Origins of the regulated secretory pathway

Alexander A. Mironov and Peter Arvan

Introduction

Modes of transport of soluble (or luminal) secretory proteins synthesized in the endoplasmic reticulum (ER) could be divided into two groups. The so-called constitutive secretory pathway (CSP) is common to all eukaryotic cells, constantly delivering constitutive soluble secretory proteins (CSSPs) linked to the rate of protein synthesis but largely independent of external stimuli. In regulated secretion, protein is sorted from the Golgi into storage/secretory granules (SGs) whose contents are released when stimuli trigger their final fusion with the plasma membrane (Hannah et al. 1999).

In highly specialized exocrine, endocrine, and neural cells, known as professional secretors, the SGs represent the dominant route of secretory protein trafficking, superimposed upon other post-Golgi protein trafficking pathways, even though regulated secretory proteins (RSPs) and some polysaccharides are released at low levels until exocytosis is amplified in response to a stimulus.

In SGs, RSPs are stored at high concentration (Castle and Castle 1996) in order to economize cytoplasmic space and prevent premature activation of zymogen forms. The regulated secretory pathway delays release of the secretory product under unstimulated conditions so that, upon demand, protein release rate can quickly exceed protein synthesis rate, potentially resulting in massive release of protein product (in most cases, regulated secretory proteins have to be converted from condensed to soluble form after fusion of SGs with the plasmalemma). Usually the stimulatory signal occurs via binding of a stimulating ligand to its cell surface receptor, which induces entry of Ca^{2+} into the cytosol from the extracellular space and from internal storage sites (see Chapter 2.8).

Regulated secretion is present in some protists but not found in plants and yeast. However, the isolation of mutants lacking SGs in multicellular animal organisms has never been reported, suggesting an important role of the regulated secretory pathway (Borgonovo et al. 2006). In *Tetrahymena thermophila*, a master gene for SG biogenesis has not yet been found, but several genes for SG cargoes have been identified, including a family of five proteins all of which are essential for the normal assembly of the granule core (Bowman et al. 2005; Cowan et al. 2005; Borgonovo et al. 2006). In endocrine, exocrine and mast cells, it is typical to have $\geq$50% of all translation devoted to the synthesis of SG proteins, and the SGs may occupy an overwhelming

fraction of cytoplasm volume. Thus, there is a veritable river of protein flow through the secretory pathway to newly made SGs.

SGs are typically distinguished by their morphological appearance and high immunoreactivity for cell-type-specific content proteins, such as peptide hormones, as well as other more widespread cargoes, such as chromogranin A (CgA), B (CgB) and secretogranins II–VI (Taupenot et al. 2003) as well as prohormone convertases 1 (PC1) and 2 (PC2) and carboxypeptidase E (CPE) (Steiner 1998). Yet deletion of various SG genes in mice (reviewed below) neither precludes expression of other SG genes nor the biogenesis of SGs (although processing of cargoes and/or their secretion can be affected, Borgonovo et al. 2006), indicating that multiple gene products contribute to granule structure and function. One feature that tends to be shared by such proteins is their ability to undergo multimerization/condensation under intraluminal conditions prevailing in young SGs, which includes a slightly acidic pH and an increased divalent cation concentration (Laine and Lebel 1999). Although condensation of RSPs has mostly been described in the *trans*-Golgi elements ("condensing vacuoles") and immature SGs (ISGs) (Tooze et al. 1987), it has occasionally also been found in *cis*- and *medial*-Golgi cisternae (Rambourg et al. 1984; Slot et al. 1997), and rarely in the ER (Geuze and Slot 1980). High Ca^{2+} concentration and a pH ≤ 6.5 in specialized parts of the *trans*-Golgi network (TGN) especially favour initiation of multimerization of selected SG cargo such as granins. These in turn may form a nidus upon which condensation of other RSPs may follow, driven by multiple interactions among RSPs and helper/assembly factors within SGs.

The origins of the RSP seem to derive from the constitutive secretory pathway (CSP). Although no visible granules are apparent, Chavez et al. (1996) suggested that constitutive secretory cells such as CHO or L cells have a cryptic regulated pathway. A significant fraction of [35S] sulfate-labelled free glycosaminoglycan chains were stored intracellularly and showed stimulated secretion after treatments with phorbol ester or those that increase cytoplasmic calcium. As for the morphological appearance of granules, the basic message is that formation of SG structures is to a large extent the result of self-organizing properties of RSP cargo. Moreover, although surely not an essential feature of the regulated secretory pathway (Arvan and Halban 2004), many RSPs are endoproteolytically processed from proprotein precursors by specialized granule processing enzymes not expressed in cells outside of the neuroendocrine system. Such processing can be critical for the biological activity of the stored products (Beuret et al. 2004) and may also change the biophysical properties of RSPs (Zhang et al. 2001), facilitating multimerization in the intragranular ionic environment.

Once formed, the majority of SGs accumulate in the cytosol, creating an undocked vesicle pool. ISGs then migrate to the cell periphery with cytoskeleton assistance (Rudolf et al. 2001). ISGs, disconnected from the TGN, acquire at least a partial stimulated exocytosis response to secretagogues (Rindler et al. 2001). Indeed, SGs are subsequently recruited to the plasma membrane

where they become tethered and primed for fusion with the plasma membrane upon external stimulation (Martin 2003), to deliver their cargo to the extracellular space. This is the process of regulated exocytosis, controlled by Ca^{2+} (using synaptotagmin) and mediated by soluble N-ethylmaleimide-sensitive factor attachment protein receptors (SNAREs), which is similar to that occurring at neuronal synapses (DeCamilli and Jahn 1990). Under unstimulated conditions, SGs are long-lived, resulting in cytoplasmic accumulation (Alberts et al. 2002).

Morphology of the regulated secretory pathway

In overview, the formation of SGs requires de novo synthesis and translocation of the RSPs and mediators into the ER and transport to the Golgi and subsequently the TGN, where secretory vesicles are formed. Nevertheless, the morphology of the RSP is different in different cells. The sizes and location of granule formation appears to be related to dilations of condensed material within the interconnected network of Golgi membranes (Rambourg et al. 1993). In the Golgi of the epithelial cells of the lactating rat mammary gland, casein submicelles could be seen already within the *cis*-Golgi, becoming more distended in *trans* cisternae. In the Golgi of principal epithelial cells of rat seminal vesicles, the first detectable aggregation of RSPs begins in the medial cisternae.

In neuroendocrine cells, peptide hormone precursors and helper/assembly proteins such as granins gradually develop into an electron-dense core in the SG lumen (Arvan and Halban 2004). These dense-core vesicles in neuroendocrine and endocrine cells show considerable size variation, have a diameter >100 nm and can contain more than one cargo molecule (Sollner 2003). In bovine adrenal chromaffin cells, atrial natriuretic factor is packaged with catecholamines in large dense-core vesicles (Nguyen et al. 1988) with a mean diameter of 380 nm (Duncan et al. 2003). The electron density of the granule core depends upon the internally stored contents and may be less evident in exocrine cells. Additionally, SGs accumulated in differentiated cell lines are typically smaller than those found in their cognate cells in vivo (Feng and Arvan 2003).

Condensing vacuoles, still attached to the Golgi stack, contain a denser aggregation of contents than found in the Golgi stacks, while mature SGs (MSGs) contain compact dense cores (Clermont et al. 1993). In one group of cells where SGs do not appear associated with the Golgi, Clermont et al. (1995) observed the TGN as the tubular network connected with last two Golgi cisternae. In a second group of cells such as prolactin-producing cells of lactating rats (Rambourg et al. 1992) ISGs form within the *trans*-most cisterna and the TGN is smaller. In a third group of cells, ISGs are formed as distensions of most Golgi cisternae and the non-attached, free TGN is even smaller. TGN tubules are almost invisible in cells in which the ISGs start forming from the *cis*-most cisternae (Clermont et al. 1995).

ISGs bud from the *trans*-Golgi network by a process that is said to exhibit superficial similarity to virus budding (Alberts et al. 2002). EM studies have

found patches of both clathrin (Andresen and Moore 2001) and coatomer (Fig. 5c in Martinez-Menarguez et al. 1999; and see Andresen and Moore 2001) on the surface of ISGs. Whether ISGs are connected to other elements of the TGN is not always evident. According to Rambourg et al. (2001), 'in the *trans*-most Golgi elements, nodular swellings reach their final size and remain interconnected by flattened anastomosed membranous-tubules'. In endothelial cells, connections between forming Weibel–Palade bodies and the TGN are more evident after high pressure freezing than after chemical fixation (Zenner et al. 2007). These images imply that liberation of ISGs may occur by rupture of the tubular areas rather than by ISG budding from saccular elements (Rambourg et al. 2001). ISGs detached from the TGN may still have some characteristics of that area, including lysosomal enzymes (Kuliawat et al. 1997) and clathrin coats attached in patches on the surface (Orci et al. 1987a,b; Tooze and Tooze 1986). In some cell types, the size of SGs is reported to increase during granule maturation, implying that immature SGs (ISGs) may fuse and increase in size while granule contents becomes denser (Clermont et al. 1992).

Birth of the secretory granule at the Golgi complex

Formation of MSGs has been divided into two main processes. The first is ISG formation from the Golgi complex and its operation as an advanced sorting station beyond the TGN. The second is the ability of ISGs to use molecular motors to move to the cell periphery that accompanies maturation to MSGs (Rudolf et al. 2001). Useful indicators of the MSG compartment includes prolonged storage in unstimulated cells, the inclusion of specific v-SNAREs (Meldolesi et al. 2004), and acquisition of special markers (e.g., cysteine string protein (Chamberlain et al. 1996)).

As ISGs mature, their intraluminal pH becomes more acidic (Duncan et al. 2003), and Ca^{2+} more elevated (Winkler and Westhead 1980). This change in ionic environment facilitates conversion of prohormones to mature peptide hormones (Orci 1985; Orci et al. 1986) and facilitates their intragranular storage (Cowley et al. 2000). Along with this, as described above (Dittie et al. 1997), certain proteins present both at the TGN and in ISGs (Orci et al. 1987a,b; Beuret et al. 2004) disappear during granule maturation (Dittie et al. 1997), linked to the loss of clathrin-coated membrane (Kuliawat et al. 1997) with a subset of removed proteins delivered to the endosome–lysosome system (Feng and Arvan 2003).

ISGs represent a minority of granules, yet are indistinguishable from MSGs by light microscopy (Rudolf et al. 2001). However, electron microscopic studies have suggested that in some cells the dense core of MSGs is larger in diameter than that of ISGs (Tooze et al. 1991) consistent with homotypic ISG fusion (Wendler et al. 2001), while in other cells MSGs are smaller than ISG (Arvan and Halban 2004). One concept invokes the formation of small free ISGs that undergo homotypic fusion to produce larger granules as a key step in MSG biogenesis (reviewed in Arvan et al. 2002). This reaction can be reconstituted

in vitro (Wendler et al. 2001), although it has been difficult to confirm in live cells (Clermont et al. 1992; Rindler et al. 2001). A second school of thought views the initial creation of condensing vacuoles at the TGN as larger dilations, which, during and after disconnection from the Golgi complex, proceed to lose membrane and selected contents (i.e., maturation, Sesso et al. 1980). Although there is a heterogeneous picture on the formation and deployment of SGs, a detailed staging of SG life cycle involves: 1. synthesis of RSPs at the ER and their intra-Golgi transport to form ISGs. 2. Maturation of ISGs. 3. Delivery of SGs to the site of the fusion. 4. Priming of SGs. 5. Fusion of SGs with the PM. 6. Elimination of SG membranes from the PM. We now review these areas in the sections that follow (Fig. 1).

Assays of intra-Golgi transport to secretory granules

One way to follow protein transport to and through the Golgi complex is to measure endo-H resistance of RSPs. For glycoproteins stored within the lumen of secretory granules, an endo-H-resistant pool of secretory protein generally
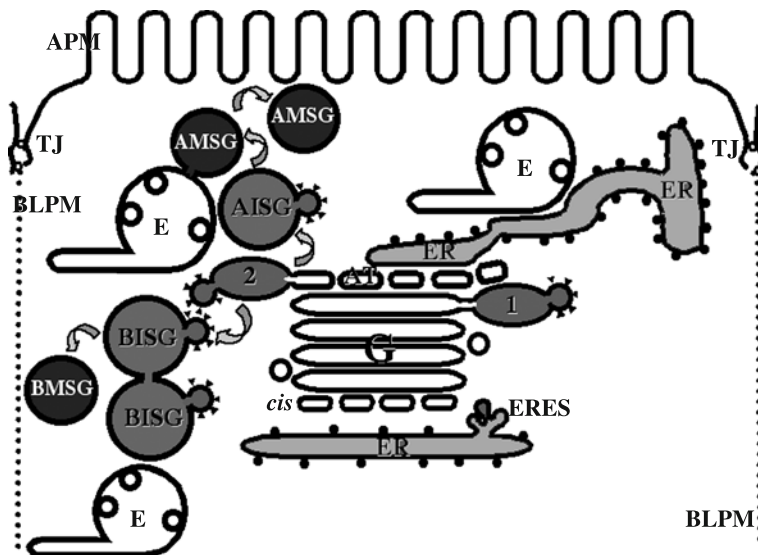

Figure 1. Formation of secretory granules (SGs) at the Golgi exit site (Two types of SGs are formed. The first (ASGs) serves for the delivery of RSPs towards the apical plasma membrane (APM). The second type of SGs (BSGs) delivers RSPs to the bosolateral plasma membrane (BLPM). In some cells, the formation of SGs starts in the distensions of COPI-positive Golgi cisternae (1). In other cells, SGs begin to form in the distensions (2) of the attached and free TGN. SGs containing clathrin-coated buds are immature (AISGs and BISGs). Movement of SGs towards the BLPM and their maturation involves interactions with endosomes (E) possibly using kiss-and-run mechanism. APM (solid line) is separated form the BLPM (dash line) by tight junctions (TJ). The ER (the structure with grey content) is marked by ribosomes (pictured as black dots) close to the *cis*-side of the Golgi contains ER exit site (ERES).

becomes increasingly evident, with the size of this pool at steady state approximating the fraction of molecules stored in secretory granules (Stahl et al. 1996). Thus, despite the slowness of ER exit for some secretory proteins (especially those that are difficult to fold), granule half-life in unstimulated cells is sufficiently long that the majority of intracellular regulated secretory proteins are endo-H-resistant (Lara-Lemus et al. 2006).

Another way to follow transport is to follow synchronous granulation or re-granulation (Handley et al. 2007). For instance in rat parotid acinar cells, existing secretory granules are almost entirely released immediately after isoproterenol injection, and 60 min later, newly formed granules are observed at the concave *trans*-face of the Golgi apparatus. Alternatively, formation of new SGs bearing fluorescent cargo has been followed after release from the 20°C block of protein exit from the TGN. This temperature block causes the *trans*-Golgi region to become markedly extended (Tamaki and Yamashina 2002). In another approach, after 60 min of restored temperature, fluorescent RSP [hCgB-GFP (S65T)] has completely exited from the TGN into ISGs (Rudolf et al. 2001). Both approaches suggest that intra-Golgi protein transport takes less than 60 min. However, precise measurements of the rate of intra-Golgi transport of RSPs have yet to be reported.

Several groups have attempted to determine requirements for SG formation from the Golgi complex using cell-free systems and in permeabilized cells (Austin and Shields 1996). For example, pro-opiomelanocortin (POMC) is packaged into granules before its endoproteolytic cleavage (Tooze et al. 1987; Tanaka et al. 1997; Andresen and Moore 2001). The requirements identified to obtain POMC processing (a marker of ISG maturation) are inclusion of cytosol and acidification of the granule lumen (at least to pH 6.2). Probably this reflects more than just a requirement for acidification, because processing of secretogranins II by PC2 cannot be activated merely by acidification alone. ARF1 enhances the POMC processing (maturation) assay, during which V-ATPase contributes compartmental acidification for activation of prohormone convertases, and further incubation at 37°C allows free granule release. Granule release is dependent on cytosol and ATP and is effectively inhibited by BFA or 100 μM GTPγS, implicating the activity of a high affinity GTP-binding protein (Dittie et al. 1997; Urbe et al. 1997a,b).

Membrane mechanisms for packaging regulated secretory proteins

It is well known that while transiting along the entire secretory pathway, RSPs become progressively concentrated. Concentration is evident already upon exit from the ER (Bendayan et al. 1980; Oprins et al. 2001), continues in the Golgi complex, and proceeds further in cells in which the proteins are efficiently packaged into SGs (Geuze et al. 1979; Geuze and Slot 1980; Slot and Geuze 1983). While prolactin may be 200 times more concentrated in dense core SGs than in the lumen of the ER (Farquhar et al. 1978), amylase

concentration in exocrine SGs is more moderate, engaging small concentration steps at the *cis*-side (Postuma et al. 1988) or *trans*-side (Bendayan 1982) of the Golgi stack.

In any event, formation of SGs is directly linked to ongoing transport of RSPs from earlier stages of the secretory pathway. While brefeldin A (BFA) does not affect already existing SGs, BFA causes marked redistribution of RSPs from the Golgi cluster to dilated cisternae of the RER, and blocks granule biogenesis. After BFA washout, Golgi stacks regenerate and shortly thereafter granule formation is re-initiated (Haang and Arvan 1994; Tamaki and Yamashina 2002).

Linked to these observations, one hypothesis has implied that concentration of RSPs might occur by COPI vesicles; however, evidence indicates that COPI-coated membranes are mostly depleted of RSPs such as proinsulin in pancreatic beta cells (Orci et al. 1997) or amylase in exocrine cells (Martinez-Menarguez et al. 1999). COPI is especially enriched in the membranous network of vesicular tubular clusters (VTCs), but this appears insufficient to explain concentration during RSP transport along the secretory pathway except perhaps to the extent that the concentration event may occur by exclusion (Martinez-Menarguez et al. 1999), resulting in increased protein concentration in the remaining *cis*-Golgi compartment. For amylase and chymotrypsinogen, an increase in concentration between ER and *cis*-Golgi compartment was reported as 3.7 and 57.6-fold, respectively (Oprins et al. 2001). If this were to occur solely from 'concentration by exclusion' (i.e., without other volume-removal mechanisms), then the initial VTC volume might need to be 57.6-fold larger than that of the final ER exit carriers, which would also need to exhibit unique surface-to-volume characteristics that render such a possibility unlikely. Indeed, after synchronization of transport, the surface area and volume of each Golgi cisterna is equal at any moment in time, and the volume of the VTC compartment is equal to the volume of the whole stack (Trucco et al. 2004). Thus, despite a definite role, Golgi cisternal maturation–progression may not be the sole concentrating mechanism for RSPs.

A detailed analysis of various models of intra-Golgi transport is described in Chapter 3.2. For the present discussion, we briefly note that increasing consideration is being given to a "kiss-and-run" model of intra-Golgi transport, which invokes a series of coordinated fusions and fission. This leads to residual connectivity along the secretory pathway, which may account for the fact that labelled lipids may diffuse through the Golgi complex even in aldehyde-fixed cells – suggesting structural continuities. In addition, this model may help to explain why SG biogenesis is blocked at 20°C (Kuliawat and Arvan 1992; Rudolf et al. 2001; Xu and Shields 1994).

The "kiss-and-run" model may be extended to include ISG biogenesis and maturation, implying that Golgi dilations (Ladinsky et al. 2002) fuse with the TGN, inducing concentration and carrier maturation by removal of non-granule resident proteins. The "kiss-and-run" hypothesis is based on the

assumption that several separate and distinct compartments co-exist in the secretory pathway and that membrane fusion events (homotypic fusions may be included, but the model especially requires heterotypic fusions) along with consecutive fissions, can be used to generate compartmental maturation.

For such a system to work: (1) compartments of distinct ionic environment must be narrowly connected to limit admixing, and (2) condensation of different RSPs along these interconnected but distinct compartmental domains must create a diffusion gradient along the tubular connections. Certainly, long processes/tubules emanating from the TGN have indeed been described (Polishchuk and Mironov 2004; Fig. 2). If intercisternal connections mediated by continuous fusion–fission events are used for granule formation
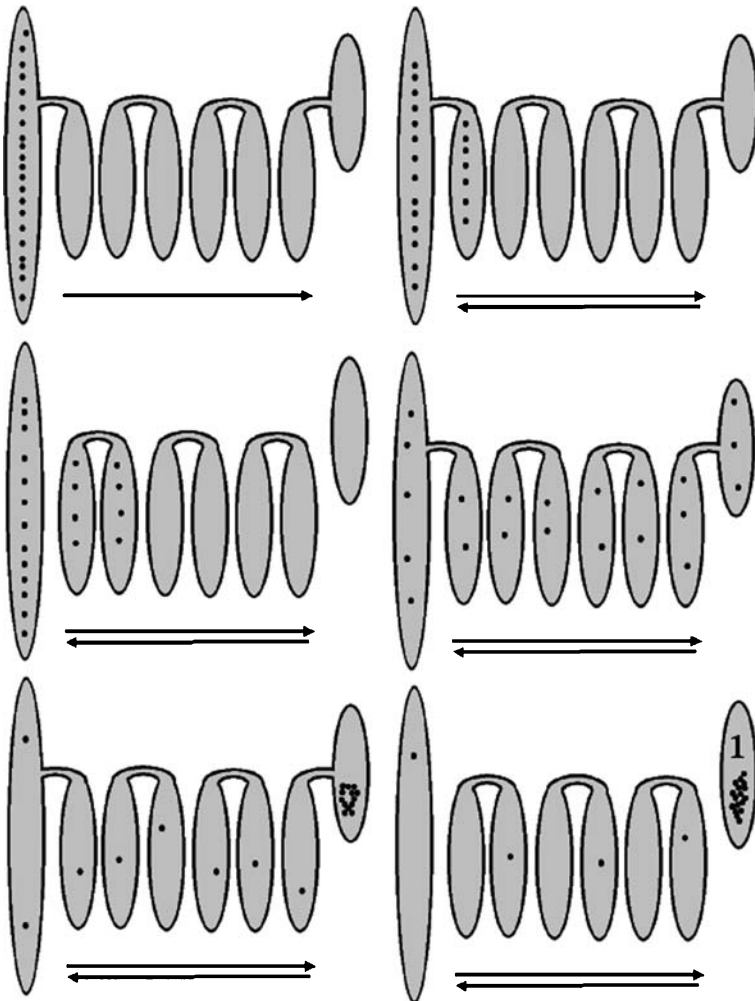
and RSP concentration, then predictions of this model may include that: (1) in properly designed studies to stage advancement of RSPs in the secretory pathway, microinjection of mutant αSNAP should be expected to block MSG formation, and (2) upon treatment with ONO or pyrophenone [inhibitors of PLA2a (see also Chapter 3.2.)], MSG generation should similarly be inhibited.

Sorting of RSPs to secretory granules

Secretory proteins ultimately follow regulated, constitutive, or constitutive-like secretory pathways. The delivery of different proteins to specific post-Golgi carriers and SGs involves processes collectively termed protein sorting and targeting (Arvan and Castle 1998). This occurs mostly at the TGN level. There are three principles driving this sorting: (1) selective entry of RSPs into nascent SGs due to their binding to membrane or cargo receptors, (2) selective retention of these proteins in maturing secretory granules due to multi-merization/condensation of RSPs in the ionic environment of SGs, and (3) refinement of membrane and content components by membrane removal mechanisms (Shennan 1996; Martinez-Menarguez et al. 1999). It seems most likely that a mosaic of these sorting mechanisms may contribute to RSP sorting in various regulated secretory cell types (Gorr et al. 2001; Day and Gorr 2003; Kim et al. 2003; Huh et al. 2003).

Figure 2. A schematic representing kiss-and-run mechanism of intra-Golgi transport of newly-synthesized RSPs. The ER-to-post-Golgi transport pathway is pictured as a row of compartments beginning from the ER (left, the most elongated oval filled with RSPs, black dots). Golgi cisternae are pictured as less elongated ovals in the middle, whereas the post-Golgi compartment (oval on the right) is placed above the Golgi cisternae. Each of these compartments could be successively connected or disconnected from each other. Thus in this model, there is alternating fusion and fission between Golgi cisternae and post-Golgi compartments. Initially (upper left panel), the Golgi cisternae and the post-Golgi compartments are shown as empty such as would be seen during a pulse-chase experiment, although in the steady state each of these compartments contains abundant protein undergoing transit. During the second stage of transport (right upper panel), fusion delivers cargo (black dots) to the most proximal Golgi cisterna. For ease of understanding the model, presentation of the ER-to-Golgi transit step is schematized and highly simplified. During the next stage (middle left panel), the first Golgi cisternae becomes disconnected from more proximal membranes whereas the first and second Golgi cisterna become transiently connected with cargo flowing between them. After several further reiterations (the middle right panel), as consecutive compartments become connected and disconnected, cargo reaches the post-Golgi compartment (black dots in the upper oval on the right). In this post-Golgi compartment where ionic pumps would be localized, cargo undergoes precipitation; the aggregated state limits cargo backflow to proximal compartments. The transient nature of the connections between the post-Golgi compartment and other compartments helps to prevent the specialized ionic environment from being dissipated. At the conclusion of intracellular transport, the near-final distributions of newly-synthesized RSPs are shown in the lower panels, with nearly all newly-synthesized cargo molecules becoming precipitated in the post-Golgi compartment (the compartment now called ISGs, or "1" in the Figure). In summary, in this model, the combination of alternating fusion and fission between Golgi cisternae, and aggregation of RSPs, are driving forces that facilitate transport of RSPs for storage in secretory granules.

The cargo receptor-based model called "sorting-for-entry" is analogous to receptor-mediated endocytosis and M6PR-dependent lysosomal proenzyme transport (Schmid 1997, see also Chapter 3.6), where cargo binds to receptors that in turn recruit a cytosolic coat. In indirect support of this model, the propeptide of prosomatostatin (Stoller and Shields 1989) a charged/hydrophobic patch (Dikeakos et al. 2007), and a disulfide-bonded loop segment of chromogranin B (CgB) (Kromer et al. 1998; Glombik et al. 1999) and dibasic processing sites (Felliciangeli and Kitabgi 2002) have been proposed as being necessary and sufficient to mediate sorting, acting as putative ligands. An amphipathic loop of POMC was also reported to be required for efficient sorting (Cool et al. 1995). Some major caveats would exist if this were to operate as a sole conserved sorting mechanism (Irminger et al. 1997). First, the failure to discover a sorting signal universal to all RSPs (Halban and Irminger 1994) excludes this as a single general mechanism. Second, luminal protein cargo abundance (often exceeding 100 mg/ml) in conjunction with restricted availability of membrane, limits accessible surface area to only a small fraction of luminal protein. Third, in 'sorting for entry' models, CSSPs are portrayed as excluded from granules (reviewed in Beuret et al. 2004; Lara-Lemus et al. 2006), although they may actually be selectively captured for entry into their own export carriers (Lara-Lemus et al. 2006; Rustom et al. 2002) as is seen in apical sorting in epithelial cells (Hansen et al. 2000; Ikonen 2001). Finally, receptor-mediated sorting for entry would lead one to anticipate a significant step-up in luminal cargo concentration during ISG formation, which has not been found, at least not in the exocrine pancreas (Oprins et al. 2001) (although this remains to be determined in AtT20 cells (Dhavantari and Loh 2000)), PC12 cells (Wendler et al. 2001; Feliciangeli and Kitabgi 2002), or Neuro2A cells (Cool et al. 1997)). While it does seem likely that at least some RSPs bind to specific sites on the luminal side of the ISG membrane, the puzzle to be solved in this model is to understand the stoichiometry of cargo receptors to the vast majority of RSPs stored in secretory granules.

To the extent that true cargo receptors exist, they must be membrane proteins or luminal proteins engaged tightly with granule lipids. The lipid composition of secretory granules is more cholesterol-rich than that of more proximal secretory pathway compartments, and this lipid composition is likely to be critical to proper formation of outbound membrane carriers (Wang et al. 2000; Tooze et al. 2001). Some RSPs interact with the luminal leaflet of the granule membrane (Dhanvantari and Loh 2000; Glombik et al. 1999; Tooze et al. 2001), including association with "lipid rafts" (Wasmeier et al. 2002; Dhavantari and Loh 2000; Blazquez et al. 2000, 2001).

In one example, SgIII association with cholesterol-rich microdomains can facilitate CgA association that can in turn associate with other RSPs (Hosaka et al. 2002; Taupenot et al. 2002a,b). The prohormone-convertases PC1, PC2, and PC5/6A also associate with lipid rafts (Blazquez et al. 2000, 2001; Dikeakos et al. 2007a,b; Lou et al. 2007) where they can contribute to sorting prohormones with dibasic processing sites (Feliciangeli et al. 2001; Brakch et al. 2002;

Lacombe et al. 2005; Garcia et al. 2005; Mulcahy et al. 2005). The most studied example is CPE, a protein that exhibits association with lipid rafts in the TGN, interacting with membranes via its C-terminal domain (Dhanvantari and Loh 2000), and had at one time been proposed as necessary to efficiently deliver proinsulin into newly forming ISGs (Dhavantari and Loh 2000; Cawley et al. 2003; Dhavantari et al. 2003). Indeed, CPE can co-aggregate with POMC, prolactin, and insulin at an acidic pH in vitro (Cool et al. 1997; Rindler 1998), which has served as a basis for proposing it as a sorting receptor for POMC (Cool et al. 1997) and other peptide hormones. However, not only is sorting of CgA not disturbed in Neuro-2a cells depleted of CPE (Normant and Loh 1998) but entry of proinsulin into ISGs is also not impaired in CPE-deficient Cpefat/Cpefat mice (Irminger et al. 1997; Varlamov et al. 1997). Moreover, in the 4 years since the homozygous CPE null mice have become available (Niamh et al. 2004), there are no indications of an inability of secretory granules to form or for newly synthesized prohormones to enter ISGs in these mice. However, CPE has more recently been proposed as a retention factor for prohormones during maturation of ISGs (Lou et al. 2005). Thus, CPE like many RSPs can serve as helper/assembly factors, facilitating protein condensation during SG biogenesis.

Granin-family members including chromogranin A (CgA) (Hosaka et al. 2005), CgB, secretogranin II (SgII), SgIII, and 7B2, are also targeted to SGs (Winkler and Fischer-Colbrie 1992; Song and Fricker 1995; Dannies 1999; Tooze et al. 2001; Day and Gorr 2003) and also have been implicated in RSP condensation (Natori and Huttner 1996). Both CgA and CgB associate with inositol 1,4,5-trisphosphate receptors in a pH-dependent manner (Thrower et al. 2003), although the significance of this is unclear. In CgA-deficient, POMC-expressing PC12 cells, storage of POMC in SGs appears impaired (Kim et al. 2001), but in tissues, granin family members probably exhibit functional redundancy. Mice lacking SgIII reveal no major obvious effects on viability, fertility, or locomotor behaviour (Kingsley et al. 1990). Perhaps this is because CgA is still efficiently targeted to SGs in cells, such as primary adrenal chromaffin cells, in which SgIII is lacking (Hosaka et al. 2002; Taupenot et al. 2002a,b). However, absence of CgA also generates a largely unremark-able mouse phenotype (Hendy et al. 2006), again speaking to redundancy between granins as well as non-granin RSPs.

By contrast, overexpression of CgA (Kim et al. 2001), CgB (Huh et al. 2003); or various RSPs can induce formation of SG-like structures with an electron-dense core even in cells lacking the traditional regulated secretory pathway (Michaux et al. 2003; Beuret et al. 2004). Indeed, transient expression in the COS-1 fibroblast cell line of several RSPs as well as SgII and CgB, but not alpha 1-antitrypsin, accumulate with varying efficiency in granule-like cytoplasmic structures devoid of markers of the endoplasmic reticulum, endosomes, and lysosomes, but partially co-localizing with TGN46 (Beuret et al. 2004). Expression of CgA (Kim et al. 2001) or CgB (Huh et al. 2003) induces related morphological structures, although ∼80%

of CgB–GFP is released from Vero cells within 2 h after synthesis (Wacker et al. 1997) with a storage of pro-Vasopressin in heterologous cells that is even lower (Beuret et al. 2004). While expression of granins or other RSPs in heterologous cells does not induce gene expression of other SG components (Kim et al. 2001; Huh et al. 2003; Beuret et al. 2004), overexpression of CgB may increase the sorting and processing of selected RSPs in regulated secretory cells (Natori and Huttner 1996). CgA forms dimers at pH 7.5 and tetramers at pH 5.5 (Yoo and Lewis 1992). The granin multimers formed in a weakly acidic, high Ca^{2+}-intragranular milieu can in turn facilitate RSP condensation (Yoo 2000; Yoo et al. 2001; Taupenot et al. 2002a,b; Dannies 1999; Thrower et al. 2003). Thus, like CPE, granins are also SG helper/assembly factors (Kim et al. 2001, 2003; Day and Gorr 2003), but endocrine cells clearly engage more than one gene product regulating the process of granule biogenesis and optimizing RSP storage efficiency (Beur et al. 2004).

RSP polymerization

RSPs appear to have evolved to lose solubility via multimerization in the intraluminal space (Kuliawat et al. 2000; Lee et al. 2001; Arvan et al. 2002; Song and Fricker 1995; Yoo 1996), indicating that differences in solubility may play an important role in protein trafficking. The main site of RSP condensation is the TGN with continuation in ISGs (although multimers can form within earlier compartments – see above sections). Condensed proteins often accumulate in dilated regions of the TGN possessing tubular interconnections (Clermont et al. 1995), which can result in formation of a concentration gradient (Bauerfeind and Huttner 1993) that, according to the "kiss-and-run" model (see above) can lead to formation of ISGs. In such a view, one might expect no important structural requirements for luminal protein entry into ISGs (El Meskini et al. 2001; Molinete et al. 2000) other than these are proteins that had not otherwise been removed via TGN tubule sequestration or fragmentation.

Multimeric protein assembly is one of the factors regulating efficiency of RSP storage by mechanisms that might be considered as the culmination of the intra-Golgi transport process. In the extreme case, it would be plausible for >99% of RSPs to be sorted by multimerization with <1% (i.e., an undetectable fraction) of molecules sorted by receptor interactions, underscoring the potential quantitative importance of passive associations in the appropriate intraluminal environment (reviewed in Arvan and Halban 2004). Thus, the bioactivity of substoichiometric amounts of SG assembly/helper factors in the Golgi complex is augmented by the predisposition of RSPs to multimerize under intraluminal TGN conditions (Chanat and Huttner 1991; Colomer et al. 1996). However, the consequences for protein sorting in the secretory pathway as a result of higher-order protein assembly may be less predictable than for receptor-mediated trafficking events. This is because there may be equilibria between soluble and multimeric states, competitions at the luminal

leaflet and in the soluble phase for both heterotypic and homotypic protein interactions, mixed complexes containing similar proteins in different stoichiometric ratios, and different post-translationally modified forms of the same gene product exhibiting different properties (Zhang et al. 2001). Remarkably, most condensed RSPs rapidly dissolve when the ionic environment and concentrations of the intragranular space are reversed (Arvan et al. 1984).

Multimerization interactions may be homotypic or heterotypic. In endocrine cells, an increase in SG density is accompanied by membrane remodelling (Eaton et al. 2000) and even crystallization of internal cargo (Greider et al. 1969; Kuliawat and Arvan 1992), although actual crystal formation is not required for storage (Arvan and Halban 2004). In exocrine cells, certain sulphated proteoglycans may facilitate efficient storage of amylase in parotid SGs (Venkatesh and Gorr 2002) and may contribute to heterotypic RSP storage in the exocrine pancreas (De Lisle 2002; Venkatesh et al. 2004), even as they play no clear role in sorting and storage of endocrine RSPs (Gorr 1996). Differences in aggregation of individual proteins (Colomer et al. 1996; Kleene et al. 1999) can result in different RSPs being sorted to distinct intracellular locations (Klumperman et al. 1996). In exocrine cells two distinct proteins initially appearing in ISGs at one stoichiometric ratio are finally stored in MSGs at a different stoichiometric ratio (Von Zastrow and Castle 1987). In somatomammotrophs of the bovine pituitary, distinct prolactin and growth hormone granules appear in the same cells, and occasionally appear in separate aggregates within single granules (Fumagalli and Zanini 1985; Hashimoto et al. 1987). In bag cell neurons of *Aplysia californica*, the precursor of egg-laying hormone is cleaved into distinct C-terminal and N-terminal products that are packaged into separate granules, with sorting that appears to occur by homotypic multimerization (Sossin et al. 1990). The partitioning of luminal volume between various outbound pathways from the TGN is likely to be a factor in these various regulated secretory cell types (Arvan and Castle 1998; Thiele et al. 1997) and between cell lines (Rindler et al. 2001) and their cognate cell types in vivo.

Protein structure optimized for the distinct intracompartmental ionic environment of SGs is an important factor in RSP multimer formation and storage. Simple engineering of hexahistidine epitope tags onto secretory proteins can enhance calcium-induced RSP condensation within the SG environment (Gorr 1996; Oliver et al. 1997; Jain et al. 2000). The "granin loop" (Song and Fricker 1995) is another feature likely designed for condensation in the high calcium, lower pH environment. This ability to undergo calcium- and low-pH-induced condensation has been demonstrated for both endocrine (Bell-Parikh et al. 2001; Jain et al. 2000), and exocrine (Dartsch et al. 1998; De Lisle 2002) RSPs. A CgA–GFP fusion protein is trafficked to dense-core granules in PC12 cells (Taupenot et al. 2002a,b) and exposure to bafilomycin A1 causes a substantial decrease in detectable granules (Taupenot et al. 2005), while such treatment of pituitary cells causes POMC and prolactin to accumulate in larger vacuolar structures (Henomatsu et al. 1993; Schoonderwoert

et al. 2000). Thus, in order to induce condensation of RSPs, SGs must generate an acidic pH (reviewed in Wu et al. 2001). Mechanisms that SGs may use to achieve a more acidic pH than the TGN may also include a low transmembrane proton leak and a favourable buffering capacity at low pH. Additionally, calcium buffering (e.g., CgB binds >90 Ca^{2+}/mol with a Kd of 1.5 mM) promotes RSP condensation as well as providing calcium for stimulated release to the cytosol (Yoo et al. 2001).

Insulin storage as an example

The "sorting by retention" model was developed from studies of trafficking of proinsulin-derived peptides (the major peptides manufactured in pancreatic beta cells) (Arvan and Halban 2004). Proinsulin forms hexamers but they have very poor self-association properties to form higher-order complexes (Orci 1985). Nevertheless the vast majority of newly synthesized proinsulin in beta cells is directed into ISGs (Rhodes and Halban 1987). Despite hypotheses to the contrary (Orci et al. 1984a,b; Fredman et al. 2000; Osterbye et al. 2001), the existence of a specific TGN-based sorting mechanism for entry into ISGs has not been found for proinsulin (Glombik and Gerdes 2000). In pancreatic beta cells, proinsulin rather than conversion intermediates or final processing products) is initially packaged in ISGs. Thus the earliest detectable granules are proinsulin-rich, replete with electron-pale material, carry a (discontinuous) clathrin coat, and have a weakly acidic milieu (Orci et al. 1984b, 1986). In the absence of stimulation, proinsulin is converted to insulin, which is retained for storage in granules, whereas in the presence of secretagogues there is stimulated release of both proinsulin and newly made insulin. The majority of endogenous proinsulin is recovered in the soluble phase prior to its conversion to insulin within ISGs. By contrast, intragranular insulin polymerizes as a function of both prevailing proton and zinc concentrations (Kuliawat and Arvan 1994). Even in yeast, endoproteolytic processing in the late Golgi to form single chain insulin from a fusion protein precursor promotes intracellular retention, preventing rapid secretion of the precursor (Zhang et al. 2001). In mammalian beta cells, after proteolytic processing, insulin becomes insoluble within granules whereas C-peptide remains completely soluble.

During the processing period, lysosomal procathepsin B, long recognized as an insulin granule component (Docherty et al. 1984), is actively removed from beta cell ISGs (Kuliawat and Arvan 1994). Nevertheless, the majority of granule C-peptide is not removed from islet ISGs but remains behind in mature granules (Kuliawat and Arvan 1992) because constitutive-like protein traffic conveys only a very minor fraction of ISG volume (reviewed in Arvan and Halban 2004). Indeed, it has been questioned in primary beta cells whether insulin is significantly better retained in granules than proinsulin (Molinete et al. 2001), although such a result does seem clear in cell culture models (Kuliawat et al. 2000). Further, a point mutant of proinsulin, His-

B10Asp, that is defective for hexamerization, tends to be associated with enhanced constitutive or constitutive-like secretion (Carroll et al. 1988; Gross et al. 1989), and is found at excessive levels in the circulation of human patients bearing the mutation (Chan et al. 1987). A proinsulin in which dibasic endoprotease cleavage sites were mutated to residues that cannot be cleaved was reported to be sorted efficiently to SGs (Halban and Irminger 2003), consistent with another report that a mutant bearing no C-peptide whatsoever is efficiently sorted to SGs (Powell et al. 1988). These findings have been used to argue in favour of selective sorting for proinsulin entry into ISGs rather than efficient storage by condensation of insulin. However upon further study, it was found that the latter molecules (lacking C-peptide) were quantitatively misfolded, bearing mispaired disulfide bonds (Liu et al. 2003). Surprisingly, this unsuspected misfolding does not prevent transit of the mutants to the TGN and beyond (Liu et al. 2003), but it would imply that a putative proinsulin sorting receptor (Dhanvantari et al. 2003) would need also to be able to recognize a completely non-native ligand. We think this is possible, but unlikely. All in all, the insulin example tends to support the sorting by retention model; favouring the view that polymeric assembly of newly synthesized insulin (Huang and Arvan 1994, 1995) helps to enhance its storage in maturing secretory granules (Kuliawat and Arvan 1992). This model is discussed further in the next section.

Entrance, exit, and avoidance of non-granule proteins in ISGs

The 'sorting by retention' model invokes both multimerization/condensation of specific RSPs to facilitate retention in ISGs along with membranous removal of proteins that are not destined to be stored in SGs (Arvan and Halban 2004). Thus, the sorting-by-retention hypothesis does not require that specific sorting information is used at the entry step into forming SGs, thereby rendering the SG itself as an important sorting station. Indeed, lysosomal proenzymes, and endosomal and lysosomal membrane proteins en route, and luminal and membrane proteins thought of as markers of the constitutive secretory pathway, can also enter ISGs (for review see (Arvan and Castle 1998)).

Newly synthesized lysosomal proenzymes en route to the endosomal system enter ISGs (Kuliawat and Arvan 1994; Klumperman et al. 1998; Turner and Arvan 2000). To refine the composition of MSGs, these proteins must be removed as ISGs mature (Kuliawat and Arvan 1994); indeed, procathepsin B is virtually quantitatively removed during ISG maturation. When lysosomal proenzymes are defective for specific M6PR-mediated recognition, their entry into granules is even more abundant – although removal from maturing granules is blocked (Kuliawat and Arvan 1992).

Although challenged by some (Rindler et al. 2001), the bulk of the evidence favours that there also can be substantial entrance of soluble secretory proteins from the TGN into ISGs (Arvan and Castle 1998). This entry seems

to require neither N-glycans (Kelly et al. 1983) nor lipid raft association (Feng and Arvan 2003). For instance, SEAP, a truncated form of alkaline phosphatase lacking the glycosyl phosphatidylinositol membrane anchor, has been described as a CSSP (Gorr 1996; Harrison et al. 1996; Molinete et al. 2000) although at least in pancreatic beta cells, SEAP abundantly enters ISGs (Lara-Lemus et al. 2006). Indeed, there are no data indicating that initial entry of SEAP into β-cell secretory granules is any less efficient than that of endogenous proinsulin, although ultimate storage of the two proteins in SGs differs (Arvan and Halban 2004). While a CgB–eGFP fusion protein is targeted to ISGs (Kaether et al. 1997), soluble secretory GFP (a protein that is not normally targeted to the secretory pathway but that can be introduced therein merely by virtue of the presence of a cleavable signal sequence) also abundantly enters SGs (El Meskini et al. 2001; Molinete et al. 2006). This form of GFP not only enters insulin secretory granules, but it also appears to be stored and secreted with efficiency that is comparable to that of authentic insulin (Molinete et al. 2006). However, the ability of eGFP to form disulfide-linked oligomers may affect the outcome of these experiments (Jain et al. 2001; Feng and Arvan 2003). Nevertheless, entrance from the TGN into ISGs of "marker proteins of the constitutive pathway" is not limited to fusion proteins or genetically engineered GFP-containing constructs (Feng and Arvan 2003).

How would CSSPs be prevented from entry into ISGs? Several experiments have been performed to date (Tooze and Huttner 1990; Chanat and Huttner 1991), and thus far, only very few identified proteins have been found to meet the criteria of failing to enter ISGs. Recently, a genetically truncated version of Cab45 (a protein that binds Ca^{2+} and normally resides in the Golgi lumen, Scherer et al. 1996) was described as the first bona fide constitutive secretory marker to be identified without demonstratably entering ISGs. Similar to other members of the CREC family, loss of information encoded in the C-terminal region of Cab45 causes the protein to lose its intracellular retention within the Golgi complex (Honore and Vorum 2000). Indeed, Cab308myc is no longer colocalizes with the GM130 Golgi marker, but rather is distributed to vesicular organelles at the cell periphery that are non-overlapping with the immunoreactive distribution of insulin (SGs) or proinsulin (ISGs) (Lara-Lemus et al. 2006). Importantly, unlike SEAP or even alpha 1-antitrypsin (Feng and Arvan 2003), at no time does Cab308myc ever enter a stimulus-releasable compartment – at least not in INS1 insulinoma cells, and apparently also not in AtT20 pituitary cells (Lara-Lemus et al. 2006). How does Cab308myc avoid entry into ISGs? Interestingly, upon permeabilization of organelle membranes with saponin, SEAP behaves as a typical soluble secretory protein, being fully extracted by the detergent treatment, whereas Cab308myc remains associated with the luminal aspect of secretory pathway membranes destined to become transport intermediates for the constitutive secretory pathway (Lara-Lemus et al. 2006). At the time of exocytosis, the membrane associations of such secretory proteins must be reversed to account for their free release to the extracellular environment upon exocytosis (Schlegel et al. 2001).

In addition to this constitutive secretory pathway conveyed directly from the TGN to the cell surface in regulated secretory cells (Orci et al. 1987a,b), the constitutive-like secretory pathway involves two limbs: one from ISGs (and the TGN) to the endosomal system followed by a second from endosomes to the cell surface (Turner and Arvan 2000). Constitutive-like secretion of lysosomal procathepsin B appears to proceed via an endosomal intermediate (Turner and Arvan 2000), although the role of endosomes as intermediates in the release of bona fide secretory proteins has been less well studied (Millar et al. 2000). In our view, the important aspect of the constitutive-like secretory pathway is not the quantitative contribution of its contents to overall secretion, but rather the notion that a membrane trafficking pathway out of ISGs is likely targeted to endosomes rather than direct retrograde transport to the Golgi/TGN. Membrane recycling between exocytic and endocytic pathways is described in greater detail in Chapter 3.10, but here, we wish to emphasize that these relationships are especially prominent in regulated secretory cells.

Once reaching endosomes, a fraction of secretory protein is likely to be channelled to lysosomes for degradation (Neerman-Arbez and Halban 1993) with another fraction conveyed to the extracellular space, creating constitutive-like secretion (Kuliawat and Arvan 1992). Support continues to grow for the idea that unstimulated secretion of proteins that had already traversed, but were no longer contained within, the secretory granule compartment (Arvan et al. 1991; Huang et al. 2001) is likely to reflect membrane trafficking events occurring via an endosomal intermediate (Feng and Arvan 2003).

Role of clathrin

In neuroendocrine cells, there is clear concentration of clathrin at regions of the TGN membrane engaging in granulogenesis (Orci et al. 1984a,b) and on parts of the membrane of ISGs. In both TGN dilations and detached ISGs, most clathrin is accumulated on clathrin-coated buds (for review see Halban and Irminger 1994). Clathrin coat, mannose phosphate receptors (M6PRs), furin, and AP-1 adaptors present at the TGN and ISGs (Orci et al. 1987a,b; Dittie et al. 1996; Klumperman et al. 1998) disappear in MSGs.

Excess membranes and proteins such as the ubiquitously expressed TGN/endosomal membrane protease furin, are thought to be removed from maturing granules by budding of clathrin-coated ISG-derived vesicles (Dittie et al. 1996, 1997; Klumperman et al. 1998). Indeed, a list of proteins removed from ISGs may include some CSSPs as well as lysosomal enzymes and endo/lysosomal membrane proteins such as M6PR. Thus, sorting of lysosomal enzymes from the ISGs uses the same system involving the M6PR as elsewhere in the TGN (Kuliawat et al. 1997). GGA (Golgi-associated g-ear-containing ADP-ribosylation-factor-binding protein) is an adaptor protein recruiting clathrin to ISGs (Kakhlon et al. 2006). Only after reaching a relatively advanced stage of morphological maturation do SGs lose the clathrin coat

(Tooze and Tooze 1986). Neither the dominant negative clathrin hub fragment (Molinete et al. 2001) nor arginine/lysine analogs (Kuliawat and Arvan 1992) block creation of new granules but both demonstrate that clathrin function is needed for ISG maturation. What might greatly advance this model of ISG maturation would be if clathrin-dependent vesicles after budding from ISGs could be isolated in vitro after incubation with cytosol and appropriate budding factors.

Clathrin might also be invoked in the sorting-for-entry model, in which RSPs bind specific membrane receptors. However, it has been shown that TGN and *trans*-Golgi-derived clathrin-coated buds contain Golgi resident proteins (Velasco et al. 1993) but excluding secretory protein cargoes (Salamero et al. 1990); and experiments with yeast mutants reveal that clathrin can play a direct role in the retention of Golgi resident proteins (Seeger and Payne 1992).

Lui-Roberts et al. (2005) have recently proposed a novel scaffolding role for an AP-1/clathrin coat in initial endothelial cell granule formation. However, unless we invoke significant cell type-specific differences, such a model cannot account for the fact that expression of the dominant negative clathrin Hub peptide does not affect the efficiency of delivery of peptide prohormones to SGs (Molinete et al. 2001); and clathrin depletion does not affect prohormone processing (Andresen and Moore 2001).

Role of cytoskeleton in transport of SG

ISGs and MSGs have somewhat different localizations. The cell cortex is the site of priming and docking of SGs (Robinson and Martin 1998). The fraction of granules docked at the plasmalemma varies with cell type (e.g., in PC12 cells, the majority of MSGs is localized in close proximity to the plasma membrane (Baneriee et al. 1996)); such granules can be recruited to undergo stimulated exocytosis (Martin and Kowalchyk 1997). While certainly not the only factors, both microtubules and F-actin synergistically and consecutively affect SG cortical localization.

Microtubules are implicated particularly in fast transport of ISGs from the TGN to the PM (Rudolf et al. 2001). Newly made ISGs may leave the TGN in straight trajectories towards the plasmalemma with maximal velocities of up to 2 μm/s (Rudolf et al. 2001). This straight, unidirectional transport contrasts with the anterograde transport of constitutive carriers which is also microtubule-dependent but may occur in a random bidirectional manner (Wacker et al. 1997) – although in NGF-differentiated PC12 cells, SGs may also move both anterogradely and retrogradely (Lochner et al. 1998; Rudolf et al. 2001). In the presence of nocodazole to disrupt microtubules, ISGs do not accumulate in the TGN area but reach the PM more indirectly (Rudolf et al. 2001).

Using a phogrin-dsRed fusion protein to follow SG movement (Wasmeier and Hutton 1996), anterograde transport along microtubules was shown to require the ATP-dependent activity of the conventional kinesin motor (Varadi

et al. 2002). Using another RSP tagged with the dsRed-E5 'timer' protein (a fluorophore that progressively shifts its fluorescence emission over 16 h, Terskikh et al. 2000), it was found that newly generated SGs are quickly mobilized to the plasma membrane (Duncan et al. 2003) and, as a function of age, their speed and direction changes with some granules moving rapidly in a saltatory manner along straight lines, indicating transport along tracks.

Actin filaments engage SGs at slightly later stages of maturation. In neuroendocrine chromaffin and PC12 cells, completion of ISG-to-MSG maturation is kinetically linked to F-actin binding (Rudolf et al. 2001). Specifically, upon shift from 20°C (a temperature at which detachment of ISGs from the TGN is blocked, Kuliawat and Arvan 1992) to 37°C, GFP-labelled ISGs move within seconds in a microtubule-dependent fashion to the F-actin-rich cell cortex where they mature as measured by progressive loss of furin signal (Rudolf et al. 2001). SGs undergo both F-actin-dependent and independent immobilization (Rudolf et al. 2001), and interaction of SGs with cortical F-actin (Trifaro and Vitale 1993) remains preserved in vitro in preparations of plasma membrane sheets (Martin and Kowalchyk 1997; Avery et al. 2000).

SNAREs and RABs in the regulated secretory pathway

A complete review of these proteins is beyond the scope of this chapter. However, it is necessary here to at least recognize that SNARE proteins move dynamically during compartmental genesis and maturation, while selected Rab proteins are acquired on the cytosolic face of the organelle.

Syntaxin 6 (Syn6) is predominantly distributed in endosomes and the TGN (Kuliawat et al. 2004) although Syn6 is also present in ISGs but not MSGs (Bock et al. 1997; Klumperman et al. 1998; Steegmaier et al. 1999; Eaton et al. 2000; Hinners et al. 2003). Homotypic ISG–ISG fusion, proposed as a step in MSG biogenesis (Tooze et al. 1991; Urbe et al. 1998) engages Syn6 (Wendler et al. 2001) and synaptotagmin IV (Ahras et al. 2006). Interestingly, the process of secretory granule maturation is SNAP-dependent (Chamberlain et al. 1995) suggesting involvement of (forward or retrograde) membrane fusion machinery.

The final stages of SG lifespan (Nemoto et al. 2001; Thorigrave;n et al. 2004; Sørensen 2004) require SNAREs to trigger exocytosis in the presence of Ca^{2+}. Synaptotagmin (a Ca^{2+} – and phospholipid-binding protein) is involved in setting the Ca^{2+} dependence of the fusion process (Sorensen 2004). In sea urchin eggs, SNARE complexes primarily increase the Ca^{2+} sensitivity of fusion between plasma membrane and cortical exocytic vesicles (Coorssen et al. 1998; Johns et al. 2001). In turn, SNARE proteins undergo assembly into functionally active complexes following a rise in Ca^{2+}, with formation of the tSNARE complex preceding formation of the trans vSNARE–tSNARE assembly. The details of tSNARE complex formation have not been characterized in vivo, thus it is not certain which represents the core complex precursor in vivo (Stojikovic 2005).

Assembly of the SG–plasma membrane SNARE complex consists of two main steps. The first involves a high affinity syntaxin interaction with the N-terminal SNARE motif of SNAP25 (An and Almers 2004). This complex assembles reversibly during transient elevations in Ca^{2+} induced by depolarization of cells and tolerates a mutation that blocks formation of other kinds of syntaxin–SNAP25 complexes. This complex might function as the plasma membrane "docking receptor" for SGs containing VAMP, to be followed by *trans*-SNARE formation during a Ca^{2+}-dependent priming step of exocytosis (Stojikovic 2005). However, at steady state, certain molecules such as VAMP4 are distributed in the TGN and ISGs but not in MSGs (Bock et al. 1997; Klumperman et al. 1998; Steegmaier et al. 1999; Eaton et al. 2000; Hinners et al. 2003) eliminating them from consideration as partners in the final stages of SG docking. Thus, some SNARE proteins in ISGs function only in a prelude to actual stimulus-dependent exocytosis (Regazzi et al. 1996; Wheeler et al. 1996).

Another important class of regulators are the Rabs, especially members of the Rab3 and Rab27 families. In PC12 cells, endogenous Rab3a colocalizes with SGII-marked SGs, and transiently expressed EGFP–Rab3a (and ECFP–Rab27a) preferentially localizes to newly made ISGs dissociated from the TGN within 20 min after release of the 20°C temperature block (Handley et al. 2007). Experiments using fluorescence recovery after photo bleaching have suggested that Rab3a might continue to rapidly exchange between granule-bound and cytosolic forms, yet exocytosis per se does not drive immediate release or dispersal of Rab3a from granule membranes (Handley et al. 2007). A potentially important effector of Rab3a is Noc2, whose deficiency in genetically altered mice results in diminished insulin secretion from pancreatic beta cells as well as significant defects in exocrine granule exocytosis (Matsumoto et al. 2004).

The Rab27 subfamily consists of two homologs with closely related but non-identical function in the regulated secretory pathway. Rab27a is known to be important for the biogenesis and function of lysosome-related organelles such as melanosomes (Barral et al. 2002) although it has also been implicated in endocrine SG docking at the plasma membrane (Kasai et al. 2005). Rab27b may be expressed in a somewhat more narrow range of tissues than Rab27a. Loss of Rab27b function seems to be associated with a prominent decrease in the numbers of platelet granules and a bleeding disorder (Tolmachova et al. 2007; Tsuboi and Fukuda 2006), as well as abnormal movement and secretion of mast cell granules (Mizuno et al. 2007). Nevertheless, Rab27b may also play a role in granule exocytosis in the brain and in certain endocrine and exocrine tissues (Zhao et al. 2002; Imai et al. 2004; Gomi et al. 2007).

Exocytosis of SGs

As with the previous section, such a topic extends well beyond the scope of this chapter. Here, we merely wish to note that design of the secretory granule

must build in how the SG will be deployed to complete the anterograde transport pathway with depletion of granule contents and selective handling of SG membranes (Borgonovo et al. 2006).

Exocytosis of SG contents to the extracellular space is mediated by complete or partial fusion ("kiss-and-run", with a variable duration of <1 s to >10 s (Rutter and Tsuboi 2004) of SG continuities with the plasma membrane (Tsuboi and Rutter 2003; Tsuboi et al. 2004). In its most minimal form, a narrow pore allows only selective release of low-molecular-mass species (ATP, GABA, etc.) from SGs. Electrophysiological recordings appear unable to detect fusion events with such a minimal pore size. In a greater form, the fusion opening proceeds to limited admixing of vesicle membrane proteins with those of the plasma membrane (Tsuboi et al. 2004) while constituents of the dense core are extruded. Live cell imaging of fluorescent probes such as the lipophilic dye FM-1-43 appears able to detect a multiplicity of such interactions between SGs and the PM (Leung et al. 2002).

To be available for either form of fusion with the plasma membrane, SG docking and priming events must take place first. Priming occurs in two stages, resulting in generation of slowly releasable and rapidly releasable pools of granules. Recent findings indicate that all transitional stages leading up to and including the fusion event itself have potential reversibility (Martin 2003). While priming requires only a moderate increase in Ca^{2+} as well as the presence of MgATP, SG exocytosis demands a more substantial elevation in Ca^{2+} and exhibits a considerably longer latency than that known for synaptic vesicles. SG exocytosis can occur from either the slowly or rapidly releasable primed pool of SGs, but with different rates and latency. Thus, the extent of content release is variable with respect to both the fraction of cellular granules involved, and the fraction of cargo of individual SGs undergoing release – both depend on the nature and strength of the secretagogue as well as key second messengers (Stojikovic 2005) and their effectors (Tsuboi et al. 2006).

One of the molecules that may limit full fusion and diffusional intermixing of SG membranes with the plasmalemma is dynamin. When simultaneously imaged with a fluorescent vesicle cargo (NPY-mRFP), dynamin-1-EGFP is observed to arrive at sites of exocytosis synchronously with the onset of release events, and to linger for 2–3 s after the peak of release (Artalejo et al. 2002), where it may be engaged in closing the fusion pore (Tsuboi et al. 2004). Dynamin recruitment is also likely to be a key feature to the nearly intact recapture of SG membranes to the cytoplasm following exocytosis (Taraska et al. 2003). However, the signals that recruit dynamin to SG exocytotic sites remain unclear. Local increases in PtdIns(4,5)P2 and increased local Ca^{2+} (Wiser et al. 1999; Emmanouilidou et al. 1999) might each play roles in recruiting dynamin to the sites of potential binding partners, including members of the SNX family (sorting nexins, especially SNX9; Soulet et al. 2005) as well as phospholipase D (Lee et al. 2006), syndapin-1/PACSIN (Anggono et al. 2006), and syntaxin-1 (Galas et al. 2000).

Conclusions

This chapter on origins of the SG highlights the creation and utilization of a novel organelle that has evolved with the increasing complexity of eukaryotic cells and been assigned a highly specialized task for regulated cargo delivery to the cell surface. Much of the task begins in the Golgi complex, and is critically linked to the specific biophysical behaviour of content proteins that helps to drive protein condensation for storage (see Fig. 1). Regardless of model invoked for the origins of the regulated secretory pathway, the specialization and refinement of the SG membrane appears to require ongoing membrane fusion and fission events. Herein, we present the idea that "kiss-and-run" – a model that has grown in popularity to account for many exocytotic events – may be equally applicable in earlier intracellular compartments along the anterograde transport pathway, and could be intimately associated with Golgi structure and sorting mechanisms for granule biogenesis (Fig. 2).

Abbreviations

BFA   brefeldin A
Cg   chromogranin
CPE   carboxypeptidase E
CS   constitutive secretory
CSSP   constitutive soluble secretory protein
ER   endoplasmic reticulum
ISG   immature SGs
MSG   mature SG
PC1   prohormone convertases 1
PC2   prohormone convertases 2
POMC   pro-opiomelanocortin
RSP   regulatory secretory protein
SG   storage/secretory granule
SgII   secretogranin II
TGN   *trans*-Golgi network
VTC   vesicular tubular cluster

References

Ahras M, Otto GP, Tooze SA (2006) Synaptotagmin IV is necessary for the maturation of secretory granules in PC12 cells. J Cell Biol 173(2): 241–251

Alberts B, Johnson A, Lewis J, Raff M, Roberts K, Walter P (2002) Molecular biology of the cell, 4th edn. Garland Science, New York

An SJ, Almers W (2004) Tracking SNARE complex formation in live endocrine cells. Science 306: 1042–1046

Andresen JM, Moore HP (2001) Biogenesis of processing-competent secretory organelles in vitro. Biochemistry 40(43): 13020–13030

Anggono V, Smillie KJ, Graham ME, Valova VA, Cousin MA, Robinson PJ (2006) Syndapin I is the phosphorylation-regulated dynamin I partner in synaptic vesicle endocytosis. Nat Neurosci 9(6): 752–760

Artalejo CR, Elhamdani A, Palfrey HC (2002) Sustained stimulation shifts the mechanism of endocytosis from dynamin-1-dependent rapid endocytosis to clathrin- and dynamin-2-mediated slow endocytosis in chromaffin cells. Proc Natl Acad Sci USA 99(9): 6358–6363

Arvan P, Rudnick G, Castle JD (1984) Osmotic properties and internal pH of isolated rat parotid secretory granules. J Biol Chem 259: 13567–13572

Arvan P, Castle D (1998) Sorting and storage during secretory granule biogenesis: looking backward and looking forward. Biochem J 332: 593–610

Arvan P, Kuliawat R, Prabakharan D, Zavacki A-M, Elahi D, Wang S, Pilkey D (1991) Protein discharge from immature secretory granules displays both regulated and constitutive characteristics. J Biol Chem 266: 14171–14174

Arvan P, Zhang BY, Feng L, Liu M, Kuliawat R (2002) Lumenal protein multimerization in the distal secretory pathway/secretory granules. Curr Opin Cell Biol 14(4): 448–453

Arvan P, Halban PA (2004) Sorting ourselves out: seeking consensus on trafficking in the beta-cell. Traffic 5: 53–61

Austin CD, Shields D (1996) Prosomatostatin processing in permeabilized cells. Calcium is required for prohormone cleavage but not formation of nascent secretory vesicles. J Biol Chem 271: 1194–1199

Avery J, Ellis D, Lang T, Holroyd P, Riedel D, Henderson R, Edwardson M, Jahn R (2000) A cell-free system for regulated exocytosis in PC12 cells. J Cell Biol 148: 317–324

Banerjee A, Barry VA, Bibhuti RD, Martin TFJ (1996) N-ethylmaleimide-sensitive factor acts at a prefusion ATP-dependent step in $Ca2+$ -activated exocytosis. J Biol Chem 271: 20223–20226

Barral DC, Ramalho JS, Anders R, Hume AN, Knapton HJ, Tolmachova T, Collinson LM, Goulding D, Authi KS, Seabra MC (2002) Functional redundancy of Rab27 proteins and the pathogenesis of Griscelli syndrome. J Clin Invest 110: 247–257

Bauerfeind R, Huttner WB (1993) Biogenesis of constitutive secretory vesicles, secretory granules and synaptic vesicles. Curr Opin Cell Biol 5(4): 628–635

Bell-Parikh LC, Eipper BA, Mains RE (2001) Response of an integral granule membrane protein to changes in pH. J Biol Chem 276: 29854–29863

Bendayan M, Roth J, Perrelet A, Orci L (1980) Quantitative immunocytochemical localization of pancreatic secretory proteins in subcellular compartments of the rat acinar cell. J Histochem Cytochem 28: 149–160

Bendayan M (1982) Double immunocytochemical labeling applying the protein A-gold technique. J Histochem Cytochem 30: 81–85

Beuret N, Stettler H, Renold A, Rutishauser J, Spiess M (2004) Expression of regulated secretory proteins is sufficient to generate granule-like structures in constitutively secreting cells. J Biol Chem 279(19): 20242–20249

Blazquez M, Thiele C, Huttner WB, Docherty K, Shennan KIJ (2000) Involvement of the membrane lipid bilayer in sorting prohormone convertase 2 into the regulated secretory pathway. Biochem J 349: 843–852

Blazquez M, Docherty K, Shennan KI (2001) Association of prohormone convertase 3 with membrane lipid rafts. J Mol Endocrinol 27(1): 107–116

Bock JB, Klumperman J, Davanger S, Scheller RH (1997) Syntaxin 6 functions in *trans*-Golgi network vesicle trafficking. Mol Biol Cell 8: 1261–1271

Borgonovo B, Ouwendijk J, Solimena M (2006) Biogenesis of secretory granules. Curr Opin Cell Biol 18: 365–370

Bowman GR, Smith DG, Siu KWM, Pearlman RE, Turkewitz AP (2005) Genomic and proteomic evidence for a second family of dense core granule cargo proteins in Tetrahymena thermophila. J Eukaryot Microbiol 52: 291–297

Brakch N, Allemandou F, Cavadas C, Grouzmann E, Brunner HR (2002) Dibasic cleavage site is required for sorting to the regulated secretory pathway for both pro- and neuropeptide Y. J Neurochem 81: 1166–1175

Carroll RJ, Hammer RE, Chan SJ, Swift HH, Rubenstein AH, Steiner DF (1988) A mutant human proinsulin is secreted from islets of Langerhans in increased amounts via an unregulated pathway. Proc Natl Acad Sci USA 85: 8943–8947

Castle JD, Castle AM (1996) Two regulated secretory pathways for newly synthesized parotid salivary proteins are distinguished by doses of secretagogues. J Cell Sci 109: 2591–2599

Cawley NX, Rodriguez YM, Maldonado A, Loh YP (2003) Trafficking of mutant carboxypeptidase E to secretory granules in a {beta}-cell line derived from Cpefat/Cpefat Mice. Endocrinology 144: 292–298

Cawley NX, Zhou J, Hill JM, Abebe D, Romboz S, Yanik T, Rodriguiz RM, Wetsel WC, Loh YP (2004) The carboxypeptidase E knockout mouse exhibits endocrinological and behavioral deficits. Endocrinology 145: 5807–5819

Chabin-Brion K, Marceiller J, Perez F, Settegrana C, Drechou A, Durand G, Pous C (2001) The Golgi complex is a microtubule-organizing organelle. Mol Biol Cell 12: 2047–2060

Chamberlain LH, Roth D, Morgan A, Burgoyne RD (1995) Distinct effects of alpha-SNAP, 14-3-3 proteins, and calmodulin on priming and triggering of regulated exocytosis. J Cell Biol 130(5): 1063–1070

Chamberlain LH, Henry J, Burgoyne RD (1996) Cysteine string proteins are associated with chromaffin granules. J Biol Chem 271(32): 19514–19517

Chan SJ, Seino S, Gruppuso PA, Schwartz R, Steiner DF (1987) A mutation in the B chain coding region is associated with impaired proinsulin conversion in a family with hyperproinsulinemia. Proc Natl Acad Sci USA 84: 2194–2197

Chanat E, Huttner WB (1991) Milieu-induced, selective aggregation of regulated secretory proteins in the trans-Golgi network. J Cell Biol 115(6): 1505–1519

Chavez RA, Miller SG, Moore HP (1996) A biosynthetic regulated secretory pathway in constitutive secretory cells. J Cell Biol 133(6): 1177–1191

Clermont Y, Rambourg A, Hermo L (1992) Segregation of secretory material in all elements of the Golgi apparatus in principal epithelial cells of the rat seminal vesicle. Anat Rec 232: 349–358

Clermont Y, Xia L, Rambourg A, Turner JD, Hermo L (1993) Transport of casein submicelles and formation of secretion granules in the Golgi apparatus of epithelial cells of the lactating mammary gland of rat. Anat Rec 235: 363–373

Clermont Y, Rambourg A, Hermo L (1995) Trans-Golgi network (TGN) of different cell types: three-dimensional structural characteristics and variability. Anat Rec 242(3): 289–301

Colomer V, Kicska GA, Rindler MJ (1996) Secretory granule content proteins and the luminal domains of granule membrane proteins aggregate in vitro at mildly acidic pH. J Biol Chem 271(1): 48–55

Cool DR, Fenger M, Snell CR, Loh YP (1995) Identification of the sorting signal motif within pro-opiomelanocortin for the regulated secretory pathway. J Biol Chem 270 (15): 8723–8729

Cool DR, Normant E, Shen FS, Chen HC, Pannel L, Zhang Y, Loh YP (1997) Carboxypeptidase E is a regulated secretory pathway sorting receptor: genetic obliteration leads to endocrine disorders in Cpefat mice. Cell 88: 73–83

Coorssen JR, Blank PS, Tahara M, Zimmerberg J (1998) Biochemical and functional studies of cortical vesicle fusion: the SNARE complex and Ca2+ sensitivity. J Cell Biol 143(7): 1845–1857

Cowan AT, Bowman GR, Edwards KF, Emerson JJ, Turkewitz AP (2005) Genetic, genomic, and functional analysis of the granule lattice proteins in Tetrahymena secretory granules. Mol Biol Cell 16: 4046–4060

Cowley DJ, Moore YR, Darling DS, Joyce PB, Gorr SU (2000) N- and C-terminal domains direct cell type-specific sorting of chromogranin A to secretory granules. J Biol Chem 275: 7743–7748

Dannies PS (1999) Protein hormone storage in secretory granules: mechanisms for concentration and sorting. Endocr Rev 20(1): 3–21

Dartsch H, Kleene R, Kern HF (1998) In vitro condensation-sorting of enzyme proteins isolated from rat pancreatic acinar cells. Eur J Cell Biol 75: 211–222

Day R, Gorr SU (2003) Secretory granule biogenesis and chromogranin A: master gene, on/off switch or assembly factor? Trends Endocrinol Metab 14(2): 10–13

De Lisle RC (2002) Role of sulfated O-linked glycoproteins in zymogen granule formation. J Cell Sci 115: 2941–2952

De Matteis MA, Luini A (2008) Exiting the Golgi complex. Nat Rev Mol Cell Biol 9(4): 273–284

DeCamili P, Jahn R (1990) Pathways to regulated exocytosis in neurons. Annu Rev Physiol 52: 625–645

Dhanvantari S, Loh YP (2000) Lipid raft association of carboxypeptidase E is necessary for its function as a regulated secretory pathway sorting receptor. J Biol Chem 275: 29887–29893

Dhanvantari S, Shen FS, Adams T, Snell CR, Zhang C, Mackin RB, Morris SJ, Loh YP (2003) Disruption of a receptor-mediated mechanism for intracellular sorting of proinsulin in familial hyperproinsulinemia. Mol Endocrinol 17: 1856–1867

Dikeakos JD, Lacombe MJ, Mercure C, Mireuta M, Reudelhuber TL (2007a) A hydrophobic patch in a charged alpha-helix is sufficient to target proteins to dense core secretory granules. J Biol Chem 282: 1136–1143

Dikeakos JD, Mercure C, Lacombe MJ, Seidah NG, Reudelhuber TL (2007b) PC1/3, PC2 and PC5/6A are targeted to dense core secretory granules by a common mechanism. FEBS J 274: 4094–4102

Dittie AS, Hajibagheri N, Tooze SA (1996) The AP-1 adaptor complex binds to immature secretory granules from PC12 cells, and is regulated by ADP-ribosylation factor. J Cell Biol 132(4): 523–536

Dittie AS, Thomas L, Thomas G, Tooze SA (1997) Furin is sorted into the regulated secretory pathway in neuroendocrine cells, interacts with the AP-1 complex, and is removed during granule maturation by a casein kinase II dependent mechanism. EMBO J 16: 4859–4870

Docherty K, Hutton JC, Steiner DF (1984) Cathepsin B-related proteases in the insulin secretory granule. J Biol Chem 259: 6041–6044

Duncan RR, Greaves J, Wiegand UK, Matskevich I, Bodammer G, Apps DK, Shipston MJ, Chow RH (2003) Functional and spatial segregation of secretory vesicle pools according to vesicle age. Nature 422(6928): 176–180

Eaton BA, Haugwitz M, Lau D, Moore HP (2000) Biogenesis of regulated exocytotic carriers in neuroendocrine cells. J Neurosci 20: 7334–7344

El Meskini R, Jin L, Marx R, Bruzzaniti A, Lee J, Emeson R, Mains R (2001) A signal sequence is sufficient for green fluorescent protein to be routed to regulated secretory granules. Endocrinology 142: 864–873

Emmanouilidou E, Teschemacher A, Pouli AE, Nicholls LI, Seward EP, Rutter GA (1999) Imaging Ca2+ concentration changes at the secretory vesicle surface with a recombinant targeted cameleon. Curr Biol 9(16): 915–918

Farquhar MG, Reid JJ, Daniell LW (1978) Intracellular transport and packaging of prolactin: a quantitative electron microscope autoradiographic study of mammotrophs dissociated from rat pituitaries. Endocrinology 102: 296–311

Feliciangeli S, Kitabgi P, Bidard JN (2001) The role of dibasic residues in prohormone sorting to the regulated secretory pathway. A study with proneurotensin. J Biol Chem 276: 6140–6150

Feliciangeli S, Kitabgi P (2002) Insertion of dibasic residues directs a constitutive protein to the regulated secretory pathway. Biochem Biophys Res Commun 290: 191–196

Feng L, Arvan P (2003) The trafficking of alpha 1-antitrypsin, a post-Golgi secretory pathway marker, in INS-1 pancreatic beta cells. J Biol Chem 278: 31486–31494

Fontijn RD, Goud B, Echard A, Jollivet F, Van Marle J, Pannekoek H, Horrevoets AJ (2001) The human kinesin-like protein RB6K is under tight cell cycle control and is essential for cytokinesis. Mol Cell Biol 21: 2944–2955

Fredman P, Mansson JE, Rynmark BM, Josefsen K, Ekblond A, Halldner L, Osterbye T, Horn T, Buschard K (2000) The glycosphingolipid sulfatide in the islets of Langerhans in rat pancreas is processed through recycling: possible involvement in insulin trafficking. Glycobiology 10: 39–50

Fumagalli G, Zanini A (1985) In cow anterior pituitary, growth hormone and prolactin can be packed in separate granules of the same cell. J Cell Biol 100: 2019–2024

Galas MC, Chasserot-Golaz S, Dirrig-Grosch S, Bader MF (2000) Presence of dynamin–syntaxin complexes associated with secretory granules in adrenal chromaffin cells. J Neurochem 75: 1511–1519

Garcia AL, Han SK, Janssen WG, Khaing ZZ, Ito T, Glucksman MJ, Benson DL, Salton SR (2005) A prohormone convertase cleavage site within a predicted alpha-helix mediates sorting of the neuronal and endocrine polypeptide VGF into the regulated secretory pathway. J Biol Chem 280: 41595–41608

Geuze HJ, Slot JW (1980) Disproportional immunostaining patterns of two secretory proteins in guinea pig and rat exocrine pancreatic cells. An immunoferritin and fluorescence study. Eur J Cell Biol 21: 93–100

Geuze HJ, Slot JW, Tokuyasu KT (1979) Immunocytochemical localization of amylase and chymotrypsinogen in the exocrine pancreatic cell with special attention to the Golgi complex. J Cell Biol 82: 697–707

Glombik MM, Krömer, A, Salm T, Huttner WB, Gerdes HH (1999) The disulfide-bonded loop of chromogranin B mediates membrane binding and directs sorting from the trans-Golgi network to secretory granules. EMBO J 18(4): 1059–1070

Glombik MM, Gerdes HH (2000) Signal-mediated sorting of neuropeptides and prohormones: secretory granule biogenesis revisited. Biochimie 82: 315–326

Gomi H, Mori K, Itohara S, Izumi T (2007) Rab27b is expressed in a wide range of exocytic cells and involved in the delivery of secretory granules near the plasma membrane. Mol Biol Cell 18: 4377–4386

Gorr SU (1996) Differential storage of prolactin, granins (chromogranin B and secretogranin II), and constitutive secretory markers in rat pituitary GH4C1 cells. J Biol Chem 271: 3575–3580

Gorr SU, Jain RK, Kuehn U, Joyce PB, Cowley DJ (2001) Comparative sorting of neuroendocrine secretory proteins: a search for common ground in a mosaic of sorting models and mechanisms. Mol Cell Endocrinol 172: 1–6

Greider MH, Howell SL, Lacy PE (1969) Isolation and properties of secretory granules from rat islets of Langerhans. II. Ultrastructure of the beta granule. J Cell Biol 41: 162–166

Gross DJ, Halban PA, Kahn CR, Weir GC, Villa-Komaroff L (1989) Partial diversion of a mutant proinsulin (B10 aspartic acid) from the regulated to the constitutive secretory pathway in transfected AtT-20 cells. Proc Natl Acad Sci USA 86(11): 4107–4111

Halban PA, Irminger JC (1994) Sorting and processing of secretory proteins. Biochem J 299(Pt 1): 1–18

Halban PA, Irminger JC (2003) Mutant proinsulin that cannot be converted is secreted efficiently from primary rat beta-cells via the regulated pathway. Mol Biol Cell 14: 1195–1203

Handley MT, Haynes LP, Burgoyne RD (2007) Differential dynamics of Rab3A and Rab27A on secretory granules. J Cell Sci 120: 973–984

Hannah MJ, Schmidt AA, Huttner WB (1999) Synaptic vesicle biogenesis. Annu Rev Cell Dev Biol 15: 733–798

Hansen GH, Nies-Christiansen L-L, Thorsen E, Immerdal L, Danielsen EM (2000) Cholesterol depletion of enterocytes. Effect on the Golgi complex and apical membrane trafficking. J Biol Chem 275(7): 5136–5142

Harrison TM, Chidgey MA, Uff S (1996) Novel markers for constitutive secretion used to show that tissue plasminogen activator is sorted to the regulated pathway in transfected PC12 cells. Cell Biol Int 20: 293–299

Hashimoto S, Fumagalli G, Zanini A, Meldolesi J (1987) Sorting of three secretory proteins to distinct secretory granules in acidophilic cells of cow anterior pituitary. J Cell Biol 105(4): 1579–1586

Hendy GN, Li T, Girard M, Feldstein RC, Mulay S, Desjardins R, Day R, Karaplis AC, Tremblay ML, Canaff L (2006) Targeted ablation of the chromogranin a (Chga) gene: normal neuroendocrine dense-core secretory granules and increased expression of other granins. Mol Endocrinol 20(8): 1935–1947

Henomatsu N, Yoshimori T, Yamamoto A, Moriyama Y, Tashiro Y (1993) Inhibition of intracellular transport of newly synthesized prolactin by bafilomycin A1 in a pituitary tumor cell line, GH3 cells. Eur J Cell Biol 62(1): 127–139

Hinners I, Wendler F, Fei H, Thomas L, Thomas G, Tooze SA (2003) AP-1 recruitment to VAMP4 is modulated by phosphorylation-dependent binding of PACS-1. EMBO Rep 4: 1–8

Honore B, Vorum H (2000) The CREC family, a novel family of multiple EF-hand, low-affinity Ca2+ -binding proteins localised to the secretory pathway of mammalian cells. FEBS Lett 466(1): 11–18

Hosaka M, Watanabe T, Sakai Y, Uchiyama Y, Takeuchi T (2002) dentification of a chromogranin A domain that mediates binding to secretogranin III and targeting to secretory granules in pituitary cells and pancreatic beta-cells. Mol Biol Cell 13(10): 3388–3399

Hosaka M, Suda M, Sakai Y, Izumi T, Watanabe T, Takeuchi T (2004) Secretogranin III binds to cholesterol in the secretory granule membrane as an adapter for chromogranin A. J Biol Chem 279: 3627–3634

Hosaka M, Watanabe T, Sakai Y, Kato T, Takeuchi T (2005) Interaction between secretogranin III and carboxypeptidase E facilitates prohormone sorting within secretory granules. J Cell Sci 118: 4785–4795

Huang IF, Arvan P (1994) Formation of the insulin-containing secretory granule core occurs within immature beta-granules. J Biol Chem 269: 20838–20844

Huang XF, Arvan P (1995) Intracellular transport of proinsulin in pancreatic beta-cells. Structural maturation probed by disulfide accessibility. J Biol Chem 270(35): 20417–20423

Huang AY, Castle AM, Hinton BT, Castle JD (2001) Resting (basal) secretion of proteins is provided by the minor regulated and constitutive-like pathways and not granule exocytosis in parotid acinar cells. J Biol Chem 276: 22296–22306

Huh YH, Jeon SH, Yoo SH (2003) Chromogranin B-induced secretory granule biogenesis: comparison with the similar role of chromogranin A. J Biol Chem 278(42): 40581–40589

Hui N, Nakamura N, Sönnichsen B, Shima DT, Nilsson T, Warren G (1997) An isoform of the Golgi t-SNARE, syntaxin 5, with an endoplasmic reticulum retrieval signal. Mol Biol Cell 8(9): 1777–1787

Ikonen E (2001) Roles of lipid rafts in membrane transport. Curr Opin Cell Biol 13(4): 470–477

Imai A, Yoshie S, Nashida T, Shimomura H, Fukuda M (2004) The small GTPase Rab27B regulates amylase release from rat parotid acinar cells. J Cell Sci 117: 1945–1953

Irminger JC, Verchere CB, Meyer K, Halban PA (1997) Proinsulin targeting to the regulated pathway is not impaired in carboxypeptidase E-deficient Cpefat/Cpefat mice. J Biol Chem 272(44): 27532–27534

Jain RK, Joyce PB, Gorr SU (2000) Aggregation chaperones enhance aggregation and storage of secretory proteins in endocrine cells. J Biol Chem 275(35): 27032–27036

Jain RK, Joyce PB, Molinete M, Halban PA, Gorr SU (2001) Oligomerization of green fluorescent protein in the secretory pathway of endocrine cells. Biochem J 360: 645–649

Johns LM, Levitan ES, Shelden EA, Holz RW, Axelrod D (2001) Restriction of secretory granule motion near the plasma membrane of chromaffin cells. J Cell Biol 153: 177–190

Kaether C, Salm T, Glombik M, Almers W, Gerdes H-H (1997) Targeting of green fluorescent protein to neuroendocrine secretory granules: a new tool for real time studies of regulated protein secretion. Eur J Cell Biol 74: 133–142

Kakhlon O, Sakya P, Larijani B, Watson R, Tooze SA (2006) GGA function is required for maturation of neuroendocrine secretory granules. EMBO J 25: 1590–1602

Kasai K, Ohara-Imaizumi M, Takahashi N, Mizutani S, Zhao S, Kikuta T, Kasai H, Nagamatsu S, Gomi H, Izumi T (2005) Rab27a mediates the tight docking of insulin granules onto the plasma membrane during glucose stimulation. J Clin Invest 115: 388–396

Kelly RB, Buckley KM, Burgess TL, Carlson SS, Caroni P, Hooper JE, Katzen A, Moore HP, Pfeffer SR, Schroer TA (1983) Membrane traffic in neurons and peptide-secreting cells. Cold Spring Harbor Symp Quant Biol 48(Pt2): 697–705

Kim T, Tao-Cheng JH, Eiden LE, Loh YP (2001) Chromogranin A, an "on/off" switch controlling dense-core secretory granule biogenesis. Cell 106(4): 499–509

Kim T, Tao-Cheng JH, Eiden LE, Loh YP (2003) The role of chromogranin A and the control of secretory granule genesis and maturation. Trends Endocrinol Metab 14 (2): 56–57

Kingsley DM, Rinchik EM, Russell LB, Ottiger H-P, Sutcliffe JG, Copeland NG, Jenkins NA (1990) Genetic ablation of a mouse gene expressed specifically in brain. EMBO J 9(2): 395–399

Kleene R, Kastner B, Rosser R, Kern H (1999) Complex formation among rat pancreatic secretory proteins under mild alkaline pH conditions. Digestion 60(4): 305–313

Klumperman J, Spijker S, Van Minnen J, Sharp-Baker H, Smit AB, Geraerts WPM (1996) Cell type-specific sorting of neuropeptides: a mechanism to modulate peptide composition of large dense-core vesicles. J Neurosci 16(24): 7930–7940

Klumperman J, Kuliawat R, Griffith JM, Geuze HJ, Arvan P (1998) Mannose 6-phosphate receptors are sorted from immature secretory granules via AP-1, clathrin, and syntaxin 6-positive vesicles. J Cell Biol 141: 359–371

Krömer A, Glombik MM, Huttner WB, Gerdes HH (1998) Essential role of the disulfide-bonded loop of chromogranin B for sorting to secretory granules is revealed by expression of a deletion mutant in the absence of endogenous granin synthesis. J Cell Biol 140(6): 1331–1346

Kuliawat R, Arvan P (1992) Protein targeting via the 'constitutive-like' secretory pathway in isolated pancreatic islets: passive sorting in the immature granule compartment. J Cell Biol 118: 521–529

Kuliawat R, Arvan P (1994) Distinct molecular mechanisms for protein sorting within immature secretory granules of pancreatic β-cells. J Cell Biol 126: 77–86

Kuliawat R, Klumperman J, Ludwig T, Arvan P (1997) Differential sorting of lysosomal enzymes out of the regulated secretory pathway in pancreatic beta-cells. J Cell Biol 137: 595–608

Kuliawat R, Prabakaran D, Arvan P (2000) Proinsulin endoproteolysis confers enhanced targeting of processed insulin to the regulated secretory pathway. Mol Biol Cell 11: 1959–1972

Kuliawat R, Kalinina E, Bock J, Fricker L, McGraw TE, Kim SR, Zhong J, Scheller R, Arvan P (2004) Syntaxin-6 SNARE involvement in secretory and endocytic pathways of cultured pancreatic β–cells. Mol Biol Cell 15(4): 1690–1701

Lacombe MJ, Mercure C, Dikeakos JD, Reudelhuber TL (2005) Modulation of secretory granule-targeting efficiency by cis and trans compounding of sorting signals. J Biol Chem 280: 4803–4807

Ladinsky MS, Wu CC, McIntosh S, McIntosh JR, Howell K (2002) Structure of the Golgi and distribution of reporter molecules at 20°C reveals the complexity of the exit compartments. Mol Biol Cell 13: 2810–2825

Lane J, Allan V (1998) Microtubule-based membrane movement. Biochim Biophys Acta 1376(1): 27–55

Laine J, Lebel D (1999) Efficient binding of regulated secretory protein aggregates to membrane phospholipids at acidic pH. Biochem J 338: 289–294

Lara-Lemus R, Liu M, Turner MD, Scherer P, Stenbeck G, Iyengar P, Arvan P (2006) Lumenal protein sorting to the constitutive secretory pathway of a regulated secretory cell. J Cell Sci 119: 1833–1842

Lee MS, Zhu YL, Chang JE, Dannies PS (2001) Acquisition of Lubrol insolubility, a common step for growth hormone and prolactin in the secretory pathway of neuroendocrine cells. J Biol Chem 276: 715–721

Lee CS, Kim IS, Park JB, Lee MN, Lee HY, Suh PG, Ryu SH (2006) The phox homology domain of phospholipase D activates dynamin GTPase activity and accelerates EGFR endocytosis. Nat Cell Biol 8(5): 477–484

Leung YM, Sheu L, Kwan E, Wang G, Tsushima R, Gaisano H (2002) Visualization of sequential exocytosis in rat pancreatic islet beta cells. Biochem Biophys Res Commun 292(9): 980–986

Liu M, Ramos-Castaneda J, Arvan P (2003) Role of the connecting peptide in insulin biosynthesis. J Biol Chem 278: 14798–14805

Lochner JE, Kingma M, Kuhn S, Meliza CD, Cutler B, Scalettar BA (1998) Real-time imaging of the axonal transport of granules containing a tissue plasminogen activator/green fluorescent protein hybrid. Mol Biol Cell 9: 2463–2476

Lou H, Kim SK, Zaitsev E, Snell CR, Lu B, Loh YP (2005) Sorting and activity-dependent secretion of BDNF require interaction of a specific motif with the sorting receptor carboxypeptidase E. Neuron 45: 245–255

Lou H, Smith AM, Coates LC, Cawley NX, Loh YP, Birch NP (2007) The transmembrane domain of the prohormone convertase PC3: a key motif for targeting to the regulated secretory pathway. Mol Cell Endocrinol 267: 17–25

Lui-Roberts WW, Collinson LM, Hewlett LJ, Michaux G, Cutler DF (2005) An AP-1/clathrin coat plays a novel and essential role in forming the Weibel–Palade bodies of endothelial cells. J Cell Biol 170: 627–636

Martin TFJ, Kowalchyk JA (1997) Docked secretory vesicles undergo Ca^{2+}-activated exocytosis in a cell-free system. J Biol Chem 272: 14447–14453

Martin TF (2003) Tuning exocytosis for speed: fast and slow modes. Biochim Biophys Acta 1641: 157–165

Martínez-Menárguez JA, Geuze HJ, Slot JW, Klumperman J (1999) Vesicular tubular clusters (VTCs) between ER and Golgi mediate concentration of soluble secretory proteins by exclusion from COPI-coated vesicles. Cell 98: 81–90

Matsumoto M, Miki T, Shibasaki T, Kawaguchi M, Shinozaki H, Nio J, Saraya A, Koseki H, Miyazaki M, Iwanaga T, Seino S (2004) Noc2 is essential in normal regulation of exocytosis in endocrine and exocrine cells. Proc Natl Acad Sci USA 101: 8313–8318

Meldolesi J, Chieregatti E, Luisa Malosio ML (2004) Requirements for the identification of dense-core granules. Trends Cell Biol 14: 13–19

Michaux G, Hewlett LJ, Messenger SL, Goodeve AC, Peake IR, Daly ME, Cutler DF (2003) Analysis of intracellular storage and regulated secretion of 3 von Willebrand disease-causing variants of von Willebrand factor. Blood 102: 2452–2458

Millar CA, Meerloo T, Martin S, Hickson GRX, Shimwell NJ, Wakelam MJO, James DE, Gould GW (2000) Adipsin and the glucose transporter GLUT4 traffic to the cell surface via independent pathways in adipocytes. Traffic 1(2): 141–151

Mizuno K, Tolmachova T, Ushakov DS, Romao M, Abrink M, Ferenczi MA, Raposo G, Seabra MC (2007) Rab27b regulates mast cell granule dynamics and secretion. Traffic 8: 883–892

Molinete M, Lilla V, Jain R, Joyce PBM, Gorr S-U, Ravazzola M, Halban PQ (2000) Trafficking of non-regulated secretory proteins in insulin secreting (INS-1) cells. Diabetologia 43: 1157–1164

Molinete M, Dupuis S, Brodsky FM, Halban PA (2001) Role of clathrin in the regulated secretory pathway of pancreatic beta-cells. J Cell Sci 114: 3059–3066

Mulcahy LR, Vaslet CA, Nillni EA (2005) Prohormone-convertase 1 processing enhances post-Golgi sorting of prothyrotropin-releasing hormone-derived peptides. J Biol Chem 280: 39818–39826

Natori S, Huttner WB (1996) Chromogranin B (secretogranin I) promotes sorting to the regulated secretory pathway of processing intermediates derived from a peptide hormone precursor. Proc Natl Acad Sci USA 93(9): 4431–4436

Neerman-Arbez M, Halban PA (1993) Novel, non-crinophagic, degradation of connecting peptide in transformed pancreatic beta cells. J Biol Chem 268(22): 16248–16252

Nemoto Y, Wenk MR, Watanabe M, Daniell L, Murakami T, Ringstad N, Yamada H, Takei K, De Camilli P (2001) Identification and characterization of a synaptojanin 2 splice isoform predominantly expressed in nerve terminals. J Biol Chem 276(44): 41133–41142

Nguyen TT, Ong H, De Lean A (1988) Secretion and biosynthesis of atrial natriuretic factor by cultured adrenal chromaffin cells. FEBS Lett 231: 393–396

Normant E, Loh YP (1998) Depletion of carboxypeptidase E, a regulated secretory pathway sorting receptor, causes misrouting and constitutive secretion of proinsulin and proenkephalin, but not chromogranin A. Endocrinology 139(4): 2137–2145

Oliver PM, Fox JE, Kim R, Rockman HA, Kim HS, Reddick RL, Pandey KN, Milgram SL, Smithies O, Maeda N (1997) Hypertension, cardiac hypertrophy, and sudden death in mice lacking natriuretic peptide receptor A. Proc Natl Acad Sci USA 94(26): 14730–14735

Oprins A, Rabouille C, Posthuma G, Klumperman J, Geuze HJ, Slot JW (2001) The ER to Golgi interface is the major concentration site of secretory proteins in the exocrine pancreatic cell. Traffic 2: 831–838

Orci L, Ravazzola M, Perrelet A (1984a) Proinsulin associates with Golgi membranes of pancreatic B cells. Proc Natl Acad Sci USA 81: 6743–6746

Orci L, Halban P, Amherdt M, Ravazzola M, Vassalli J-D, Perrelet A (1984b) A clathrin-coated, Golgi-related compartment of the insulin secreting cell accumulates proinsulin in the presence of monensin. Cell 39: 39–47

Orci L (1985) The insulin factory. a tour of the plant surroundings and a visit to the assembly line. Diabetologia 28: 528–546

Orci L, Ravazzola M, Amherdt M, Madsen O, Perrelet A, Vassalli JD, Anderson RGW (1986) Conversion of proinsulin to insulin occurs coordinately with acidification of maturing secretory vesicles. J Cell Biol 103: 2273–2281

Orci L, Ravazzola M, Storch MJ, Anderson RG, Vassalli JD, Perrelet A (1987a) Proteolytic maturation of insulin is a post-Golgi event which occurs in acidifying clathrin-coated secretory vesicles. Cell 49(6): 865–868

Orci L, Ravazzola M, Amherdt M, Perrelet A, Powell SK, Quinn DL, Moore H-PH (1987b) The trans-most cisternae of the Golgi complex: a compartment for sorting of secretory and plasma membrane proteins. Cell 51(6): 1039–1051

Orci L, Stamnes M, Ravazzola M, Amherdt M, Perrelet A, Sollner TH, Rothman JE (1997) Bidirectional transport by distinct populations of COPI-coated vesicles. Cell 90(2): 335–349

Osterbye T, Jorgensen KH, Fredman P, Tranum-Jensen J, Kaas A, Brange J, Whittingham JL, Buschard K (2001) Sulfatide promotes the folding of proinsulin, preserves insulin crystals, and mediates its monomerization. Glycobiology 11: 473–479

Polishchuk RS, Mironov AA (2004) Structural aspects of Golgi function. Cell Mol Life Sci 61(2): 146–158

Posthuma G, Slot JW, Geuze HJ (1988) Immunogold determination of amylase concentrations in pancreatic subcellular compartments. Eur J Cell Biol 46: 327–335

Powell SK, Orci L, Craik CS, Moore HPH (1988) Efficient targeting to storage granules of human proinsulins with altered propeptide domain. J Cell Biol 106: 1843–1851

Rambourg A, Segretain D, Clermont Y (1984) Tridimensional architecture of the Golgi apparatus in the atrial muscle cell of the rat. Am J Anat 170: 163–179

Rambourg A, Clermont Y, Chretien M, Olivier L (1992) Formation of secretory granules in the Golgi apparatus of prolactin cells in the rat pituitary gland: a stereoscopic study. Anat Rec 232: 169–179

Rambourg A, Clermont Y, Chrétien M, Olivier L (1993) Modulation of the Golgi apparatus in stimulated and nonstimulated prolactin cells of female rats. Anat Rec 235(3): 353–362

Rambourg A, Jackson CL, Clermont Y (2001) Three dimensional configuration of the secretory pathway and segregation of secretion granules in the yeast *Saccharomyces cerevisiae*. J Cell Sci 114(Pt 12): 2231–2239

Regazzi R, Sadoul K, Meda P, Kelly RB, Halban PA, Wollheim CB (1996) Mutational analysis of VAMP domains implicated in Ca^{2+}-induced insulin exocytosis. EMBO J 15: 6951–6959

Rhodes CJ, Halban PA (1987) Newly synthesized proinsulin/insulin and stored insulin are released from pancreatic B cells predominantly via a regulated, rather than constitutive, pathway. J Cell Biol 105: 145–153

Rindler MJ (1998) Carboxypeptidase E, a peripheral membrane protein implicated in the targeting of hormones to secretory granules, co-aggregates with granule content proteins at acidic pH. J Biol Chem 273(47): 31180–31185

Rindler MJ, Colomer V, Jin Y (2001) Immature granules are not major sites for segregation of constitutively secreted granule content proteins in NIT-1 insulinoma cells. Biochem Biophys Res Commun 288: 1071–1077

Robinson LJ, Martin TFJ (1998) Docking and fusion in neurosecretion. Curr Opin Cell Biol 10: 483–492

Rudolf R, Salm T, Rustom A, Gerdes HH (2001) Dynamics of immature secretory granules: role of cytoskeletal elements during transport, cortical restriction, and F-actin-dependent tethering. Mol Biol Cell 12(5): 1353–1365

Rustom A, Bajohrs M, Kaether C, Keller P, Toomre D, Corbeil D, Gerdes HH (2002) Selective delivery of secretory cargo in Golgi-derived carriers of nonepithelial cells. Traffic 3: 279–288

Rutter GA, Tsuboi T (2004) Kiss and run exocytosis of dense core secretory vesicles. Neuroreport 15(1): 79–81

Salamero J, Sztul E, Howell K (1990) Exocytic transport vesicles generated in vitro from the *trans*-Golgi network carry secretory and plasma membrane proteins. Proc Natl Acad Sci USA 87(19): 7717–7721

Schlegel A, Arvan P, Lisanti MP (2001) Caveolin-1 binding to endoplasmic reticulum (ER) membranes and entry into the regulated secretory pathway are regulated by serine phosphorylation. Protein sorting at the level of the ER. J Biol Chem 276: 4398–4408

Schmid SL (1997) Clathrin-coated vesicle formation and protein sorting: an integrated process. Annu Rev Biochem 66: 511–548

Scherer PE, Lederkremer GZ, Williams S, Fogliano M, Baldini M, Lodish HF (1996) Cab45, a novel (Ca2+)-binding protein localized to the Golgi lumen. J Cell Biol 133(2): 257–268

Schoonderwoert VT, Holthuis JC, Tanaka S, Tooze SA, Martens GJ (2000) Inhibition of the vacuolar H + -ATPase perturbs the transport, sorting, processing and release of regulated secretory proteins. Eur J Biochem 267(17): 5646–5654

Seeger M, Payne G (1992) Selective and immediate effects of clathrin heavy chain mutations on Golgi membrane protein retention in *Saccharomyces cerevisiae*. J Cell Biol 118(3): 531–540

Sesso A, Assis JE, Kuwajima VY, Kachar B (1980) Freeze-fracture and thin-section study of condensing vacuoles in rat pancreatic acinar cells. Acta Anat 108: 521–539

Shennan KI (1996) Intracellular targeting of secretory proteins in neuroendocrine cells. Biochem Soc Trans 24: 535–539

Slot JW, Geuze HJ (1983) Immunoelectron microscopic exploration of the Golgi complex. J Histochem Cytochem 31: 1049–1056

Slot JW, Garutti G, Martin S, Oorschot V, Posthuma G, Kraegen EW, Laybutt R, Thibault G, James DE (1997) Glucose transporter (GLUT-4) is targeted to secretory granules in rat atrial cardiomyocytes. J Cell Biol 137: 1243–1254

Sollner TH (2003) Regulated exocytosis and SNARE function. Mol Membr Biol 20: 209–220

Song L, Fricker LD (1995) Calcium- and pH-dependent aggregation of carboxypeptidase E. J Biol Chem 270(14): 7963–7967

Sørensen JB (2004) Formation, stabilisation and fusion of the readily releasable pool of secretory vesicles. Pflugers Arch 448(4): 347–362

Sossin WS, Fisher JM, Scheller RH (1990) Sorting within the regulated secretory pathway occurs in the *trans*-Golgi network. J Cell Biol 110: 1–12

Soulet F, Yarar D, Leonard M, Schmid SL (2005) SNX9 regulates dynamin assembly and is required for efficient clathrin-mediated endocytosis. Mol Biol Cell 16(4): 2058–2067

Stahl LE, Wright RL, Castle JD, Castle AM (1996) The unique proline-rich domain of parotid proline-rich proteins functions in secretory sorting. J Cell Sci 109: 1637–1645

Steegmaier M, Klumperman J, Foletti DL, Yoo JS, Scheller RH (1999) Vesicle-associated membrane protein 4 is implicated in *trans*-Golgi network vesicle trafficking. Mol Biol Cell 10: 1957–1972

Steiner DF (1998) The proprotein convertases. Curr Opin Chem Biol 2(2): 31–39

Stojilkovic SS (2005) Ca^{2+}-regulated exocytosis and SNARE function. Trends Endocrinol Metabol 16(3): 81–83

Stoller TJ, Shields D (1989) The propeptide of preprosomatostatin mediates intracellular transport and secretion of alpha-globin from mammalian cells. J Cell Biol 108(5): 1647–1655

Tamaki H, Yamashina S (2002) Structural integrity of the Golgi stack is essential for normal secretory functions of rat parotid acinar cells: effects of brefeldin A and okadaic acid. J Histochem Cytochem 50: 1611–1623

Tanaka S, Yora T, Nakayama K, Inoue K, Kurosumu K (1997) Proteolytic processing of pro-opiomelanocortin occurs in acidifying secretory granules of AtT-20 cells. J Histochem Cytochem 45: 425–436

Taraska JW, Perrais D, Ohara-Imaizumi M, Nagamatsu S, Almers W (2003) Secretory granules are recaptured largely intact after stimulated exocytosis in cultured endocrine cells. Proc Natl Acad Sci USA 100: 2070–2075

Taupenot L, Harper KL, Mahapatra NR, Parmer RJ, O'Connor DT (2002a) Intracellular protein trafficking into catecholamine storage vesicles: novel chimeric photoproteins visualized by deconvolution fluorescence microscopy. Ann NY Acad Sci 971: 262–265

Taupenot L, Harper KL, Mahapatra NR, Parmer RJ, Mahata SK, O'Connor DT (2002b) Identification of a novel sorting determinant for the regulated pathway in the secretory protein chromogranin A. J Cell Sci 115: 4827–4841

Taupenot L, Harper KL, O'Connor DT (2003) The chromogranin–secretogranin family. N Engl J Med 348: 1134–1149

Taupenot L, Harper KL, O'Connor DT (2005) Role of H + -ATPase-mediated acidification in sorting and release of the regulated secretory protein chromogranin A: evidence for a vesiculogenic function. J Biol Chem 280(5): 3885–3897

Terskikh A, Fradkov A, Ermakova G, Zaraisky A, Tan P, Kajava AV, Zhao X, Lukyanov S, Matz M, Kim S, Weissman I, Siebert P (2000) Fluorescent timer: protein that changes color with time. Science 290(5496): 1585–1588

Thiele C, Gerdes HH, Huttner WB (1997) Protein secretion: puzzling receptors. Curr Biol 7(8): R496–R500

Thorn P, Fogarty KE, Parker I (2004) Zymogen granule exocytosis is characterized by long fusion pore openings and preservation of vesicle lipid identity. Proc Natl Acad Sci USA 101(17): 6774–6779

Thrower EC, Choe CU, So SH, Jeon SH, Ehrlich BE, Yoo SH (2003) A functional interaction between chromogranin B and the inositol 1,4,5-trisphosphate receptor/Ca2+ channel. J Biol Chem 278(50): 49699–49706

Tolmachova T, Abrink M, Futter CE, Authi KS, Seabra MC (2007) Rab27b regulates number and secretion of platelet dense granules. Proc Natl Acad Sci USA 104(4): 5872–5877

Tooze J, Tooze SA (1986) Clathrin-coated vesicular transport of secretory proteins during the formation of ACTH-containing secretory granules in AtT20 cells. J Cell Biol 103: 839–850

Tooze J, Hollinshead M, Frank R, Burke B (1987) An antibody specific for an endoproteolytic cleavage site provides evidence that pro-opiomelanocortin is packaged into secretory granules in AtT20 cells before its cleavage. J Cell Biol 105: 155–162

Tooze J, Tooze SA, Fuller SD (1987) Sorting of progeny coronavirus from condensed secretory proteins at the exit from the *trans*-Golgi network of AtT2O cells. J Cell Biol 105: 1215–1226

Tooze SA, Huttner WB (1990) Cell-free protein sorting to the regulated and constitutive secretory pathways. Cell 60: 837–847

Tooze S, Flatmark T, Tooze J, Huttner WB (1991) Characterization of the immature secretory granule, an intermediate in granule biogenesis. J Cell Biol 115(6): 1491–1503

Tooze S, Martens GJM, Huttner WB (2001) Secretory granule biogenesis: rafting to the SNARE. Trends Cell Biol 11(3): 116–122

Trifaró J-M, Vitale ML (1993) Cytoskeleton dynamics during neurotransmitter release. Trends Neurobiol Sci 16: 466–472

Trucco A, Polishchuk RS, Martella O, Di Pentima A, Fusella A, Di Giandomenico D, San Pietro E, Beznoussenko GV, Polishchuk EV, Baldassarre M, Buccione R, Geerts WJ, Koster AJ, Burger KN, Mironov AA, Luini A (2004) Secretory traffic triggers the formation of tubular continuities across Golgi sub-compartments. Nat Cell Biol 6 (11): 1071–1081

Tsuboi T, Rutter GA (2003) Multiple forms of "kiss-and-run" exocytosis revealed by evanescent wave microscopy. Curr Biol 13(7): 563–567

Tsuboi T, McMahon HT, Rutter GA (2004) Mechanisms of dense core vesicle recapture following "kiss-and-run" ("cavicapture") exocytosis in insulin-secreting cells. J Biol Chem 279(45): 47115–47124

Tsuboi T, Ravier MA, Parton LE, Rutter GA (2006) Sustained exposure to high glucose concentrations modifies glucose signaling and the mechanics of secretory vesicle fusion in primary rat pancreatic β-cells. Diabetes 55(4): 1057–1065

Tsuboi T, Fukuda M (2006) Rab3A and Rab27A cooperatively regulate the docking step of dense-core vesicle exocytosis in PC12 cells. J Cell Sci 119: 2196–2203

Turner MD, Arvan P (2000) Protein traffic from the secretory pathway to the endosomal system in pancreatic β-cells. J Biol Chem 275: 14025–14030

Urbe S, Tooze SA, Barr FA (1997a) Formation of secretory vesicles in the biosynthetic pathway. Biochim Biophys Acta 1358(1): 6–22

Urbe S, Dittie AS, Tooze SA (1997b) pH-dependent processing of secretogranin II by the endopeptidase PC2 in isolated immature secretory granules. Biochem J 321(Pt 1): 65–74

Urbe S, Page LJ, Tooze SA (1998) Homotypic fusion of immature secretory granules during maturation in a cell-free assay. J Cell Biol 143: 1831–1844

Varadi A, Ainscow EK, Allan VJ, Rutter GA (2002) Involvement of conventional kinesin in glucose-stimulated secretory granule movements and exocytosis in clonal pancreatic β-cells. J Cell Sci 115(Pt 21): 4177–4189

Varlamov O, Fricker LD, Furukawa H, Steiner DF, Langley SH, Leiter EH (1997) Beta-cell lines derived from transgenic Cpe(fat)/Cpe(fat) mice are defective in carboxypeptidase E and proinsulin processing. Endocrinology 138: 4883–4892

Velasco A, Hendricks L, Moreman KW, Tulsiani DRP, Touster O, Farquhar MG (1993) Cell-type dependent variations in the subcellular distribution of a-mannosidase I and II. J Cell Biol 122: 39–51

Venkatesh SG, Gorr SU (2002) A sulfated proteoglycan is necessary for storage of exocrine secretory proteins in the rat parotid gland. Am J Physiol Cell Physiol 283: C438–C445

Venkatesh SG, Cowley DJ, Gorr SU (2004) Differential aggregation properties of secretory proteins that are stored in exocrine secretory granules of the pancreas and parotid glands. Am J Physiol Cell Physiol 286(2): C365–C371

Von Zastrow M, Castle JD (1987) Protein sorting among two distinct export pathways occurs from the content of maturing exocrine storage granules. J Cell Biol 105: 2675–2684

Wacker I, Kaether C, Krömer A, Migala A, Almers W, Gerdes HH (1997) Microtubule-dependent transport of secretory vesicles visualized in real time with a GFP-tagged secretory protein. J Cell Sci 110(Pt 13): 1453–1463

Wang Y, Thiele C, Huttner WB (2000) Cholesterol is required for the formation of regulated and constitutive secretory vesicles from the *trans*-Golgi network. Traffic 1: 952–962

Wasmeier C, Hutton JC (1996) Molecular cloning of phogrin, a protein-tyrosine phosphatase homologue localized to insulin secretory granule membranes. J Biol Chem 271: 18161–18170

Wasmeier C, Bright NA, Hutton JC (2002) The lumenal domain of the integral membrane protein phogrin mediates targeting to secretory granules. Traffic 3(9): 654–665

Wendler F, Page L, Urbe S, Tooze SA (2001) Homotypic fusion of immature secretory granules during maturation requires syntaxin 6. Mol Biol Cell 12: 1699–1709

Wheeler MB, Sheu L, Ghai M, Bouquillon A, Grondin G, Weller U, Beaudoin AR, Bennett MK, Trimble WS, Gaisano H (1996) Characterization of SNARE protein expression in beta cell lines and pancreatic islets. Endocrinology 137: 1340–1348

Winkler H, Westhead E (1980) The molecular organization of adrenal chromaffin granules. Neuroscience 5(11): 1803–1823

Winkler H, Fischer-Colbrie R (1992) The chromogranins A and B: the first 25 years and future perspectives. Neuroscience 49(3): 497–528

Wiser O, Trus M, Hernandez A, Renstrom E, Barg S, Rorsman P, Atlas D (1999) The voltage sensitive Lc-type Ca^{2+} channel is functionally coupled to the exocytotic machinery. Proc Natl Acad Sci USA 96(1): 248–253

Wu MM, Grabe M, Adams S, Tsien RY, Moore HP, Machen TE (2001) Mechanisms of pH regulation in the regulated secretory pathway. J Biol Chem 276: 33027–33035

Xu H, Shields D (1994) Prosomatostatin processing in permeabilized cells. J Biol Chem 269: 22875–22881

Yoo SH, Lewis MS (1992) Effects of pH and Ca^{2+} on monomer–dimer and monomer–tetramer equilibria of chromogranin A. J Biol Chem 267(16): 11236–11241

Yoo SH (1996) pH- and Ca$^{(2+)}$-dependent aggregation property of secretory vesicle matrix proteins and the potential role of chromogranins A and B in secretory vesicle biogenesis. J Biol Chem 271(3): 1558–1565

Yoo SH (2000) Coupling of the IP3 receptor/Ca^{2+} channel with Ca^{2+} storage proteins chromogranins A and B in secretory granules. Trends Neurosci 23(9): 424–428

Yoo S, Oh Y, Kang M, Huh Y, So S, Park H, Park H (2001) Localization of three types of the inositol 1,4,5-trisphosphate receptor/Ca$^{(2+)}$ channel in the secretory granules and coupling with the Ca$^{(2+)}$ storage proteins chromogranins A and B. J Biol Chem 276 (49): 45806–45812

Zenner HL, Collinson LM, Michaux G, Cutler DF (2007) High-pressure freezing provides insights into Weibel-Palade body biogenesis. J Cell Sci 120(Pt12): 2117–2125

Zhang B-Z, Chang A, Kjeldsen TB, Arvan P (2001) Intracellular retention of newly-synthesized insulin in yeast is caused by endoproteolytic processing in the Golgi complex. J Cell Biol 153: 1187–1197

Zhao S, Torii S, Yokota-Hashimoto H, Takeuchi T, Izumi T (2002) Involvement of Rab27b in the regulated secretion of pituitary hormones. Endocrinology 143(5): 1817–1824