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Review

Revisiting the regulated secretory pathway: From frogs to human

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ABSTRACT

The regulated secretory pathway is a hallmark of endocrine and neuroendocrine cells. This process comprises different sequential steps, including ER-associated protein synthesis, ER-to-Golgi protein transport, Golgi-associated posttranslational modification, sorting and packing of secretory proteins into carrier granules, cytoskeleton-based granule transport towards the plasma membrane and tethering, docking and fusion of granules with specialized releasing zones in the plasma membrane. Each one of these steps is tightly regulated by a large number of factors that function in a spatially and temporarily coordinated fashion. During the past three decades, much effort has been devoted to characterize the precise role of the yet-known proteins participating in the different steps of this process and to identify new regulatory factors in order to obtain a unifying picture of the secretory pathway. In spite of this and given the enormous complexity of the process, certain steps are not fully understood yet and many players remain to be identified. In this review, we offer a summary of the current knowledge on the main molecular mechanisms that govern and ensure the correct release of secretory proteins. In addition, we have integrated the advance on the field made possible by studies carried out in non-mammalian vertebrates, which, although not very numerous, have substantially contributed to acquire a mechanistic understanding of the regulated secretory pathway.

1. Introduction

The secretory pathway is a fundamental cellular process that entails synthesis, modification, sorting and release of secretory proteins to the extracellular milieu or delivery of different components to the cell surface. These proteins are synthesized on endoplasmic reticulum (ER)-bound ribosomes and translocated into the ER lumen wherein they are folded, assembled, and glycosylated [19,101]. Cargo proteins (either soluble or membrane-associated) are transported from ER exit sites to the entry side of the Golgi complex by the tubular-vesicular elements of the ER-Golgi complex intermediate compartment (ERGIC) [also termed VTCs (vesicular-tubular clusters)] [56] and, subsequently, pass through the distinct cisternae forming the Golgi stacks (*cis*-, *medial*- and *trans*-cisternae), wherein proteins undergo further processing and maturation [145]. At the most distal region of the Golgi complex, the *trans*-Golgi network (TGN), proteins are sorted into different vesicles or pleiomorphic carriers for trafficking and delivery to their final destinations, including the endosomal system, the plasma membrane or the cell surface, by the constitutive or regulated exocytic pathway [44,50]. In the constitutive pathway, a process common to all cell types that enables recycling of membrane and extracellular matrix components, newly synthesized proteins are packaged into constitutive secretory vesicles that are transported directly to the cell surface and secreted independently of any extracellular or intracellular stimuli [25,99]. In contrast, the regulated pathway is a hallmark of specialized secretory cells such as neurons, endocrine and exocrine cells, and involves the concentration and condensation of the secretory products into specialized organelles, the secretory granules, which are stored in the cytoplasm, wherein they await exocytosis following cell surface stimulation [25]. Hence, intracellular vesicles carry different cargo, use different trafficking routes and have specific destinations. The cartoon in Fig. 1 summarizes the distinct intracellular routes that vesicles from different compartments can engage and the coating components that decorate their surface and that confer them identity in the early stages of the vesicle's life.

This review aims to provide an overview on the structure and function of the regulated secretory pathway and to integrate the data obtained on this field in non-mammalian vertebrates, which, though relatively scarce, have significantly contributed to understand the underlying mechanisms for hormone secretion.

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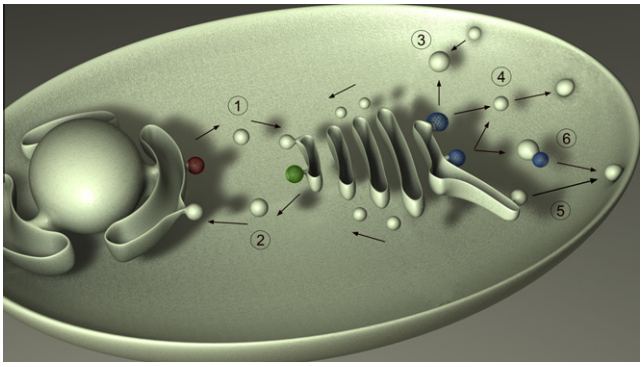

Fig. 1. The secretory pathway and intracellular vesicle traffic. The cartoon depicts the main vesicle-based intracellular routes that secretory peptides undertake, from their ER-associated synthesis to their arrival to the plasma membrane. (1) After synthesis, secretory peptides are packed into COPII-coated vesicles (red) derived from the ER membrane. COPII vesicles unload their content into the ERGIC compartment, from where they are directed to the most-*trans* cisternae of the Golgi complex. (2) Protein sorting receptors and ER-resident proteins mistakenly directed to the ERGIC are recycled back to the ER within COPI-coated vesicles (green). Once in the TGN, proteins are sorted in specific subdomains of this compartment. (3) Lysosomal hydrolytic enzymes are packed within clathrin-coated vesicles (blue) that are transported to and fuse with late endosomes. (4) Other TGN-derived clathrin-coated vesicles are loaded with secretory peptides (immature secretory granules), which undergo further processing and aggregation to form mature secretory granules, a hallmark of neuroendocrine cells. Upon extracellular stimulation, these granules fuse with the plasma membrane and release their content in a process referred to as regulated secretion. (5) In turn, the molecular components of the plasma membrane and extracellular matrix are packed within uncoated vesicles, which are directed towards the cell surface in a constitutive manner (constitutive secretory pathway). (6) Alternatively, it has been proposed the existence of intermediate compartments in which regulated and constitutive secretion diverge based on the capacity of secretory proteins to sort by aggregation. In this case, constitutive secretory proteins would be packed within clathrin-coated vesicles in a process named as constitutive-like secretion.

2. The endoplasmic reticulum

The endoplasmic reticulum (ER) is the entry point of proteins into the secretory pathway. In endocrine cells, these proteins are generally synthesized as inactive precursor polypeptides (prohormones) that become activated later by prohormone convertase-driven proteolytic cleavage. At the ER lumen, secretory proteins encounter the molecular machines (i.e. chaperones and oxidoreductases) that help proteins to fold and evaluate their conformations, so that only properly folded or assembled proteins will travel further along the secretory pathway [28,72,135]. Two main classes of chaperones have been identified, BiP (also known as GPR78) and calnexin/calreticulin, which form complexes with several co-chaperones and redox proteins to prevent protein aggregation and thereby allow more efficient folding [72]. Available evidence supports the view that, as for other proteins transiting the secretory pathway, BiP is required for proper folding and processing of prohormones (i.e. insulin) [152].

Molecular chaperones and associated factors also recognize and target non-native and unassembled subunits of multimeric proteins for retrotranslocation to the cytoplasm, where they are degraded by the ubiquitin–proteasome machinery [135]. This ER-associated degradation (ERAD) pathway acts in coordination with the unfolded protein response (UPR) signaling pathway, which regulates gene expression in response to accumulation of unfolded or misfolded proteins in the ER [124]. Both pathways are critical components of the protein quality control machinery in the ER and their dysfunction has been associated to the development of several endocrine diseases, including diabetes and hypothyroidism [11,111].

In addition to the general helper complexes for protein folding, neuroendocrine cells are endowed with non-classical chaperones such as the protein 7B2, a highly conserved secretory protein distantly related to a subclass of molecular chaperones, the so-called chaperonins [21]. Specifically, 7B2 has been proposed to function as a chaperone for the proprotein convertase (PC) 2 [84], a key enzyme involved in the tissue-specific endoproteolytic posttranslational processing of many hormonal precursors, including pro-opiomelanocortin (POMC) [139], pro-thyrotropin-releasing hormone (TRH) [109], glucagon [12], cholecystokinin [102] or vasopressin [43]. Most of our current knowledge on the role of 7B2 on PC2 maturation and activation has been obtained from studies on the intermediate pituitary lobe of the toad *Xenopus laevis*, whose cells (i.e. melanotrope cells) produce and secrete α-melanocyte stimulating hormone (α-MSH) upon proteolytic cleavage of the prohormone precursor POMC [66,130,139]. Thus, in a series of elegant studies Dr. Martens' group at the University of Nijmegen demonstrated that 7B2 binds the proform of PC2 (proPC2) in the ER of *Xenopus* melanotrope cells to prevent premature activation of the convertase until the appropriate site in the secretory pathway is reached [21,22,128]. Thereafter, the inactive complex is transported to the TGN and proPC2 subsequently gets autocatalytically cleaved after the prodomain, dissociates from 7B2 and becomes activated as the complex is transferred into secretory granules [reviewed in 86].

After proper folding and oligomer assembly, proteins destined for the secretory pathway are selectively separated from ER resident proteins. This occurs at specific ER exit sites (ERESs), which are highly organized membrane domains (1–2 μm in diameter) that are scattered over the surface of the ER and adjacent to the ERGIC [reviewed in 80]. Secretory cargo is actively sorted and concentrated at ER exit sites through the activity of the cytosolic coat protein complex known as COPII and the small GTPase Sar1 that regulates COPII coat assembly, which will finally give rise to COPII-coated vesicles that bud from the ER containing the selected cargo (Fig. 1) [80,110]. The process of COPII assembly onto membranes at ER exit sites has been extensively characterized in yeast and mammalian cells [for review see [116]] and is beyond the scope of this review. Of note, some components of the COPII coat (*i.e.* the Sec24 protein family) contain multiple independent cargo recognition sites and have been reported to interact with specific sets of cargo proteins [35,116,142]. Likewise, other proteins that bind to the COPII complex, such as the transmembrane proteins of the p24 family (α, β, γ, and δ subfamilies) play a role in soluble cargo selection at the ER [119]. Indeed, studies on *Xenopus* melanotrope cells support a role for at least some members of the p24 family in the ER-to-Golgi transport of POMC [105]. Thus, in melanotrope cells from black background-adapted *Xenopus*, which exhibit high levels of POMC production to sustain skin darkening [106], the propeptide partially colocalizes with p24 proteins at ER subdomains likely corresponding to ERESs [75]. Moreover, the expression of a selective set of p24 proteins (p24α3, -β1, -γ3, -δ2) is induced in coordination with POMC [105] and, in particular, p24δ2 binding to COP proteins has been shown necessary for proper POMC processing [119]. Despite these exciting findings, whether and how these or other cargo receptor proteins contribute to the selective recruitment of specific secretory cargo at ERESs in endocrine cells remains thus far unclear.

After budding from the ER, COPII transport vesicles loose their coat and fuse with pre-Golgi intermediates to release their cargo, whereas protein sorting receptors are recycled back to the ER for additional rounds of cargo export (Fig. 1) [35]. Pre-Golgi intermediates move along microtubules toward the Golgi complex for protein delivery to the *cis* face of this organelle [80]. Besides the cytoskeletal network, trafficking between the ER and the Golgi complex requires the participation of a variety of proteins that associate with the cytoplasmic face of transport carriers, including Rab GTPases and tethering factors, which target carrier vesicles to Golgi membranes, as well as SNARE (soluble N-ethylmaleimide-sensitive