Saving the neck from scission

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ecretry granule biogenesis is a pivotal process for regulated release of hormones and neurotransmitters. A prominent example is the pancreatic β cell that secretes insulin, a major abonic hormone controlling cellular metabolism upon nutrient availability. We recently described a checkpoint mechanism that halts scission of nascent secretory granules at the trans-Golgi network (TGN) until complete loading of insulin is achieved. We demonstrated that the Bin/Amphiphysin/Rvs (BAR) domain-containing protein Arfaptin-1 prevents granule scission until it is phosphorylated by Protein Kinase D (PKD). Arfaptin-1 phosphorylation releases its binding to ADP-rybosylation factor (ARF) allowing scission to occur. Lack of this control mechanism in β cells resulted in premature scission, generation of dysfunctional insulin granules and impaired regulated insulin secretion without affecting constitutive release of other transport carriers. Here we discuss two important questions related to this work: How might completion of granule loading be sensed by PKD, and how does Arfaptin-1 specifically regulate insulin granule formation in beta cells? Endocrine cells are highly specialized factories tailored for production and release of hormones and signaling molecules. The pancreatic β cell constitutes one prominent member of this cell type that dedicates a significant portion of its total protein synthesis to production of the hormone insulin, a key regulator of metabolic homeostasis. The whole process from synthesis of preproinsulin at the rough endoplasmatic reticulum to packaging and storing of mature insulin in secretory granules (Fig. 1A) is remarkably efficient in β cells. The majority of cells degrade around 33% of newly synthesized protein due to translation, folding, processing or targeting errors. In β cells, however, more than 99% of newly synthesized preproinsulin are successfully routed and packaged into secretory granules. This extremely effective machinery in β cells suggests that a series of quality control checkpoints safeguards proper granule generation from preproinsulin translation and folding up to proinsulin sorting and formation of secretory granules. Recent work of our laboratory unveiled how β cells ensure proper secretory granule biogenesis at the trans-Golgi network (TGN). We showed that Arfaptin-1 controls insulin granule scission and proposed that it stabilizes the neck of budding secretory granules (Fig. 1B). Arfaptin-1 was reported to bind to curved membrane structures with its crescent shaped Bin/Amphiphysin/Rvs (BAR) domain. In addition to curved membranes, Arfaptin-1 also binds to activated small GTPases of the ARF family. Binding of Arfaptin-1 to ARF blocks the small GTPase’s ability to recruit and activate downstream effectors. Thus, the effect of Arfaptin-1 on neck stability can be 2-fold. On the one hand, Arfaptin-1 could generate a scaffold on the vesicle neck that provides mechanical support as has been suggested for other BAR domain proteins. On the other hand, it prevents active ARFs to induce fission by dimerization or recruitment of a fission complex. Once granule loading is complete and all necessary components have been
necessary for PKD-dependent release of nascent secretory granules from the TGN. Today more than 130 insulin granule proteins have been described, half of which are transmembrane proteins. These proteins may help to concentrate insulin in granules (e.g., granins) or recruit the partial clathrin coat to the growing bud (e.g., Phogrin). The changing surface protein composition during nascent granule growth may induce PKD activity by clustering of PKC interaction partners. PKCs rely on receptors for activated C kinases (RACKs) for proper localization to specific subcellular compartments. One example is coatomer protein complex subunit β-2 (COPB2), which has been shown to recruit active novel PKCs to COPI-coated vesicles. A similar mechanism could apply for secretory granules, although a granule specific RACK has not yet been identified.

Figure 1. Insulin granule biogenesis—Model for PKD activation and Arfaptin-1 action. (A) Preproinsulin is cotranslationally inserted into the endoplasmatic reticulum (ER) and cleaved into proinsulin. Proinsulin is transported to the Golgi apparatus, where it is packaged into secretory granules at the trans-Golgi network (TGN). Immature insulin granules are covered by a discontinuous clathrin coat, which facilitates outsorting of granule components during the maturation process. Constitutive-like vesicles bud off the maturing granules and return to the Golgi or are degraded by lysosomes. During the maturation process, which is accompanied by increasing acidification of the granule, proinsulin is converted into c-peptide and insulin, which crystallizes in the mature granule. (B) Arfaptin-1 dimers bind to active, GTP-bound ARF at the neck of nascent granule precursors and are likely to form a scaffold that provides mechanical support. At the same time ARFs are shielded from dimerization and interaction with downstream effectors. (C) During loading of the nascent granule precursor an unknown receptor of activated C kinase (RACK) could accumulate at the granule surface, which binds active novel Protein Kinase C (nPKC). nPKC phosphorylates and activates Protein Kinase D (PKD), which is recruited upon diacylglycerol (DAG) accumulation in the neck. PKD phosphorylates in turn Arfaptin-1 and disrupts the Arfaptin-1-ARF complex. ARFs are free to dimerize and interact with downstream partners leading to neck destabilization and granule fission.

How Can PKD Induce Scission of Insulin Granules at the Right Time?

A mechanism that is to trigger timely release of a growing secretory granule bud from the TGN should sense one or more features that indicate the loading status of the transport carrier. Such features include surface protein composition, lipid composition and granule size. Hence, the question arises how PKD may sense these features of a growing insulin granule and how its activation might occur at the right time? PKD is recruited to membranes by diacylglycerol (DAG). At the same time, DAG activates novel Protein Kinase Cs (nPKCs) the main upstream activators of PKD. nPKCs phosphorylate PKD at two regulatory sites and induce full enzymatic activity. Thus, both presence of DAG and phosphorylation by upstream kinases are necessary for PKD-dependent release of nascent secretory granules from the TGN.

Today more than 130 insulin granule proteins have been described, half of which are transmembrane proteins. These proteins may help to concentrate insulin in granules (e.g., granins) or recruit the partial clathrin coat to the growing bud (e.g., Phogrin). The changing surface protein composition during nascent granule growth may induce PKD activity by clustering of PKC interaction partners. PKCs rely on receptors for activated C kinases (RACKs) for proper localization to specific subcellular compartments. One example is coatomer protein complex subunit β-2 (COPB2), which has been shown to recruit active novel PKCs to COPI-coated vesicles. A similar mechanism could apply for secretory granules, although a granule specific RACK has not yet been identified.
PKD activity at the granule neck can be regulated at the level of upstream activation by PKC and/or at the level of PKD recruitment. Both changed lipid composition and size-dependent steric properties of the vesicle neck could directly control PKD recruitment. The best candidate molecule in this process is DAG. Due to its unique cone-like shape, DAG is preferably found in membranes with an extremely large negative curvature.25,26 The negative curvature at the neck of a growing granule bud increases with the size of the vesicle, which allows for local DAG incorporation. Even without elevated DAG production, the tropism of residual DAG for the vesicle neck would cause local accumulation by diffusion. Growth of the granule bud by continuous cargo incorporation could induce local PKD recruitment at the neck of the granule once a certain size and thus membrane curvature has been reached. PKD activation releases Arfaptin-1 from ARF and scission can occur. Such indirect size sensing mechanism would guarantee that insulin granule precursors are not prematurely released from the TGN and contain the appropriate amount of peptide hormone.

The origin of DAG at the Golgi is still a matter of debate. However, a recent report corroborates conversion of phosphatidylcholine and ceramide into DAG and sphingomyelin as one important source.27 Interestingly, ceramide homeostasis at the Golgi may be under direct control of sphingomyelin as one important source.27

How is Specificity for Regulated Secretion Achieved in β Cells?

Interestingly, our data shows that Arfaptin-1 is necessary for regulated release of insulin but not constitutive secretion in β cells.1 Yet, both regulated and constitutive transport carriers generate neck structures during their budding from the TGN. Thus, the question is how the BAR domain-containing protein can distinguish one vesicle neck from the other. While the involvement of other proteins in specific targeting of Arfaptin-1 cannot be excluded at this point, the unique binding characteristics of Arfaptin-1 might provide an answer. A structural study on the closely related protein Arfaptin-2 has revealed that Arfaptins can cooperatively bind small GTPases of the ARF family and curved membranes.29 In addition, our own and others’ data have shown that Arfaptins bind to ARFs only in their active, GTP-bound state.34,2 Yet, binding to active ARFs cannot help Arfaptin-1 to distinguish an insulin granule bud from a constitutive vesicle neck, as ARFs play an important role in formation and scission of both types of transport carriers.3,12-14,30,31 However, a clear difference of vesicles that are constitutively released from the Golgi and insulin granules is their size and thus membrane curvature. While Golgi-derived transport carriers, that are constitutively released, have an approximate diameter of 60–80 nm,32,33 insulin secretory granules are around 350 nm.34 Consequently, the neck structures also differ in size. When expressing the non-phosphorylatable Arfaptin-1 S132A mutant in β cells, we observed incompletely separated insulin granules with stabilized neck structures that connected them with each other or to the TGN. These necks were around 50–60 nm in diameter and thus much bigger than the neck of the average constitutive transport carrier. Interestingly, Arfaptin has been shown to shape liposomes into tubules of approximately the same diameter.4 Therefore, Arfaptin-1’s affinity for curved membranes of a certain diameter could explain the observed specificity for growing insulin granules.

Although it is established that Arfaptin-1 can bind both curved membranes and ARF family members, the order of events is not clear yet. One possibility is that Arfaptin-1 is recruited first to the neck and recruits ARF-GTP in a second step. Although this model cannot be fully excluded at this point, some experimental evidence speaks against requirement of Arfaptin-1 to localize ARFs to the vesicle neck. Arfaptin-1 knockdown leads to the formation of smaller, non-functional granules that in majority are rapidly degraded. This is most likely a result of an uncontrolled scission machinery.3 If Arfaptin-1-mediated ARF recruitment to the vesicle neck was required for membrane separation, blockage of granule scission would have been expected. Thus, it is more probable that Arfaptin-1 binds initially to ARFs, which are already present on the granule neck. As a single Arfaptin dimer can bind two ARFs,29 more active ARFs would be recruited to the neck while the scaffold is growing. The scaffold organizes GTP bound ARF in a regular pattern, while keeping it inactive. Once the Arfaptin-1 is released by PKD phosphorylation, active ARFs remain in close proximity to each other around the granule neck, which could induce rapid membrane fission by ARF dimerization or fission complex recruitment.

Interestingly, dense-core secretory granule biogenesis in many endocrine cells and neurons closely resembles insulin granule formation.35 Thus, a role of Arfaptin-1 in quality control of other secretory granules is quite likely. Arfaptin-1 could also be involved in constitutive secretion of more bulky cargoes such as collagen that require much bigger transport carriers. Studies in Arfaptin-1 knockout mice will be useful to shed light on the role of Arfaptin-1 in different tissues and the physiologic consequences of its loss.

We are only starting to grasp the importance of BAR domain proteins in different cellular processes. Understanding the mechanisms that provide a specific function for Arfaptin-1 in insulin granule formation and that regulate PKD activity at the neck will provide new insights into the pivotal, yet elusive, process of secretory granule biogenesis at the TGN.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.
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