Ride the wave: Retrograde trafficking becomes Ca^{2+} dependent with BAIAP3

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The functions of four of the five proteins in the mammalian uncoordinated-13 (Munc13) family have been identified as priming factors in SNARE-dependent exocytosis. In this issue, Zhang et al. (2017. *J. Cell Biol.* https://doi.org/10.1083/jcb.201702099) show that the fifth member, BAIAP3 (brain-specific angiogenesis inhibitor I–associated protein 3), acts in retrograde trafficking by returning secretory vesicle material to the trans-Golgi network. In its absence, secretory vesicle formation is impaired, leading to accumulation of immature vesicles, or lysosomal vesicle degradation.

That regulated exocytosis of the dense-core vesicle is triggered by Ca^{2+} is common knowledge. However, fusion of secretory vesicles with the plasma membrane is but one step in a circular trafficking pathway that involves the return of vesicle components to the Golgi/TGN. Thus, the ability of Ca^{2+} to stimulate massive fusion of vesicles with the plasma membrane puts a high load on the retrograde pathway, which potentially could be overwhelmed during strong stimulation. One possible way to prevent such overload would be to render the retrograde pathway Ca^{2+} dependent as well. In this issue, Zhang et al. report that such a Ca^{2+} dependence might be conferred by BAIAP3 (brain-specific angiogenesis inhibitor I–associated protein 3). Very little was known about the cellular function of BAIAP3 before this investigation, but in the brain it was known to be expressed in the amygdala (associated with aversive and fear responses), the hypothalamus (involved in autonomous functions), and in the periaqueductal gray (involved in descending pain regulation). Its deletion in mice was shown previously to cause seizures, increased anxiety in females, and benzodiazepine tolerance in males (Wojcik et al., 2013).

Zhang et al. (2017) started by performing an siRNA screen of all human C2 domain proteins for their involvement in Ca^{2+}-dependent secretion. This resulted in the identification of 40 inhibitory siRNA pools, among them many of the usual suspects: for instance, the Ca^{2+} sensor for fusion synaptotagmin-10, as well as CAPS1 and Unc13B, two vesicle-priming proteins. *BAIAP3* was identified as a new gene potentially involved in secretion. This was unexpected because recent data obtained in adrenal chromaffin cells of the BAIAP knockout mouse identified no secretion defects (Man et al., 2015). Zhang et al. (2017) studied two different secretory cell lines: serotonin-secreting human carcinoid BON cells and insulin-secreting rat INS-1 cells. Knockdown of BAIAP3 reduced Ca^{2+}-dependent secretion in both cell types, but in different ways. In BON cells, reduction of spontaneous release resulted in an accumulation of secretory granules, which were less fusogenic and remained in an immature state as revealed by increased colocalization with VAMP4 and impaired trafficking to the plasma membrane. In INS-1 cells, BAIAP3 deficiency reduced the level of prohormone convertase 2 (PC2) and insulin and the number of insulin-positive granules. Interestingly, blocking lysosomal degradation recovered the level of PC2 and insulin- and synaptotagmin-9–positive structures, indicating that insulin granules had been lost as a result of lysosomal degradation. Overall, therefore, even though results in the two cell types differed—possibly because BAIAP3 knockdown was more complete in INS-1 cells than in BON cells—BAIAP3 is necessary for secretory granule maturation.

Further experiments showed that BAIAP3 colocalizes with endosomal markers Rab9 and Rab11 and binds to the endosomal SNAREs syntaxin-6, syntaxin-16, VAMP3, and VAMP4 in a Ca^{2+}-dependent manner (Zhang et al., 2017). This set of SNAREs are known to form a SNARE complex together with vti1a (the SNARE complex would include either VAMP3 or VAMP4) and mediate fusion of endosomes to the TGN (Mallard et al., 2002). Thus, the interaction of BAIAP3 with this set of SNAREs strongly points to a function in endosome–TGN fusion, although these presumed fusion events were not visualized directly, but inferred from mislocalization of TGN46 and Golgin-97 in cells where BAIAP3 had been knocked down (Zhang et al., 2017). Strikingly, recent work from the same laboratory showed that Munc13-4—the mammalian protein most closely related to BAIAP3—acts alternatively in the fusion of secretory granules with the plasma membrane and in homotypic fusion of secretory granules with each other in a mast cell line (Woo et al., 2017). Another group identified a function of Munc13-4 in late endosome maturation (He et al., 2016). Thus, whereas Munc13-1, -2, and -3 act exclusively in priming vesicles for exocytosis at the plasma membrane, Munc13-4 takes on functions in both exocytosis and intracellular trafficking events, and BAIAP3 so far appears to be specialized for the latter. The result is that those intracellular trafficking events become Ca^{2+} dependent (Fig. 1; Woo et al., 2017; Zhang et al., 2017). Exactly how Munc13-4 and BAIAP3 act on their respective SNARE complexes will have to be addressed in future experiments. This work will no doubt be informed by the ever more detailed

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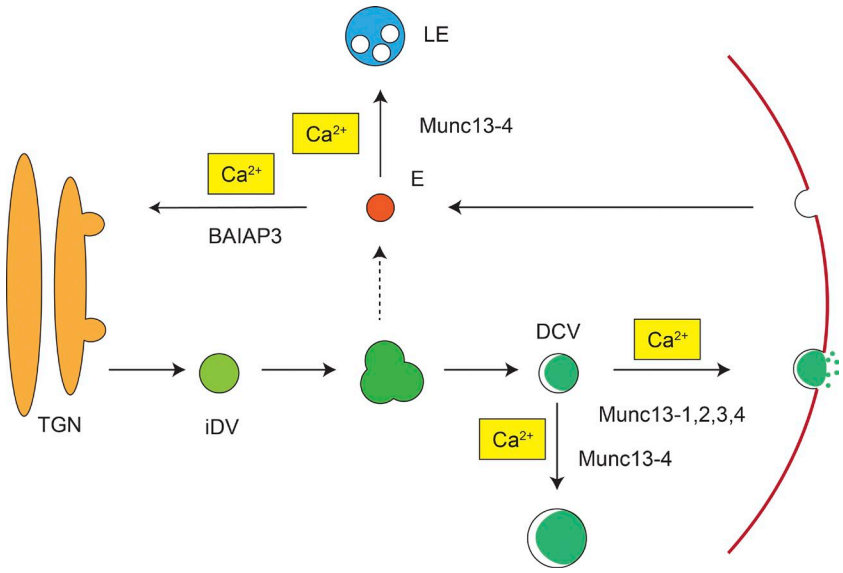

Figure 1. **The roles of the Unc13 family in post-Golgi trafficking.** Munc13-1, 2, 3, and 4 execute functions in exocytosis in different cell types. In addition, Munc13-4 acts in the homotypic fusion of secretory vesicles (Woo et al., 2017) and in the maturation of late endosomes (He et al., 2016). In this issue, Zhang et al. (2017) identify the function of BAIAP3 in endosome to TGN trafficking. All Unc13 protein members confer Ca^{2+} dependence to the fusion reaction they support. E, endosome; LE, late endosome; iDV, immature dense-core vesicle; DCV, mature dense-core vesicle. Drawn with inspiration from Zhang et al. (2017) to include the function of other Unc13 family members.

picture of Munc13-1–stimulated SNARE complex formation during neuronal exocytosis. In short, all Unc13 proteins contain two tandem Munc13 homology domains (MHDs), flanked by two Ca^{2+}-binding C2 domains. In addition, Munc13-1, -2, and -3 contain a diacylglycerol-binding C1 domain, important for membrane recruitment, and Munc13-1 and -2 contain another N-terminal C2 domain (denoted as C2A; Pinheiro et al., 2016). The MHDs are weakly homologous and structurally similar to tethering factors for intracellular trafficking, e.g., the exocyst, conserved oligomeric Golgi complex (COP), Golgi-associated retrograde protein complex (GARP), and Dsl1p complexes (Pei et al., 2009). It is possible that MHDs and tethering factors play similar roles, which might include direct functions in SNARE complex assembly or indirect functions by inhibiting SNARE disassembly by the ATPase NSF (Rizo and Südhof, 2012). BAIAP3 and Munc13-4 might have evolved to perform non-exocytosis functions in order to furnish intracellular fusion reactions with regulation by Ca^{2+} (Fig. 1).

Important questions to be answered include, where does the Ca^{2+} come from to activate BAIAP3 (and Munc13-4)? What are the Ca^{2+} dependency, kinetics, and capacity of the retrograde trafficking pathway? Does it match exocytosis under different stimulation conditions, and when does mismatch occur? As a starting point, Zhang et al. (2017) showed that ionomycin treatment to cause Ca^{2+} influx made BAIAP3 accumulate in the TGN area and, curiously, at the plasma membrane. However, during milder, more physiological stimulation, it remains unclear whether BAIAP3 would "ride the wave" of the Ca^{2+} entering through voltage-gated channels to drive retrograde trafficking or whether local sources of Ca^{2+} are recruited. In support of the latter possibility, earlier work showed that BAPTA was more effective in blocking retrograde transport of Shiga toxin than EGTA (Chen et al., 2002). Because BAPTA displays faster kinetics of Ca^{2+} binding than EGTA, the standard interpretation of this result is that a local source of Ca^{2+} is involved, although other possibilities remain. Answering these questions might involve combining high resolution live imaging with accurate manipulation and measurement of Ca^{2+} concentrations. Another open question is the exact link between deficient endosome to TGN trafficking and the appearance of immature granules in the cell. Which factor is responsible for the defect? The answers to these questions should be of wide interest to cell biologists, but also to researchers working with neurodegenerative diseases, which are often attributed to defects in the endosomal pathways, including endosome to TGN trafficking (Schreij et al., 2016).

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