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WPBs: making a mark and leaving a trail.

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Abstract:

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1 **WPBs: making a mark and leaving a trail**

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8 In this issue of *Blood*, Holthenrich et al¹ used a proximity labelling approach to pull, from out of the
9 crowded intracellular milieu, proteins that specifically interact with the Weibel-Palade body (WPB).

10 From the resulting catalogue of proteins the authors identified Munc13-2 as a novel WPB-associated
11 SNARE-interacting protein that positively regulates hormone-evoked WPB exocytosis.

12

13 WPBs are specialized secretory granules containing the adhesive glycoprotein von Willebrand factor
14 (VWF), the leukocyte adhesion molecule P-selectin and a host of angiogenic and inflammatory
15 mediators.² These unusual organelles have been likened to a “vascular emergency kit” that functions to
16 minimize blood loss, to fight infections and repair damaged blood vessels at sites of injury. Correct
17 formation, trafficking and exocytosis of WPBs involves a huge workforce comprising a great many
18 proteins, most of which we know very little about. In their study Holthenrich et al¹ have attempted to
19 identify these proteins and so narrow this major gap in our knowledge.

20

21 To do this Holthenrich et al¹ applied a fast and highly sensitive proximity-labelling methodology based
22 on fusions of WPB specific Rab GTPases, Rab27a or Rab3b, and a modified ascorbate peroxidase,
23 APEX2.³ In the presence of its substrate, biotin-phenol, and hydrogen peroxide, APEX2 catalyzes the
24 biotinylation of proteins located in its immediate vicinity (10-20 nm).³ The speed (seconds) and

1 efficiency of APEX2 makes it particularly useful in catching weak or transient protein interactions, and
2 the biotin-tagged proteins can then be extracted, purified and identified using mass spectroscopy to
3 yield a protein interactome for the Rab-APEX2-labelled WPBs.

4

5 The choice of Rab27a and Rab3b was important for several reasons. First, these Rab proteins are
6 recruited in a highly specific fashion to WPBs soon after they bud from the trans-Golgi network and
7 remain with the organelle till its destruction upon exocytosis. This allowed the identification of
8 interactors at all stages in the life cycle of the WPB. Second, both Rab proteins are fairly promiscuous in
9 their interactions with cytosolic effectors and so provided an ideal base from which to sample and catch
10 direct interactions with potential effectors. Third, because both Rabs use a C-terminal lipid anchor to
11 attach to the outer leaflet of the WPB membrane they are highly mobile ⁴, rapidly (sub second) exploring
12 the surface of the WPB to provide a dynamic “active zone” for APEX2 to “mark” proteins on or close to
13 the organelle. Finally, Rab27a and Rab3b are likely to mediate very distinct functions on the WPB that
14 may potentially be revealed through the specific protein interactomes.

15

16 The approach was successful. First, Holthenrich et al ¹ were able to identify most of the known WPB
17 regulators including integral membrane proteins incorporated into the organelle during biogenesis and
18 maturation (VAMP3, syntaxin-3, SLEP, ATP6V02), effectors recruited to the organelle from the
19 cytoplasm (e.g. Rab27a, Rab3a/b/c, Slp4-a, MyRIP) or through interactions with endomembrane
20 compartments or the plasma membrane (e.g. CD63, PLD1, STXBP1).

21

22 More important was that this elegant study revealed that the “known” components are just the tip of
23 the “iceberg” that comprises the WPB interactome. In total 183 proteins were identified of which 158
24 were common to both Rab27a and Rab3b. Amongst these was a new component of the WPB secretory

1 machinery, Munc13-2, a SNARE-interacting protein that was shown to promote WPB exocytosis. Newly
2 formed WPBs remain within the crowded environment of the trans-Golgi network as they acquire
3 Rab27a or Rab3b, a process that takes up to 30 minutes.⁵ It is perhaps not surprising therefore that a
4 number of the 183 proteins identified by both Rabs comprised ER or Golgi components, including the
5 Golgi-specific brefeldin A-resistance guanine nucleotide exchange factor 1 (GBF1), recently identified as
6 a master regulator of environmental cue driven WPB formation.⁶ Whether these ER/Golgi proteins
7 reflect “innocent bystanders” labelled within the dense confines of the cellular biosynthetic factory, or
8 in fact play a direct role in the itinerary of the WPB remains to be established. Certainly, en route to the
9 plasma membrane the WPB must navigate a cytoplasm crowded with endomembrane compartments
10 and cytoskeletal components, most graphically illustrated in recent cryo-electron microscopy studies of
11 WPBs in intact vitrified endothelial cells.^{7,8} Incredibly close physical interactions are observed between
12 WPBs and a variety of endomembrane components that may account for APEX2 detection of Rab7,
13 Rab11, SNAP29, Rab14 and the Rab5 effector Rab11FIP5. Many of these close interactions are likely to
14 be of functional importance, for example, in the delivery of CD63 and possibly other components to the
15 WPB.⁸⁻¹⁰ Indeed, the pattern of detected proteins is almost akin to a “ghost” of the path taken by the
16 organelle as it moves through the cell.

17

18 Of interest was the apparent absence of molecules implicated in the final stages of WPB fusion at the
19 plasma membrane (e.g. S100A10/AnnexinA2, syntaxin-2, syntaxin-4, STXBP3, STXBP5, RalGDS, RalA).
20 The reason for this may lie in the requirement for active translocation of such molecules to the plasma
21 membrane during cellular activation, or because WPBs, unlike secretory organelles of many
22 neuroendocrine cells, do not appear to be docked in close proximity to the plasma membrane prior to
23 activation. The current study was carried out in resting cells, and one can envisage that future studies in

1 cells stimulated with a range of agonists operating through different signalling pathways may yet reveal
2 these and novel components of the machinery regulating WPB trafficking and exocytosis.

3

4 In conclusion, the study by Holthenrich et al ¹ has provided a wealth of new information about the
5 itinerary and interaction of WPBs as they traverse the intracellular milieu. The catalogue of new
6 interactors identified will provide fertile ground for new studies, and a detailed analysis of the molecules
7 captured by Rab27a or Rab3b may provide clues to functions not previously recognized.

8

9

10 **Figure Legend**

11 Proximity ligation identifies the Weibel-Palade body (WPB) interactome. Newly formed WPBs acquire
12 the Rab3b-APEX2 or Rab27a-APEX2 fusion proteins which, upon addition of biotin, generates an active
13 zone of biotinylation around the organelle, randomly labelling proteins that are associated with the
14 granule or that are in its immediate vicinity. As the WPB makes its way through the cell it encounters
15 (and interacts with) various subcellular organelles and intracellular membranes, a path which is
16 illuminated through the identification of sets of proteins that are found on the ER, Golgi, mitochondria
17 and endosomal compartments such as the late endosomes or multivesicular bodies (LE/MVB), recycling
18 endosomes (RE) and early endosomes (EE).

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