Dense-Core Secretory Granule Biogenesis

The dense-core secretory granule is a key organelle for secretion of hormones and neuropeptides in endocrine cells and neurons, in response to stimulation. Cholesterol and granins are critical for the assembly of these organelles at the trans-Golgi network, and their biogenesis is regulated quantitatively by posttranscriptional and posttranslational mechanisms.

Dense-core secretory granules (DCGs) are a hallmark of endocrine, neuroendocrine cells, and other secretory cells, such as exocrine and hematopoietic cells that are responsible for regulated secretion of cargo molecules. In this review, we will focus on DCG biogenesis specifically in endocrine and neuroendocrine cells.

Peptide hormones and peptide neurotransmitters (neuropeptides) are synthesized as larger precursors at the rough endoplasmic reticulum (ER), inserted into the ER cisternae, and subsequently transported to the Golgi apparatus. The precursors, along with other regulated secretory pathway proteins, are packaged into secretory granules at the trans-Golgi network (TGN). The precursors are then processed to active peptides and secreted in a regulated manner in response to stimuli. The exact mechanism of DCG formation at the TGN is not fully understood. The vesicular trafficking model (34, 56, 60, 62) for transport of secretory proteins through the Golgi stack suggests that DCGs are formed by active budding from the TGN along with constitutive vesicles. Such an active budding of DCGs appears to require lipid and protein components for induction of membrane curvature at the TGN, similar to constitutive vesicle budding. Additionally, an alternative passive model has been proposed for DCG formation, which does not require active vesicle budding from the TGN. Instead, polynodular tubular progranules are first formed as a continuum at the TGN, which eventually segregate from the network to generate DCGs (57).

The regulated secretory pathway is unique to endocrine, neuronal, and exocrine cells (FIGURE 1). Since Bennett (4) first described the formation of DCGs in chromaffin cells in 1941, much has been learned about the process of DCG biogenesis. DCG biogenesis involves the budding of the immature secretory granules (ISGs) from the TGN followed by a number of maturation steps to form the mature secretory granules (MSGs). These include acidification of the newly formed ISGs to activate the prohormone convertases (PCs) and carboxypeptidases necessary for the processing of the regulated secretory pathway proteins, removal of constitutive secretory proteins and lysosomal enzymes inadvertently packaged into ISGs, loss of the clathrin coat and other coat proteins of the ISGs, and removal of water and condensation of granule contents to form the MSGs. The MSGs then move out to the cell periphery, awaiting release on stimulation of the cell (FIGURE 1). However, the molecular players and mechanism governing each of the steps is not fully understood. In this brief review, we will focus on the role of lipids and granulogenic proteins for the physical formation of the ISGs at the TGN. In addition, recent advancement in understanding the mechanisms involved in the maturation and regulation of DCG biogenesis in endocrine and neuroendocrine cells will be presented.

Lipids Necessary for Vesicle Budding at the TGN

A role for lipids in the biogenesis and trafficking of vesicles from the TGN to the plasma membrane has long been proposed and has been the subject of many reviews (8, 15, 59). Although the role of lipids in vesicle budding was studied mainly in the constitutive secretory pathway, we expect that much of what is known is applicable to the regulated secretory pathway. Therefore, we will highlight mechanisms recently proposed for lipid-mediated vesicular budding from the TGN in the constitutive secretory pathway in mammalian cells and current knowledge of some special features that are involved in the biogenesis of DCGs destined for the regulated secretory pathway in endocrine cells. These processes not only involve lipids but also are dependent on lipid interactions with enzymes and other membrane-associated proteins.

Within the constitutive secretory pathway present in all cell types (FIGURE 1), the transiently membrane-associated lipid diacylglycerol (DAG) is important for vesicle budding and secretion (66, 88). The levels of DAG at the TGN are maintained by many enzymatic reactions, including the conversion of phosphatidylincholine to DAG via phosphatase activity. Protein kinase D (PKD), which is activated by the β and γ subunits of G protein (35), has a high affinity binding site for DAG (79). PKD recruitment to the Golgi membrane is DAG-dependent and is necessary for vesicular biogenesis and protein transport to the cell surface (3, 88). Although the precise contribution of PKD to budding behavior is not clear, activated PKD can recruit downstream targets to facilitate membrane fission.
The constitutive secretory pathway present in all cell types does not require stimulation for exocytosis.

FIGURE 1. Steps involved in DCG biogenesis

Two distinctive secretory pathways are present in neuroendocrine and endocrine cells for constitutive and regulated secretion of proteins.

from DAG or phosphatidylcholine by DAG kinase or phosphatidylcholine-specific phospholipase D (PLD1), respectively, appear to be involved in budding of secretory vesicles for both the constitutive and regulated secretory pathways (13, 65, 66). When the concentration of PAs was increased by DAG kinase or PLD1 in endocrine GH3 cells, there was significant increase in secretory vesicle budding (13, 66). Moreover, the inhibition of PA synthesis quantitatively diminished secretion of growth hormone-containing vesicles in GH3 cells (65). These studies by Shields and colleagues suggest that PA plays an important role in budding of hormone-containing vesicles from the TGN. However, it is not clear whether the secretory vesicles formed using their in vitro assay system are equivalent to DCGs found in the regulated secretory pathway. Nevertheless, it is expected that DAG and PA play similar roles in both constitutive vesicle formation and DCG budding from the TGN in endocrine and neuroendocrine cells. The molecular shape of DAG and PAs is preferentially cylindrical under neutral pH. PAs assume a cone structure under low pH and submillimolar calcium concentrations, conditions similar to that in the lumen of the Golgi complex (45). Although it is not clearly understood how DAG and PAs initiate vesicle budding from the TGN, it appears that the conical molecular structure of these two molecules provides sufficient force to induce a negative curvature of tubular Golgi. This leads to pearling of its membrane bilayer followed by fission (15, 64). Thus increased amounts of PAs along with DAG in the Golgi complex seem to facilitate budding of vesicles.

Phosphoinositides are significant contributors to the initiation of budding of constitutive vesicles, although their role in regulated secretory vesicle formation is not known. Similar to DAG-mediated recruitment of proteins to the TGN, phosphatidylinoso-
it ol 4-phosphate [PtdIns(4)P] also recruits Golgi-localized FAPPs, adaptor proteins for PtdIns(4)P, to the TGN. A kinase-dependent, tightly regulated association between PtdIns(4)P and the PH domain of FAPPs was demonstrated in constitutive budding and fission reactions at TGN sites. Through the FAPP PH domain, the activated, small GTPase ARF-GTP was shown to be recruited to membranes and facilitated membrane traffic (25). Furthermore, phosphoinositol kinases differentially localized across the Golgi complex can control local production of PtdIns(4)P at the subgolral level to provide additional control of cargo selection and vesicular subtype specification during vesicle budding from the TGN (85).

Cholesterol, an integral part of the membrane, plays a significant role in all aspects of vesicular membrane formation in both the constitutive and the regulated secretory pathways. Especially important and specific is its role in formation of DCGs destined for the regulated secretory pathway. Together with glycosphin-golipids, sphingomyelin, and other lipids, cholesterol creates tightly packaged lipid microdomains known also as “lipid rafts,” from which granules bud at the TGN (19). This is described as a liquid-ordered (L_0) phase in model membranes (2, 7).

The maturing face of the Golgi complex and the secretary granules can contain 10–50 times more cholesterol than the forming Golgi complex in endocrine pancreas (53). Recently, pituitary neural lobe secretary granule membranes have been reported to contain 65 mol % cholesterol compared to all other lipids (19). In actuality, the TGN and the plasma membrane have the highest composition of cholesterol compared to the ER, and cis and medial Golgi (80, 81). Lipid raft microdomains in the TGN, which will eventually become the secretary granule, are not only composed of lipids but also of recruited proteins with a sorting signal for the regulated secretory pathway (see Granulogenic Proteins: Driving Force for Budding at the TGN) (6, 19). The budded vesicle is then composed of high levels of cholesterol and densely packed with secretary proteins. When cholesterol synthesis was blocked in AtT-20 cells, a pituitary cell line, condensed dense cores were accumulated at the TGN, but no DCGs were formed. This phenomenon was reversible on addition of cholesterol (83), indicating the importance of cholesterol in formation of DCGs.

Recently, the role of cholesterol in curvature formation for DCG biogenesis was investigated in model and biological membranes. Cholesterol, like DAG, possesses a conical structure that promotes both negative curvature and pinching of the lipid-containing domain from the rest of the vesicle (2, 15, 52). Many studies have reported that addition of cholesterol alone can orchestrate Golgi vesiculation (27, 89). Substitution of certain, but not all, sterols for cholesterol can also cause L_0 phases. In fact, other sterols structurally analogous to cholesterol, but lacking the double bond in the sterol ring, can cause positive curvature, small interspersed microdomains, or no phase separation at all (2). Lathosterol and 7-dehydrocholesterol (7-DHC), immediate precursors of cholesterol, are structurally analogous to cholesterol except for the location or number of double bond(s) in the sterol ring, respectively. In inborn errors of cholesterol biosynthesis where the enzymes that catalyze the conversion of lathosterol and 7-DHC to cholesterol are defective, these precursors, which are normally undetectable, are 250–2,000-fold elevated and become the dominant lipids in the bilayer (84). Not surprisingly, individuals who are genetically defective in cholesterol biosynthesis exhibit multiple malformations, possibly due to improper signaling in the absence of cholesterol (84). From a biochemical standpoint, 7-DHC forms more stable microdomains than cholesterol (37), but biophysically both 7-DHC and lathosterol form less rigid membranes and have lower intrinsic curvature values than cholesterol (26). This is reflected in the cholesterol-defective mouse model of Smith-Lemli-Opitz syndrome and lathosterolosis, where budding of DCGs from the TGN is impaired (26). Thus sterol structure can determine the preferred curvature of the bilayer, and cholesterol induces budding capability due to the negative, intrinsic curvature and membrane bending rigidity conferred (2, 29).

It is evident from the studies reviewed here that several factors are necessary for recruitment of cargo as well as vesiculation of cargo-containing regions of the TGN. These include not only the lipids but also the enzymes that modify them, such as localized substrate-specific kinases. Additionally, proteins that stabilize the lipid-raft microdomains at the TGN and those that provide the physical driving force for the formation of granules are necessary.

Granulogenic Proteins: Driving Force for Budding at the TGN

Granins represent a class of proteins abundant in DCGs in (neuro)endocrine cells. These include chro-mogranin A (CgA), B (CgB), secretogranin II–IV (SglII–IV) (72). These proteins have been proposed to play a granulogenic role by driving budding at the TGN to form DCGs. CgA and CgB have been shown to physically induce the formation of DCG-like structures when overexpressed in fibroblasts (32, 41). Subsequently, prohormones such as pro-vasopressin, pro-oxytocin, and pro-opiolanocortin (POMC), major DCG cargo proteins in endocrine cells, were shown to induce the formation of DCG-like vesicles in fibroblasts, suggesting that these proteins may also act as assembly factors and contribute to the physical formation of DCGs (5, 32). DCG-like vesicles formed in fibroblasts by these granulogenic proteins appear to be not only morphologically similar to bona fide DCGs from the neuroendocrine and endocrine cells but
were also capable of secreting their contents in a stimulated manner (5, 32). The common biophysical property of these proteins is that they aggregate at low pH and in the presence of Ca\(^{2+}\), conditions present in the lumen of the TGN, thus allowing them to be segregated from constitutively secreted proteins (11). These aggregates are then directly or indirectly associated with lipid rafts at the TGN to induce budding and formation of the ISG. A sorting motif consisting of an intramolecular disulfide loop domain bounded by cysteine residues and containing a number of aliphatic hydrophobic residues has been identified to be sufficient and necessary for CgB membrane association in the TGN and, hence, sorting of CgB to the regulated secretory pathway in the PC12 neuroendocrine cell line (12, 24). Although this loop structure is also present in CgA, it does not appear to be necessary for sorting of CgA to the regulated secretory pathway in PC12 cells (71). Instead, a domain (CgA\(^{48-111}\)) of the mature protein appears to be necessary, although not sufficient for trafficking of CgA to the regulated secretory pathway. Interestingly, Hosaka et al. (31) have reported that a CgA domain comprising of residues 48–111 interacted specifically with residues 214–373 in SgIII, a lipid raft-associated protein, to effect sorting to secretory granules. Deletion of the CgA\(^{48-111}\) segment resulted in missorting of CgA to the constitutive pathway in pituitary (AtT-20) and β-pancreatic (INS-1) cell lines (31). This CgA domain contains the sequence identified by Taupenot et al. (71) to be necessary for targeting CgA to the regulated secretory pathway. Because SgIII was found to be associated with lipid rafts in secretory granule membranes (31), SgIII-CgA interaction would facilitate granulogenesis by CgA aggregates and entry into the budding ISGs.

In endocrine cells, prohormones such as POMC have been shown to aggregate and bind to carboxypeptidase E (CPE), a lipid raft-associated protein, at the TGN (10, 14, 90). Sorting motifs found on several prohormones comprising of two acidic and two hydrophobic residues have been shown to be necessity...
sary for interaction with two basic residues in CPE (90). Such interaction may also facilitate granulogenesis at the TGN, as suggested from the studies demonstrating the ability of prohormones to induce granule-like structures in fibroblasts (5). We propose that the association of granins and prohormone aggregates at lipid rafts is essential to provide the driving force for granule budding at the TGN, whereas lipid components such as DAG, PA, and cholesterol facilitate formation of membrane curvature (FIGURE 2).

**Maturation of Secretory Granules**

New ISGs formed from budding of the TGN undergo several steps necessary to convert them into MSGs. As shown in FIGURE 1, immediately after budding of ISGs from the TGN, acidification within the milieu of these organelles occurs. It has been reported that the TGN, ISGs, and MSGs have increasingly acidic pH (pH\textsubscript{TGN} ~ 6.5–6.2; pH\textsubscript{ISG} ~ 6.3–5.7; pH\textsubscript{MSG} ~ 5.5–5.0) (33, 55, 77). The steady-state pH decrease from the TGN to the MSG is achieved and regulated by gradual increases in the active H\textsuperscript{+} pump density and decreases in H\textsuperscript{+} permeability of organelle membranes (87).

Maintaining acidic pH is critical for the proper association of granulogenic proteins with lipid rafts and formation of DCGs. Taupenot et al. (73) reported that treatment of PC12 cells with the V-ATPase blocker, bafilomycin A1, resulted in the significant reduction in sorting of chromogranin A to DCGs. Furthermore, electron microscopic analyses of these bafilomycin A1-treated PC12 cells, as well as AtT-20 cells, revealed reduced numbers of secretory granules with dense cores (68, 73), suggesting that the acidification mediated by V-ATPase is crucial for the formation of DCGs.

Initial processing of prohormones, for example, proinsulin, POMC, and secretogranin II, begins in the ISGs (54, 63, 77). Continued acidification in the ISGs is necessary to activate the PCs (67) and processing of the prohormones and proneuropeptides. Indeed, when the pH gradient was abolished by a V-ATPase blocker, but not by chloroquine, in AtT-20 cells, processing of POMC was severely inhibited, and it was secreted through the constitutive secretory pathway (68).

During budding from the TGN, it is known that constitutive secretory proteins, lysosomal enzymes, and some membrane proteins are inadvertently co-packaged into the ISGs of endocrine cells (1, 9, 46), and their removal is a critical step for the maturation of ISGs. This occurs by budding off of constitutive-like vesicles from the ISGs (FIGURE 1). This process involves recruitment of AP-1 adaptor complex in a casein kinase II-phosphorylation-dependent manner and is regulated by ARF1 (20, 21, 43, 46). The fates of the constitutive-like vesicles are multiple. In some cases, the contents are secreted constitutively and the vesicles recycled to endosomes. Others are destined for the TGN, carrying with them the processing enzyme furin, which is a TGN resident protein (21, 51).

During ISG maturation, a morphological transformation of the clathrin coat occurs between ISGs and MSGs. Although the clathrin coat is present on the ISGs, it is absent on the MSG membranes. It was also shown that clathrin was not equally distributed on the membrane of ISGs but was often found covering the small constitutive-like vesicles budding from ISGs to remove missorted proteins (54, 74). Budding of these constitutive-like vesicles from ISGs removes the clathrin coat and excess membranes from the MSGs.

Maturation of secretory granules in some cells requires not only budding of constitutive-like vesicles but also fusion events. ISGs in neuroendocrine PC12 cells have different sizes and density compared to mature granules. Homotypic fusion of ISGs is responsible for the increase in size of ISGs during maturation (75, 78). Studies performed in a cell-free system indicate that ISG fusion requires the SNARE protein synaptotagmin 6, which is subsequently removed by the budding of the clathrin-coated constitutive-like vesicles and not present on mature granules (86). In the maturation process, other proteins, such as synaptotagmin IV and VAMP4, present on the ISGs, are also removed in a similar manner and are absent on the MSGs (22).

MSGs are stored until release on stimulation of the (neuro)endocrine cell. The ability of ISGs to release their contents in a regulated manner is questionable and varies with different cell types (22, 75). However, it is clear that release of MSGs is much more responsive to a stimulus, compared to freshly formed ISGs, and this switch has been attributed to the removal of synaptotagmin IV and VAMP4 from the ISGs (22).

Finally, cargo molecules in maturing granules undergo condensation, which requires acidification and removal of water.

**Regulation of Dense-Core Secretory Granule Biogenesis**

Exocytosis of DCGs from (neuro)endocrine cells to release hormones and neuropeptides triggered by extracellular stimulation is immediately followed by temporal augmentation of DCG biogenesis to replenish the DCG pool for subsequent secretory events. Regulation of DCG biogenesis is governed by mechanisms networked at multiple levels (18, 40). To form intact secretion-competent DCGs in a timely manner, biosynthesis of proteins involved in the physical formation of DCGs (e.g., the granins) as well as cargo (i.e., neurotransmitters, neuropeptides, and hormones) has to be coordinately upregulated. Each mechanism at the different regulatory levels eventually determines the amount of DCG proteins available in the cells and subsequently the quantity of DCGs formed in neuroendocrine and endocrine cells. Thus the overall levels of DCG proteins appear to be a key determinant for
the quantitative and qualitative biogenesis of DCGs in these professional secretory cells (40).

It has been shown that signals triggering exocytosis of DCGs initiate transcriptional activation of mRNAs encoding DCG proteins at the nucleus (23, 47, 49, 69, 70). Stimulation by various secretagogues (e.g., nicotine, corticosterone, depolarization by K+’ triggers secretion of DCG contents such as catecholamines and CgA from bovine sympathoadrenal chromaffin as well as PC12 cells (23, 58, 70). Concurrently, mRNAs encoding CgA, tyrosine hydroxylase, phenylethanolamine-N-methyltransferase, proenkephalin and dopamine β-hydroxylase were observed to be upregulated in these cells after stimulation (23, 30, 38, 58, 61, 69, 82). Although there is no direct indication that the transcriptional upregulation of these proteins after stimulation resulted in augmentation of DCG biogenesis, it is highly likely that such a process occurs. Transcriptional activation of many different DCG protein genes to increase DCG protein levels, however, may not be an efficient way to regulate DCG formation.

A posttranscriptional regulatory mechanism for DCG biogenesis utilizing polypyrimidine-tract binding protein (PTB), recently reported by Knoch et al. (44), can provide a faster response. PTB, a nuclear-resident protein, was translocated to the cytoplasm within 2 h of glucose stimulation in insulin-secreting pancreatic β-cells and bound to a specific sequence on 3′-UTR of mRNAs encoding DCG proteins (44). This...
binding enabled stabilization of these mRNAs, preventing them from early degradation and resulting in increased translation of DCG proteins and DCG formation. Several DCG proteins, including insulin, ICA512/IA-2, CgA, PC1/3, and PC2, were shown to possess a PTB binding site in their 3'-UTR (44). Thus PTB plays a role at the posttranscriptional level to increase the quantity of DCG proteins, which then promotes DCG biogenesis in insulin-secreting β-cells. However, whether PTB is present in other types of endocrine cells to regulate DCG biogenesis remains to be determined.

Recent studies indicate that CgA is actively involved in the regulation of DCG formation at the posttranslational level in endocrine and neuroendocrine cells. Downregulation of CgA in neuroendocrine PC12 cells resulted in severely impaired DCG formation (39, 41). Additionally, endocrine 6T3 cells, which lack CgA expression, showed poor DCG formation but recovered after CAMP treatment in a reversible manner (17). When CgA was expressed exogenously in these 6T3 cells, DCG biogenesis and the regulated secretory pathway were restored (41). However, in cells that have permanently lost the genetic program for regulated secretion, such as the PC12-27 cells (16, 36), CgA expression could not restore the lost regulated secretory phenotype (50). Two recent animal studies by Mahapatra et al. (48) and Kim et al. (42) have confirmed the cell studies showing that CgA plays an important role in DCG biogenesis in vivo. CgA knockout mice generated by Mahapatra et al. (48) exhibited a phenotype showing significant reduction in DCG formation in adrenal chromaffin cells. Additionally, a similar phenotype was observed in CgA antisense transgenic mice reported by Kim et al. (42). In this latter study, partial depletion of CgA resulted in the decrease in the number of DCGs formed in adrenal chromaffin cells in a CgA dose-dependent manner. Interestingly, the size of DCGs was significantly increased, whereas the size of dense cores was only slightly changed in these transgenic mice. The mechanism by which CgA mediates DCG biogenesis is not fully understood at the present time. However, we have observed active degradation of DCG proteins in CgA-deficient PC12 cells, as well as endocrine 6T3 cells lacking CgA (41). Moreover, our recent studies showed that the degradation of the DCG proteins occurred at the Golgi complex but was prevented, along with the recovery of DCG biogenesis, by reexpression of CgA into these cells. Granule protein degradation was prevented by a CgA-dependent induction of a protease inhibitor, protease nexin-1 (PN-1) (39). By preventing degradation of granule proteins at the posttranslational level, the quantity of granule proteins would be increased for the formation of DCGs. Thus CgA regulates DCG biogenesis at the posttranslational level by protecting DCG protein degradation. Since CgA is ubiquitously distributed in various neuroendocrine and endocrine cells, CgA's role in promoting DCG biogenesis through protection of granule proteins from degradation may be general to all cells expressing CgA.

Recently, insulinoma-associated protein 2 (IA-2 or ICA512), a transmembrane protein present on DCG membranes, has also been implicated in the regulation of formation of insulin-containing DCGs in insulin-secreting β-cells (28). When IA-2 was overexpressed in an insulin-secreting β-cell line, MIN-6, there was a significant increase in insulin content and the quantity of DCGs in these cells (28). This suggests that IA-2 might stabilize insulin for the production of more DCGs in these cells. Upon stimulated secretion of granules, IA-2 is inserted into the plasma membrane, and the cytoplasmic domain is then cleaved off in a Ca2+-dependent manner. The cleaved cytosolic fragment of IA-2 is subsequently translocated into the nucleus, inducing insulin expression in β-cells (76). Since IA-2 is present in many different neuroendocrine cells, the role of IA-2 in granule biogenesis may not be restricted to β-cells.

Regulatory mechanisms for DCG biogenesis in (neuro)endocrine cells discussed above indicate control by modulating DCG protein levels at the transcriptional, posttranscriptional, and posttranslation levels. Figure 3 summarizes the regulatory mechanisms for DCG biogenesis in (neuro)endocrine cells based on the current literature. Transcriptional control of DCG protein levels would require multiple players and steps of signaling to replenish DCGs after repetitive stimulation. However, posttranscriptional and posttranslational regulation of a single protein (PTB and CgA, respectively) can provide a more efficient response for upregulating DCG protein synthesis and replenishing of DCGs after stimulation. The different mechanisms working together ensure timely upregulation of DCG biogenesis after exocytotic events in (neuro)endocrine cells.

Concluding Remarks

DCG formation is a critical step for the proper storage and secretion of hormones, neuropeptides, and neurotransmitters in endocrine and neuroendocrine cells. Until recently, studies on the biogenesis of these organelles have been sparse. Most of the work on the role of lipids has focused on constitutive vesicle budding and secretion in non-endocrine cells. Such studies have revealed the importance of a number of lipids, including DAG, phosphatidic acids, phosphoinositides, and cholesterol in vesicle formation. These lipids play a critical role in curvature formation and budding in the membrane at the TGN. Fundamental discoveries of the roles of various lipids in vesicle formation gleaned from studies in non-endocrine cells are likely applicable to the formation of DCGs in endocrine and exocrine cells. Indeed, a role of DAG,
PAs, and cholesterol in DCG biogenesis in endocrine cells has been demonstrated. Cholesterol seems to be a major player, not only in membrane curvature formation in the budding granules, but as a component, together with sphingolipids, to form “lipid raft” microdomains at the TGN. There, granulogenic protein aggregates associate to provide the driving force for granule formation in (neuro)endocrine cells. Granulogenic proteins such as the granins and prohormones bind directly or indirectly to lipid raft-associated proteins such as SgIII and CPE to trigger budding. Newly formed ISGs undergo maturation steps, such as acidification, prohormone processing, condensation of cargo content, and loss of clathrin coat, and certain SNARE proteins prime the MSGs for release. These processes are fairly well understood in (neuro)endocrine cells. However, the elucidation of the regulatory mechanisms involved in secretion-coupled signaling of DCG biogenesis is just emerging. In this respect, the recent discoveries of posttranscriptional and posttranslational mechanisms involving PTB and CgA, respectively, in regulating the quantitative production of DCG proteins and DCGs are very exciting. Such studies revealed a new insight into the physiological function of the (neuro)endocrine cell in that synthesis of DCG proteins and their mRNAs appears to be occurring at a robust rate but are constantly degraded unless stabilized. To maintain steady-state levels of DCG biogenesis in the unstimulated cell, presumably some degree of stabilization occurs. However, stimulation, triggering secretion, changes the dynamics, resulting in greater stabilization of DCG proteins and mRNAs, which increases DCG protein levels to promote granule biogenesis for the replenishment of the released pool of DCGs. Although players involved in the regulation of DCG numbers have been identified, the elucidation of the signaling mechanisms linking stimulus-coupled secretion at the plasma membrane and the activation of the intracellular players to upregulate DCG biogenesis in (neuro)endocrine cells awaits future studies.

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References


36. Keller RK, Arnold TP, and Fliesler SJ. Formation of cholesterol deficiency diseases and decreased mechanism.


