7.
[67] Dong X, Springer TA. Disulfide exchange in multimerization of von Willebrand factor and gel-forming mucins. *Blood*. 2021;137:1263–7.
[68] Mayadas TN, Wagner DD. In vitro multimerization of von Willebrand factor is triggered by low pH. *J Biol Chem*. 1989;264:13497–503.
[69] Erent M, Meli A, Moisoi N, Babich V, Hannah MJ, Skehel P, Knipe L, Zupančič G, Ogden D, Carter T. Rate, extent and concentration dependence of histamine-evoked Weibel-Palade body exocytosis determined from individual fusion events in human endothelial cells. *J Physiol*. 2007;583:195–212.
[70] Michaux G, Abbitt KB, Collinson LM, Haberichter SL, Norman KE, Cutler DF. The physiological function of von Willebrand's factor depends on its tubular storage in endothelial Weibel-Palade bodies. *Dev Cell*. 2006;10:223–32.
[71] Forgac M. Vacuolar ATPases: rotary proton pumps in physiology and pathophysiology. *Nat Rev Mol Cell Biol*. 2007;8:917–29.
[72] Van Breevoort D, Snijders AP, Hellen N, Weckhuysen S, Van Hooren KWEM, Eikenboom J, Valentijn K, Fernandez-Borja M, Ceulemans B, De Jonghe P, Voorberg J, Hannah M, Carter T, Bierings R. STXBP1 promotes Weibel-Palade body exocytosis through its interaction with the Rab27A effector Slp4-a. *Blood*. 2014;123:3185–94.
[73] Holthenrich A, Drexler HCA, Chehab T, Naß J, Gerke V. Proximity proteomics of endothelial Weibel-Palade bodies identifies novel regulator of von Willebrand factor secretion. *Blood*. 2019;134:979–82.
[74] Karacan İ, Diz Küçükkaya R, Karakuş FN, Solakoğlu S, Tolun A, Hançer VS, Turanlı ET. A novel ATP6V0A2 mutation causing recessive cutis laxa with unusual manifestations of bleeding diathesis and defective wound healing. *Turkish J Haematol*. 2019;36:29–36.
[75] Beyens A, Moreno-Artero E, Bodemer C, Cox H, Gezdirici A, Yilmaz Gulec E, Kahloul N, Khau Van Kien P, Ogur G, Harroche A, Vasse M, Salhi A, Symoens S, Hadj-Rabia S, Callewaert B. ATP6V0A2-related cutis laxa in 10 novel patients: focus on clinical variability and expansion of the phenotype. *Exp Dermatol*. 2019;28:1142–5.
[76] Bierings R, Hellen N, Kiskin N, Knipe L, Fonseca AV, Patel B, Meli A, Rose M, Hannah MJ, Carter T. The interplay between the Rab27A effectors Slp4-a and MyRIP controls hormone-evoked Weibel-Palade body exocytosis. *Blood*. 2012;120:2757–67.
[77] Hannah MJ, Hume AN, Arribas M, Williams R, Hewlett LJ, Seabra MC, Cutler DF. Weibel-Palade bodies recruit Rab27 by a content-driven, maturation-dependent mechanism that is independent of cell type. *J Cell Sci*. 2003;116:3939–48.
[78] Knop M, Aareskjold E, Bode G, Gerke V. Rab3D and annexin A2 play a role in regulated of vWF, but not tPA, from endothelial cells. *EMBO J*. 2004;23:2982–92.
[79] Kat M, Bürgisser PE, Janssen H, De Cuyper IM, Conte IL, Hume AN, Carter T, Voorberg J, Margadant C, Bierings R. GDP/GTP exchange factor MADD drives activation and recruitment of secretory Rab GTPases to Weibel-Palade bodies. *Blood Adv*. 2021;5:5116–27.
[80] Francis CR, Claflin S, Kushner EJ. Synaptotagmin-like protein 2a regulates angiogenic lumen formation via Weibel-Palade body apical secretion of angiopoietin-2. *Arterioscler Thromb Vasc Biol*. 2021;41:1972–86.

[81] Naß J, Koerdt SN, Biesemann A, Chehab T, Yasuda T, Fukuda M, Martín-Belmonte F, Gerke V. Tip-end fusion of a rod-shaped secretory organelle. *Cell Mol Life Sci*. 2022;79:344.
[82] Chehab T, Santos NC, Holthenrich A, Koerdt SN, Disse J, Schuberth C, Nazmi AR, Neeft M, Koch H, Man KNM, Wojcik SM, Martin TFJ, Van Der Sluijs P, Brose N, Gerke V. A novel Munc13-4/S100A10/annexin A2 complex promotes Weibel-Palade body exocytosis in endothelial cells. *Mol Biol Cell*. 2017;28:1688–700.
[83] Schillemans M, Karampini E, Hoogendijk AJ, Wahedi M, van Alphen FPJ, van den Biggelaar M, Voorberg J, Bierings R. Interaction networks of Weibel-Palade body regulators syntaxin-3 and syntaxin binding protein 5 in endothelial cells. *J Proteomics*. 2019;205:103417.
[84] El-Mansi S, Robinson CL, Kostelnik KB, McCormack JJ, Mitchell TP, Lobato-Márquez D, Rajeeve V, Cutillas P, Cutler DF, Mostowy S, Nightingale TD. Proximity proteomics identifies septins and PAK2 as decisive regulators of actomyosin-mediated expulsion of von Willebrand factor. *Blood*. 2023;141:930–44.
[85] Disse J, Vitale N, Bader MF, Gerke V. Phospholipase D1 is specifically required for regulated secretion of von Willebrand factor from endothelial cells. *Blood*. 2009;113:973–80.
[86] Schillemans M, Karampini E, Van Den Eshof BL, Gangaev A, Hofman M, Van Breevoort D, Meems H, Janssen H, Mulder AA, Jost CR, Escher JC, Adam R, Carter T, Koster AJ, Van Den Biggelaar M, Voorberg J, Bierings R. Weibel-palade body localized syntaxin-3 modulates von willebrand factor secretion from endothelial cells. *Arterioscler Thromb Vasc Biol*. 2018;38:1549–61.
[87] Zhu Q, Yamakuchi M, Lowenstein CJ. SNAP23 regulates endothelial exocytosis of von Willebrand factor. *PLoS One*. 2015;10:e0118737.
[88] Pulido IR, Jahn R, Gerke V. VAMP3 is associated with endothelial Weibel-Palade bodies and participates in their Ca2+-dependent exocytosis. *Biochim Biophys Acta Mol Cell Res*. 2011;1813:1038–44.
[89] Russell FD, Skepper JN, Davenport AP. Human endothelial cell storage granules: a novel intracellular site for isoforms of the endothelin-converting enzyme. *Circ Res*. 1998;83:314–21.
[90] Nightingale TD, Pattni K, Hume AN, Seabra MC, Cutler DF. Rab27a and MyRIP regulate the amount and multimeric state of VWF released from endothelial cells. *Blood*. 2009;113:5010–8.
[91] Kat M, van Moort I, Bürgisser PE, Kuijpers TW, Hofman M, Favier M, Favier R, Margadant C, Voorberg J, Bierings R. Mutations in neurobeachin-like 2 do not impact Weibel-Palade body biogenesis and von Willebrand factor secretion in gray platelet syndrome endothelial colony forming cells. *Res Pract Thromb Haemost*. 2023;7:100086.
[92] Holthenrich A, Mietkowska M, Kerkhoff E, Gerke V. The actin regulators Spire1 and Myosin Vc localize to endothelial Weibel-Palade bodies and facilitate calcium evoked von Willebrand factor surface presentation. *FEBS Open Bio*. 2021;11:378.
[93] van Hooren KWEM, van Breevoort D, Fernandez-Borja M, Meijer AB, Eikenboom J, Bierings R, Voorberg J. Phosphatidylinositol-3,4,5-triphosphate-dependent rac exchange factor 1 regulates epinephrine-induced exocytosis of weibel-palade bodies. *J Thromb Haemost*. 2014;12:273–81.
[94] Cintra VP, Dohrn MF, Tomaselli PJ, Figueiredo FB, Marques SE, Camargos ST, Barbosa LSM, P. Rebelo A, Abreu L, Danzi M, Marques W, Züchner S. Rare mutations in ATL3, SPTLC2 and SCN9A explaining hereditary sensory neuropathy and congenital insensitivity to pain in a Brazilian cohort. *J Neurol Sci*. 2021;427:117498.
[95] Donkervoort S, Krause N, Dergai M, Yun P, Koliwer J, Gorokhova S, Geist Hauserman J, Cummings BB, Hu Y, Smith R, Uapinyoying P, Ganesh VS, Ghosh PS, Monaghan KG, Edassery SL, Ferle PE, Silverstein S, Chao KR, Snyder M, Ellingwood S, et al. BET1 variants establish impaired vesicular transport as a cause for muscular dystrophy with epilepsy. *EMBO Mol Med*. 2021;13:e13787.
[96] Thomas Q, Gautier T, Marafi D, Besnard T, Willems M, Moutton S, Isidor B, Cogné B, Conrad S, Tenconi R, Iascone M, Sorlin A, Masurel A, Dabir T, Jackson A, Banka S, Delanne J, Lupski JR, Saadi NW, Alkuraya FS, et al. Haploinsufficiency of ARFGEF1 is associated with developmental delay, intellectual disability, and epilepsy with variable expressivity. *Genet Med*. 2021;23:1901–11.
[97] Haag N, Tan EC, Begemann M, Buschmann L, Kraft F, Holschbach P, Lai AHM, Brett M, Mochida GH, DiTroia S, Pais L, Neil JE, Al-Saffar M, Bastaki L, Walsh CA, Kurth I, Knopp C. Biallelic loss-of-function variants in WDR11 are associated with microcephaly and intellectual disability. *Eur J Hum Genet*. 2021;29:1663–8.
[98] Conte IL, Hellen N, Bierings R, Mashanov GI, Manneville JB, Kiskin NI, Hannah MJ, Molloy JE, Carter T. Interaction between MyRIP and the actin cytoskeleton regulates Weibel-Palade body trafficking and exocytosis. *J Cell Sci*. 2016;129:592–603.
[99] Rojo Pulido I, Nightingale TD, Darchen F, Seabra MC, Cutler DF, Gerke V. Myosin Va acts in concert with Rab27a and MyRIP to regulate acute von-Willebrand factor release from endothelial cells. *Traffic*. 2011;12:1371–82.
[100] Nguyen TTN, Koerdt SN, Gerke V. Plasma membrane phosphatidylinositol (4,5)-bisphosphate promotes Weibel-Palade body exocytosis. *Life Sci Alliance*. 2020;3:e202000788.
[101] Zografou S, Basagiannis D, Papafotika A, Shirakawa R, Horiuchi H, Auerbach D, Fukuda M, Christoforidis S. A complete rab screening reveals novel insights in Weibel-Palade body exocytosis. *J Cell Sci*. 2012;125:4780–90.
[102] Fu J, Naren AP, Gao X, Ahmmed GU, Malik AB. Protease-activated receptor-1 activation of endothelial cells induces protein kinase Calpha-dependent phosphorylation of syntaxin 4 and Munc18c: role in signaling p-selectin expression. *J Biol Chem*. 2005;280:3178–84.
[103] Huang J, Huffman JE, Yamkauchi M, Trompet S, Asselbergs FW, Sabater-Lleal M, Trégouët DA, Chen WM, Smith NL, Kleber ME, Shin SY, Becker DM, Tang W, Dehghan A, Johnson AD, Truong V, Folkersen L, Yang Q, Oudot-Mellkah T, Buckley BM, et al. Genome-wide association study for circulating tissue plasminogen activator levels and functional follow-up implicates endothelial STXBP5 and STX2. *Arterioscler Thromb Vasc Biol*. 2014;34:1093–101.
[104] van Loon JE, Sanders YV, de Wee EM, Kruip MJHA, de Maat MPM, Leebeek FWG. Effect of genetic variation in STXBP5 and STX2 on von Willebrand factor and bleeding phenotype in type 1 von Willebrand disease patients. *PLoS One*. 2012;7:e40624.
[105] Lenzi C, Stevens J, Osborn D, Hannah MJ, Bierings R, Carter T. Synaptotagmin 5 regulates calcium-dependent Weibel-Palade body exocytosis in human endothelial cells. *J Cell Sci*. 2019;132:jcs221952.
[106] Miteva KT, Pedicini L, Wilson LA, Jayasinghe I, Slip RG, Marszalek K, Gaunt HJ, Bartoli F, Deivasigamani S, Sobradillo D, Beech DJ, McKeown L. Rab46 integrates Ca2+ and histamine signaling to regulate selective cargo release from Weibel-Palade bodies. *J Cell Biol*. 2019;218:2232–46.
[107] Lopes da Silva M, Cutler DF. Von Willebrand factor multimerization and the polarity of secretory pathways in endothelial cells. *Blood*. 2016;128:277–85.
[108] Sakariassen KS, Bolhuis PA, Sixma JJ. Human blood platelet adhesion to artery subendothelium is mediated by factor VIII-Von Willebrand factor bound to the subendothelium. *Nature*. 1979;279:636–8.
[109] Giblin JP, Hewlett LJ, Hannah MJ. Basal secretion of von willebrand factor from human endothelial cells. *Blood*. 2008;112:957–64.
[110] Conte IL, Cookson E, Hellen N, Bierings R, Mashanov G, Carter T. Is there more than one way to unpack a Weibel-Palade body? *Blood*. 2015;126:2165–7.
[111] Mietkowska M, Schuberth C, Wedlich-Söldner R, Gerke V. Actin dynamics during Ca2+-dependent exocytosis of endothelial Weibel-Palade bodies. *Biochim Biophys Acta Mol Cell Res*. 2019;1866:1218–29.
[112] Nightingale TD, White IJ, Doyle EL, Turmaine M, Harrison-Lavoie KJ, Webb KF, Cramer LP, Cutler DF. Actomyosin II contractility expels von Willebrand factor from Weibel-Palade bodies during exocytosis. *J Cell Biol*. 2011;194:613–29.

[113] Han X, Li P, Yang Z, Huang X, Wei G, Sun Y, Kang X, Hu X, Deng Q, Chen L, He A, Huo Y, Li D, Betzig E, Luo J. Zyxin regulates endothelial von Willebrand factor secretion by reorganizing actin filaments around exocytic granules. *Nat Commun*. 2017;8:14639.

[114] Li P, Wei G, Cao Y, Deng Q, Han X, Huang X, Huo Y, He Y, Chen L, Luo J. Myosin IIa is critical for cAMP-mediated endothelial secretion of von Willebrand factor. *Blood*. 2018;131:686–98.

[115] Holthenrich A, Terglane J, Naß J, Mietkowska M, Kerkhoff E, Gerke V. Spire1 and Myosin Vc promote Ca2+-evoked externalization of von Willebrand factor in endothelial cells. *Cell Mol Life Sci*. 2022;79:96.

[116] Tran DT, Masedunskas A, Weigert R, Ten Hagen KG. Arp2/3-mediated F-actin formation controls regulated exocytosis in vivo. *Nat Commun*. 2015;6:10098.