CHAPTER 1

The secretory process of salivary glands and pancreas

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1. Introduction

The study of secretory cells and the definition of the secretory process have been at the forefront of modern cell biology since the inception of this field in the early 1950s. Secretory cells, particularly the acinar cells of the exocrine pancreas, presented a unique opportunity for cell biologists, who were in the process of marrying biochemistry and morphology: Here was a cell with virtually a single-minded mission, to produce and release large amounts of digestive enzymes, whose appearance in the microscope could be correlated with reactions in the test tube and that could be induced to perform its functions by physiological manipulations or the application of readily available drugs. Since almost all cells secrete proteins, these pioneering studies found wide application, and they spawned the development of methodological approaches and technological advances too numerous to mention.

Much has been learned in the 35 years that ensued after the first studies of secretory cells, and details of the progress made are available in several reviews [1–4]. The purpose of this chapter is not to reiterate the history of the development of this field, but to summarize the current status of the secretory process, with the intention of providing a framework for the subsequent chapters of this book. No attempt will be made to describe the mechanisms involved in fluid and electrolyte secretion by the salivary glands and pancreas; the interested reader may consult a number of recent reviews [5–7].

The secretory process consists of a series of interrelated steps, beginning with the synthesis of a secretory protein and culminating in the release of the mature product to the extracellular environment (Table 1-1). Although the process is continuous, and in one cell all of the different steps may occur simultaneously, it is customary to divide the secretory process into several discrete stages [1]. These stages are largely defined by the major biochemical processes occurring during secretory protein production and the cellular compartments in which they take place. Figure 1-1 shows the ultrastructure of a typical salivary-gland secretory cell, and Fig. 1-2 illustrates the basic pathway and general features of the secretory process: 1) synthesis of secretory proteins in the endoplasmic reticulum (ER) and their segregation in the lumen of the ER cisternae, 2) migration of the proteins to the Golgi apparatus, 3) concentration and packaging of the proteins in secretory granules, 4) intracellular storage, and 5) release of the granule content into the extracellular space.

2. Protein synthesis and segregation

The first step in the secretory process is the synthesis of secretory proteins. As with all other cellular proteins, secretory proteins are assembled on ribosomes from precursor amino acids under the direction of messenger ribonucleic acid (mRNA). Encoded within the mRNA, as part of the primary amino-acid sequence of the protein, is specific information that directs the protein to

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Table 1-1. Steps in the secretory process

Protein synthesis and segregation
Post-translational modifications
Intracellular transport and sorting
Secretory granule formation
Exocytosis

an appropriate cellular destination. In the case of secretory proteins, the most important information is contained in a region at the amino-terminal end of the protein, known as the *signal sequence* [9]. The signal sequence, consisting of about 15–30, mostly hydrophobic, amino acids, directs the protein to the ER and aids in its translocation across the ER membrane into the cisternal space of the ER. The signal sequences of different proteins differ in their amino acid sequences, but share common tertiary structural features, which most likely specify recognition and translocation of the nascent polypeptide chain by the ER. This essential process segregates those proteins destined for secretion from the cytoplasmic and many of the organellar proteins. The

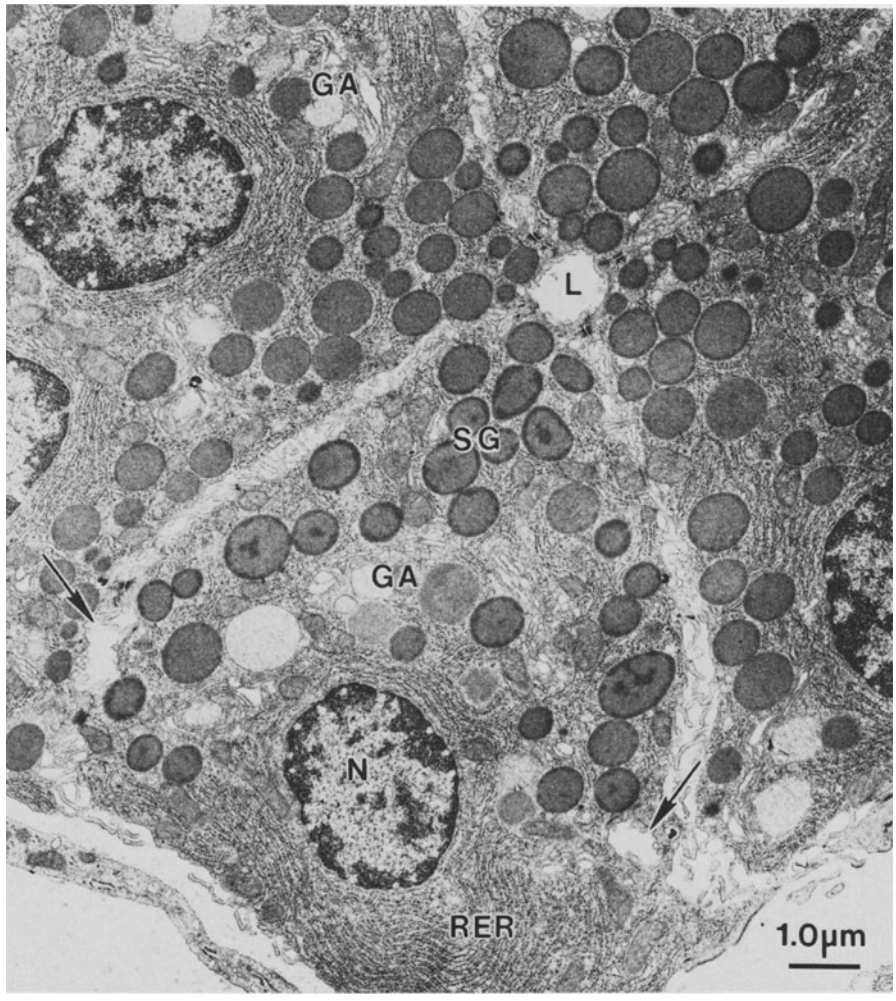

Fig. 1-1. Acinar cells of the rat parotid gland. A spherical nucleus (N) and abundant rough ER (RER) are present in the basal portion of the cell, a large Golgi apparatus (GA) is located apical or lateral to the nucleus, and numerous electron-dense secretory granules (SG) are stored in the apical cytoplasm. Lumen (L), intercellular canaliculi (arrows). (From [8]; reproduced with permission of Pergamon Press, Oxford.)

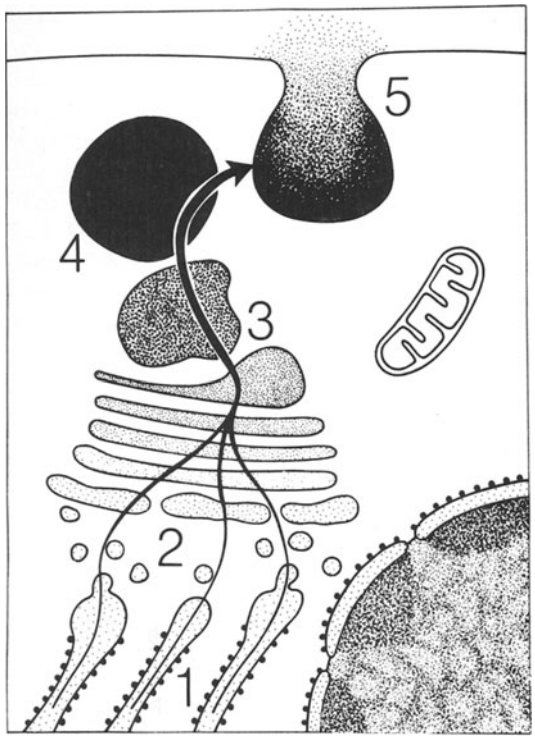

Fig. 1-2. Basic pathway (→) and general features of the secretory process in exocrine cells. 1) synthesis of proteins in the endoplasmic reticulum (ER) and segregation in the lumen of the ER; 2) migration to the Golgi apparatus; 3) concentration and packaging of the proteins in secretory granules; 4) intracellular storage; 5) discharge of the granule content by exocytosis.

mechanism by which this is achieved has been elucidated principally by Blobel and others [9–13] over the last dozen years. As the amino terminus of the protein emerges from the ribosome, when the nascent protein is about 70 amino acids long, it interacts with the signal recognition particle (SRP), an 11S cytoplasmic particle made up of proteins and RNA. Binding of the SRP to the signal sequence and the ribosome causes translation of the protein to stop. The SRP-ribosome complex then binds to an SRP receptor and (possibly) to other binding proteins or receptors on the ER membrane, the hydrophobic signal sequence inserts into the membrane, the SRP dissociates from the ribosome and SRP receptor, and translation resumes. Translocation of the protein across the ER membrane normally occurs cotranslationally (at least in most eukaryotic cells) and appears to require energy [14]. Soon after the amino-terminal end of the protein reaches the cisternal space, the signal sequence is removed by a specific signal peptidase.

This same basic mechanism is used to segregate membrane proteins and proteins destined for lysosomes from proteins resident in the cytoplasm or in other organelles. In the case of membrane proteins, other signals incorporated into the amino acid sequence of the protein prevent its complete transfer to the cisternal space and specify its positioning in the membrane [12]. Lysosomal proteins, which eventually must be removed from the secretory pathway, undergo a specific post-translational modification and are sorted from secretory proteins at a later stage [15, 16].

3. Post-translational modifications

After their synthesis, secretory, membrane, and lysosomal proteins undergo a number of covalent modifications. These modifications are essential for the protein to assume its proper configuration, to perform its biological functions, and to reach its final destination. The different modifications that various proteins may undergo are listed in Table 1-2. The first group of modifications are those that occur in the ER, many of which actually take place cotranslationally, i.e., as the protein is being synthesized and translocated across the ER membrane. These include signal-sequence cleavage, hydroxylation, disulfide bond formation, and N-linked glycosylation. Of these, the latter has been the most thoroughly investigated [17, 18] and begins with the assembly and subsequent transfer of an oligosaccharide precursor, $Glc_3Man_9GlcNAc_2$, from dolichol phosphate, a large ER-associated lipid, to asparagine residues in the acceptor glycoprotein. This precursor oligosaccharide is subsequently trimmed and rebuilt as the glycoprotein travels through the ER and Golgi apparatus by enzymatic removal of the glucose residues and some of the mannose residues, and addition of N-acetylglucosamine, galactose, fucose, and sialic acid. The final structure of the oligosaccharide groups varies among different proteins, as well as within the same protein, resulting in a microheterogeneity of glycoprotein structure.

Table 1-2. Post-translational modifications of secretory proteins

Modification	Intracellular Location
Signal sequence removal N-linked glycosylation Hydroxylation Disulfide bond formation	ER cisternae
Oligosaccharide trimming	ER and Golgi apparatus
Completion of N-linked oligosaccharides O-linked glycosylation Mannose-6-phosphate formation Fatty acylation Sulfation Phosphorylation	Golgi apparatus
Proteolytic processing	Immature and mature secretory granules Extracellular space

Subsequent modifications to the secretory proteins occur in the Golgi apparatus and may include O-glycosylation, fatty acylation, sulfation, and phosphorylation. The sequence of these modifications is specified by the topographic distribution of the modifying enzymes in the Golgi apparatus [18–20]. The structure of the Golgi apparatus (Fig. 1-3) reflects these biochemical requirements: a series of discrete, flattened, membranous saccules, providing maximum surface area for the interaction of membrane-bound enzymes and secretory proteins and providing separation of successive modification steps. Thus, as the proteins traverse the Golgi apparatus, they sequentially encounter the compartments containing the various enzymes, and their structure is modified accordingly. At present, it is not known how some proteins escape modification, even though their primary structure contains potential sites for the action of these enzymes.

The Golgi apparatus is also the location of modification and sorting steps involved in removal of lysosomal proteins from the secretory pathway and their routing to lysosomes. N-acetylglucosamine-1-phosphate is added to mannose residues on the N-linked oligosaccharides of the lysosomal proteins, then the N-acetylglucosamine residues are removed by a phosphodiesterase, leaving mannose-6-phosphate groups on the oligosaccharide [22–24]. Mannose-6-phosphate serves as a specific recognition marker of lysosomal enzymes [15, 16]; the mannose-6-phosphate-bearing enzymes bind to a receptor present in the Golgi apparatus and are subsequently routed to lysosomes [25–27]. Other, as yet unidentified, mechanisms for routing of proteins to lysosomes must also exist. For example, in I-cell disease, due to the absence of N-acetylglucosamine-1-phosphotransferase, lysosomal enzymes lack the mannose-6-phosphate recognition marker, yet a variable percentage of some enzymes reach the lysosomes [16].

Specific intracellular proteolytic cleavage of secretory protein precursors is an important post-translational modification in many cells, especially hormone-secreting cells [28, 29]. Many hormones, such as insulin, adrenocorticotrophic hormone, and vasopressin, are synthesized as larger, generally inactive, precursors (often called *prohormones*). Cleavage of the precursor by an endogenous protease is required to liberate the active hormone. Analysis of purified granules of endocrine cells [30] and comparison of the immunocytochemical localization of precursor and mature hormone [31], indicate that proteolytic processing probably begins at an early post-Golgi step and continues in the mature granule. In the exocrine pancreas, many of the digestive enzymes are synthesized as inactive proenzymes; under normal conditions their activation by proteolysis occurs only after they reach the lumen of the intestine. Specific intracellular proteolysis of exocrine secretory proteins has been less well studied, although a few examples may be cited. In the granular convoluted tubule cells of the male mouse submandibular gland, renin is synthesized as a larger precursor and appears to be cleaved to its active form intracellularly [32]. Nerve growth factor and epidermal growth factor are also synthesized as high-molecular-weight precursors by these cells and are found as complexes with proteases [33, 34]. The amino acid sequences of the human salivary acidic proline-rich proteins suggest that proteins III and IV are proteolytic products of proteins I and II, respectively [35]. A family of glutamic-acid/glutamine-rich proteins present in rat submandibular gland and saliva has recently been described [36]. Comparison of the

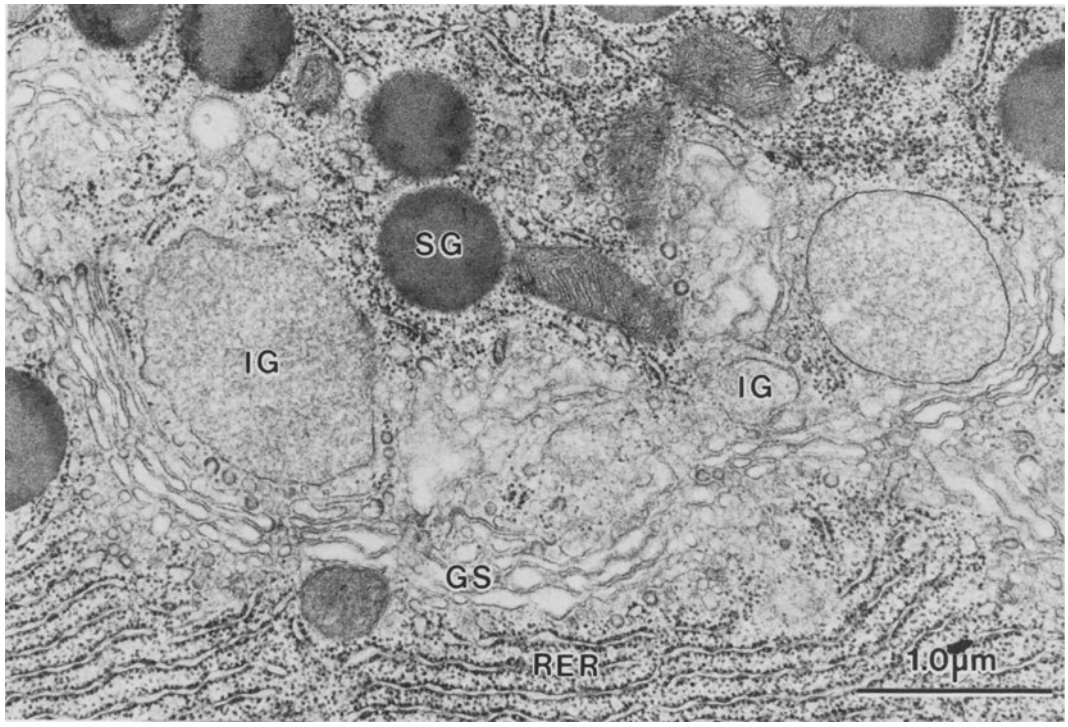

Fig. 1-3. Golgi apparatus of a rat parotid acinar cell. The Golgi apparatus is well developed and consists of 4–6 stacked saccules (GS), numerous vesicles, and immature granules (IG) of various sizes forming at the *trans* face. The rough ER (RER) closely approaches the *cis* face of the Golgi apparatus. SG = mature secretory granules. (From [21]; reproduced with permission of C.V. Mosby, St. Louis.)

protein species purified from glandular extracts with immunoprecipitated, in-vitro translation products suggests that these unusual secretory proteins may undergo post-translational proteolytic processing. Except for renin, however, the cellular location of the proteolytic processing reactions of these salivary secretory proteins has not been established. Secretory granules of rat parotid acinar cells contain a protease with similar specificity as those involved in prohormone processing in endocrine cells [37], but the protein substrates for this enzyme have not been identified.

4. Intracellular transport and sorting

Enzyme and immunocytochemical studies suggest that the entire complement of the cell's secretory proteins is synthesized and is present throughout the ER [38–40]. The movement of newly synthesized proteins within the ER probably occurs, for the most part, by simple diffusion through the cisternae. Regional morphological specialization of the ER is found principally in the transitional zone, where the ER approaches the *cis* face of the Golgi apparatus (Fig. 1-4). The transfer of proteins from the transitional ER to the Golgi apparatus occurs via small vesicles, which are abundant in this region and which apparently form by budding from the transitional ER [42–44]. The requirement of energy for movement of newly synthesized protein from the ER to the Golgi apparatus [45–47] and the disruption of transport by microtubule inhibitors [46, 48–50] are consistent with a vesicular transport mechanism.

Transport of secretory proteins through the Golgi apparatus occurs from the *cis* to the *trans* face, where the formation of secretory granules

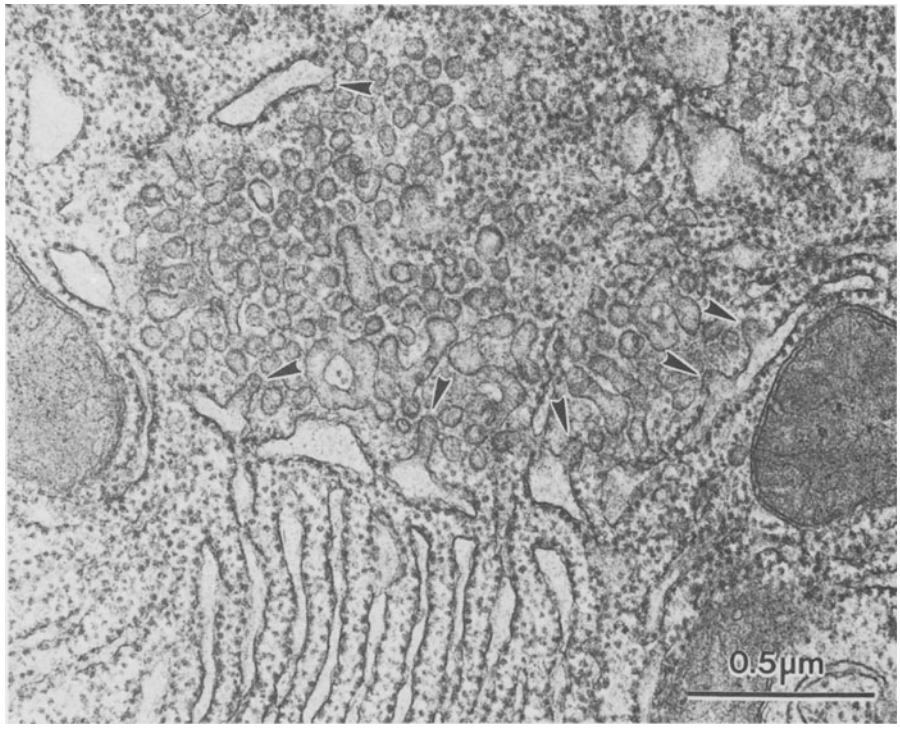

Fig. 1-4. Transitional ER in an acinar cell of the rat pancreas. Small vesicles appear to form (arrowheads) from dilated portions of the rough ER near the *cis* face of the Golgi apparatus (located out of the section plane). Numerous vesicles and tubules are present between the transitional ER and the Golgi apparatus. (From [41]; reproduced with permission of Lea & Febiger, Philadelphia.)

occurs. The mechanisms underlying this vectorial movement remain controversial. Morphological observations suggest that the Golgi saccules migrate intact toward the *trans* face, carrying their content of secretory proteins acquired at the *cis* face [39, 51–53]. In some cells that secrete large structures whose assembly begins in the *cis* Golgi saccules [54, 55], few alternatives seem possible. A more limited migration of saccules towards the *trans* face has been proposed for cells of Brunner's glands of the mouse [56]. The difficulty in reconciling the evidence for biochemical and functional heterogeneity of Golgi subcompartments, as well as the slow turnover of membrane proteins compared to that of the transported secretory proteins [57–60], with the model of saccule progression has led to the proposal that a system of vesicular shuttles operates between the Golgi saccules [1, 61]. Experiments demonstrating normal transport and processing of viral membrane proteins in recombinations of Golgi apparatus fractions from wild-type and glycosylation-deficient mutant cells [62, 63] provide additional evidence for a vesicular transport mechanism.

Vesicular transport of proteins between intracellular compartments, including the ER and Golgi apparatus, may involve receptor-mediated events rather than being a simple bulk-transfer process. Receptor recognition and binding would allow for efficient sorting and routing of proteins to different destinations. Several proteins have been shown to move between the ER and Golgi at different rates [64–66], suggesting that receptors for specific proteins either facilitate the transfer process or cause retention of the proteins in the ER. The transport of lysosomal proteins bearing the mannose-6-phosphate marker to lysosomes involves recognition and binding by a specific mannose-6-phosphate receptor [15, 16]. Biochemical and immunocytochemical evidence

indicates that lysosomal proteins diverge from the secretory pathway at the *trans* face of the Golgi apparatus [20, 26, 27].

Clathrin-coated vesicles are often seen in the Golgi region, and it seems clear that they participate in the delivery of acid hydrolases to lysosomes [26, 27, 67]. Although in some cells clathrin-coated vesicles may contain secretory proteins, it has been postulated that this is related to the maturation of secretory granules [68]. In contrast, the "coat" of coated vesicles present in an in-vitro reconstituted Golgi transport system does not appear to consist of clathrin [69]. Microinjection of clathrin antibodies into cultured cells has little or no effect on the exocytotic pathway [70], and yeast mutants defective in clathrin synthesis exhibit near-normal growth rates, suggesting that this protein is not required for intracellular protein transport and secretion [71].

Recently, the concept of constitutive versus regulated secretion has been developed by Kelly and his colleagues [4, 72]. In cells that secrete by the regulated pathway, secretory proteins are typically concentrated and stored intracellularly in granules, and are released in response to a specific stimulus. Secretion via the constitutive pathway occurs continuously, little or no concentration of the proteins occurs, and the process is unaffected by cellular stimulation. Both constitutive and regulated secretion may occur in a single cell, such as an exocrine cell, with certain proteins following the constitutive pathway (e.g., basement membrane components and plasma membrane proteins) and others following the regulated pathway (e.g., secretory proteins). Disruption of the regulated pathway, for example, by certain ionophores or weak bases that dissipate pH gradients, results in diversion of regulated proteins into the constitutive pathway.

The divergence of the constitutive and regulated pathways occurs at the *trans* face of the Golgi apparatus (probably in the *trans* Golgi network [73, 74] and involves additional mechanisms for protein sorting and the targeting of vesicular carriers to different destinations. Expression of foreign proteins in cultured cell lines and their secretion by the correct pathway indicates that the sorting information is universal and is contained in the protein. It is believed that the sorting process is receptor mediated, but specific receptors or carrier proteins involved have not been identified.

5. Secretory granule formation

Exocrine glands, such as the salivary glands and pancreas, must secrete at very high rates for short periods of time. The protein synthetic capacity of these glands is insufficient to concurrently meet these demands. Thus, secretory proteins are synthesized more or less continuously and are stored in concentrated form within the cell until their release is required. The containers for the stored proteins, the secretory granules, are the characteristic feature of the acinar cells of exocrine glands. Exocrine secretory granules vary in size from about 0.5 μm to 2 μm, and their content may appear extremely electron dense or almost electron lucent, depending upon the cell type and the nature of the secretory product. In many cells, as described in other chapters, the granule content may appear extremely electron-dense or almost electron-lucent, depending upon the cell type and

Secretory granule formation occurs at the *trans* face of the Golgi apparatus (Figs. 1-3 and 1-5), where smaller, irregularly shaped, less-dense immature granules or condensing vacuoles are found in continuity with Golgi membranes. As the immature granules fill with secretory protein, they increase in size and density until they reach maturity, then they lose their connection with the Golgi apparatus. This simplified description, however, belies the fact that the mechanisms underlying granule formation and growth, and concentration of the content, remain poorly understood.

The source of the membrane surrounding the secretory granules has been a subject of intense interest. The continuity of Golgi membranes with the forming granules, and the similarity in enzyme cytochemical reactivity between the forming granule and the confluent saccule [39, 76–78], suggest that the granule membranes are derived from the Golgi apparatus. This scheme would require replacement of the membrane donated by components of the *trans* Golgi region; however, as noted above, the earlier models of saccule progression and Golgi apparatus turnover are inconsistent with data on the composition and

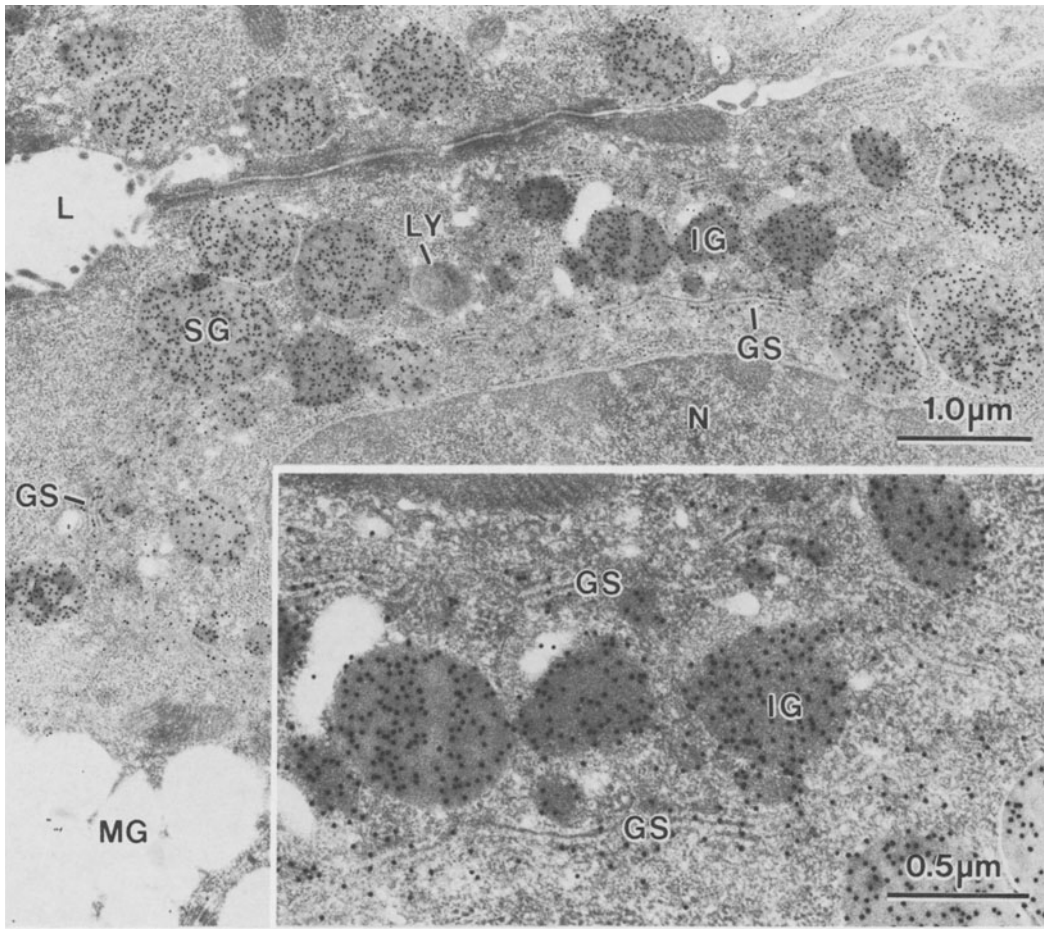

Fig. 1-5. Localization of secretory protein (B_1-immunoreactive protein) in serous demilune cells of the rat sublingual gland using the post embedding immunogold labeling technique. The thin section was incubated with an antibody to protein B_1, a secretory protein of the neonatal rat submandibular gland [75], and then with 15 nm protein A-gold. Gold particles indicating the presence of B_1-immunoreactive proteins are located over the Golgi saccules (GS) and over immature (IG) and mature (SG) secretory granules. The inset shows part of the Golgi apparatus at higher magnification. The labeling intensity (gold particles/unit area) of the granules is greater than that of the Golgi saccules. N = nucleus, LY = lysosome; L = lumen; MG = granules of adjacent mucous acinar cell.

turnover of cellular membranes. A number of studies demonstrating that membranes shuttle between the cell surface and various intracellular compartments [79–81] support the general notion that cells extensively recycle their membranes. Experiments employing electron-dense markers bound to secretory granule membranes transiently inserted into the plasma membrane during exocytosis have shown that, in some cells, the Golgi saccules and forming granules become labeled [82–85], implying that secretory granule membranes are reutilized during the formation and growth of new granules.

The pathway taken by the secretory proteins from the Golgi saccules to the forming granules has not been clearly defined. In some cells, granule formation appears to be initiated by the *trans* Golgi saccules, suggesting that the granules fill directly from these saccules. In acinar cells of the pancreas, salivary, and lacrimal glands, small forming granules are frequently seen in continuity with cisternal or tubular elements at the *trans*

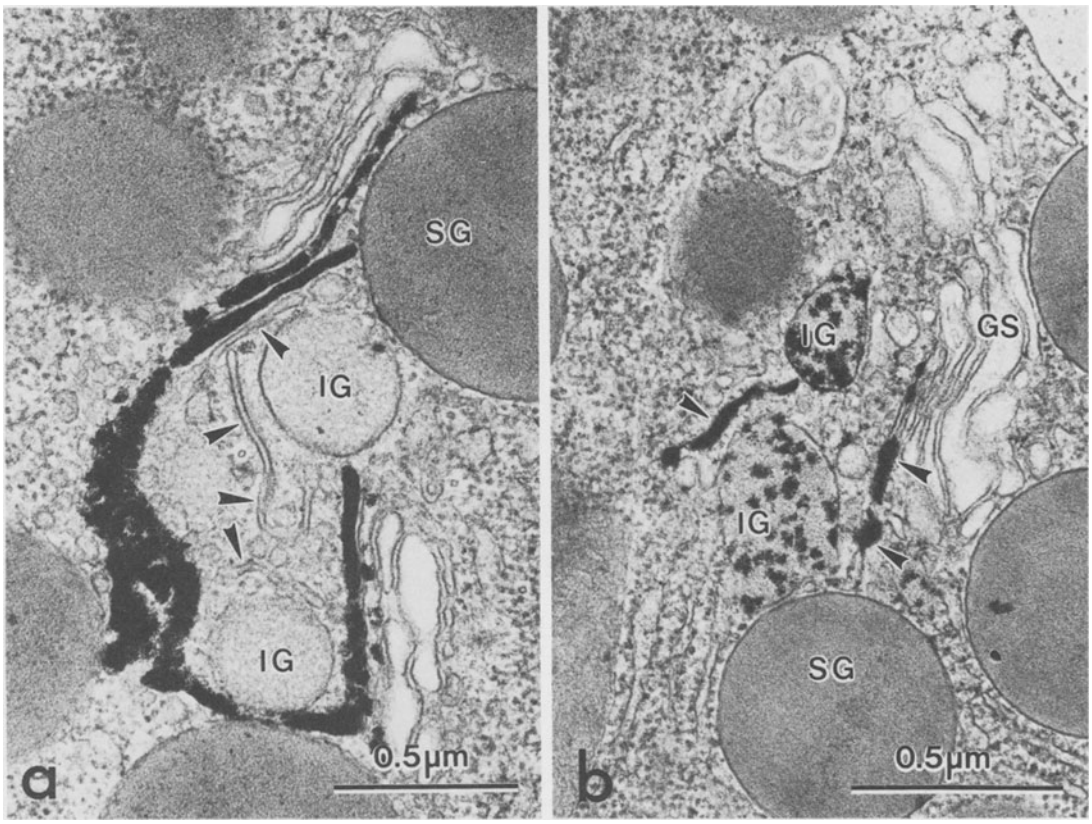

Fig. 1-6. Heterogeneity of the Golgi apparatus is demonstrated by enzyme cytochemistry. **a**: Dense reaction product for thiamine pyrophosphatase activity is present in two *trans* saccules of the Golgi apparatus of a rat parotid acinar cell. The medial and *cis* saccules, GERL or *trans* Golgi network (arrowheads), and immature granules (IG) are unreactive. SG = mature secretory granules. **b**: Reaction product for acid phosphatase activity is present in GERL (arrowheads) and immature granules (IG) of an acinar cell of the rat lingual serous glands. The Golgi saccules (GS) and mature secretory granules (SG) are unreactive. (From [86]; reproduced with permission of Alan R. Liss, New York.)

face (GERL [76] or *trans* Golgi network [73]; Fig. 1-6) that by enzyme [39] or immunocytochemistry [40, 87] lack detectable secretory proteins. In other cells, secretory proteins have been localized immunocytochemically in elements of the *trans* Golgi network that give rise to secretory granules [26, 27, 68]. Images of small vesicles either fusing with or budding from immature granules suggest that vesicular transport may also occur between the Golgi saccules and the forming granules.

In exocrine cells, the granules contain a mixture of all of the secretory proteins produced by the cells [88, 89]. The secretory proteins are concentrated during packaging by the Golgi apparatus, as indicated by increases in electron density associated with granule maturation, in immunocytochemical reactivity [87] (Fig. 1-5), and in the number of radioautographic grains seen in precursor incorporation studies [90,91]. Early studies demonstrating continued concentration in the absence of cellular respiration suggested that the process was passive rather than energy requiring [45], and directed attention toward the possible role of ionic interactions producing large aggregates with reduced osmotic activity [1]. Divalent cations, such as calcium, are present in relatively large amounts in some secretory granules [92–94] and could participate in the packaging process. Large anionic mole-

cules, such as sulfated glycosaminoglycans [95, 96], which could form a structural basis for the intragranular aggregation of basic proteins, are also present in many secretory granules. In some granules, such as the insulin granule of the pancreatic B cells, crystallization of the secretory proteins also serves the function of concentration. The exact mechanisms involved in the concentration of secretory proteins in most cells have yet to be determined.

Studies of granules isolated from several cell types have revealed that the granule interior has an acidic pH and that a proton pump (H^+-ATPase) is present in the granule membrane [97]. Using a specific molecular probe to immunocytochemically identify acidic compartments, it was shown that a progressive fall in pH occurs from the *cis* to the *trans* face of the Golgi apparatus [98]. The low pH in the Golgi saccules and immature granules may be important for proper sorting of different secretory products [4]. In some cells, the acidic environment in secretory granules may be required for the accumulation and storage of certain components, e.g., biogenic amines [97]. Mature secretory granules of the rat parotid gland and exocrine pancreas, however, have little or no detectable H^+-ATPase, and their interior pH is near neutrality [99–101].

6. Exocytosis

Secretion of the content of stored secretory granules occurs by exocytosis (Fig. 1-7). During this process, granules approach the cell membrane, make close contact with it, and the lipid bilayers of the granule and cell membrane fuse; subsequent rupture of the fused portion of the membranes leaves the granule membrane in continuity with the cell membrane and results in the exteriorization of the content. The granule membrane is then retrieved by the cell by endocytosis. Although the regulation of exocytosis has been extensively studied (see below), the intracellular factors mediating the granule discharge process are less well defined.

Cytoskeletal elements, probably microtubules, seem to be involved in movement of the granules to the cell surface. Interactions between microtubules and granule membranes have been demonstrated in vitro [102], and microtubule poisons inhibit the release of newly synthesized proteins, although they do not block exocytosis of stored granules located near the plasma membrane [48, 50]. The role of actin and actin-associated proteins in exocytosis is poorly understood. Agents that inhibit actin filaments often cause an enhanced secretion [103], presumably due to disruption of the submembrane web of actin and related proteins usually present at the luminal surface [104] (Fig. 1-7). Actin also appears to be associated with the granule membranes [105].

Since granule discharge occurs only at the luminal surface of exocrine cells, there must be a specific recognition of the luminal membrane by components of, or associated with, the granule membrane. Studies of granule membranes isolated from several different cell types have revealed striking similarities in their protein composition [106]. Presumably, these proteins serve common functions related to exocytosis, such as recognition of luminal fusion sites or interaction with the cytoskeleton. Once a granule fuses with the cell membrane and the two membranes have become confluent, the granule membrane becomes an acceptable partner for subsequent fusion events, resulting in compound exocytosis [107, 108]. The mechanism by which the granule membrane develops fusion competency so rapidly is unknown.

Some freeze-fracture studies of exocytosis in stimulated cells suggest that there is a rapid clearing of intramembrane particles from the area of the apposed granule and cell membranes about to undergo fusion [109, 110]. Presumably, this facilitates the fusion of the lipid bilayers. Other studies of systems in which rapid freezing without chemical prefixation was employed indicate that the intramembrane particles are not cleared from the site of fusion and that the formation of a small pore in the apposed membranes is the first sign of fusion [111, 112]. Whether these contradictory observations are due to artifacts induced by the preparative methods or represent differences in the systems studied remains to be resolved. Observations of living cells (mast cells [113]) and in-vitro systems (isolated cortices of sea urchin eggs [114]) have shown that swelling of the granules occurs during exocytosis. Osmotic swelling was felt to be important in initiating membrane fusion, but evidence obtained from studies of chromaffin granule exocytosis [115] appears to be inconsis-

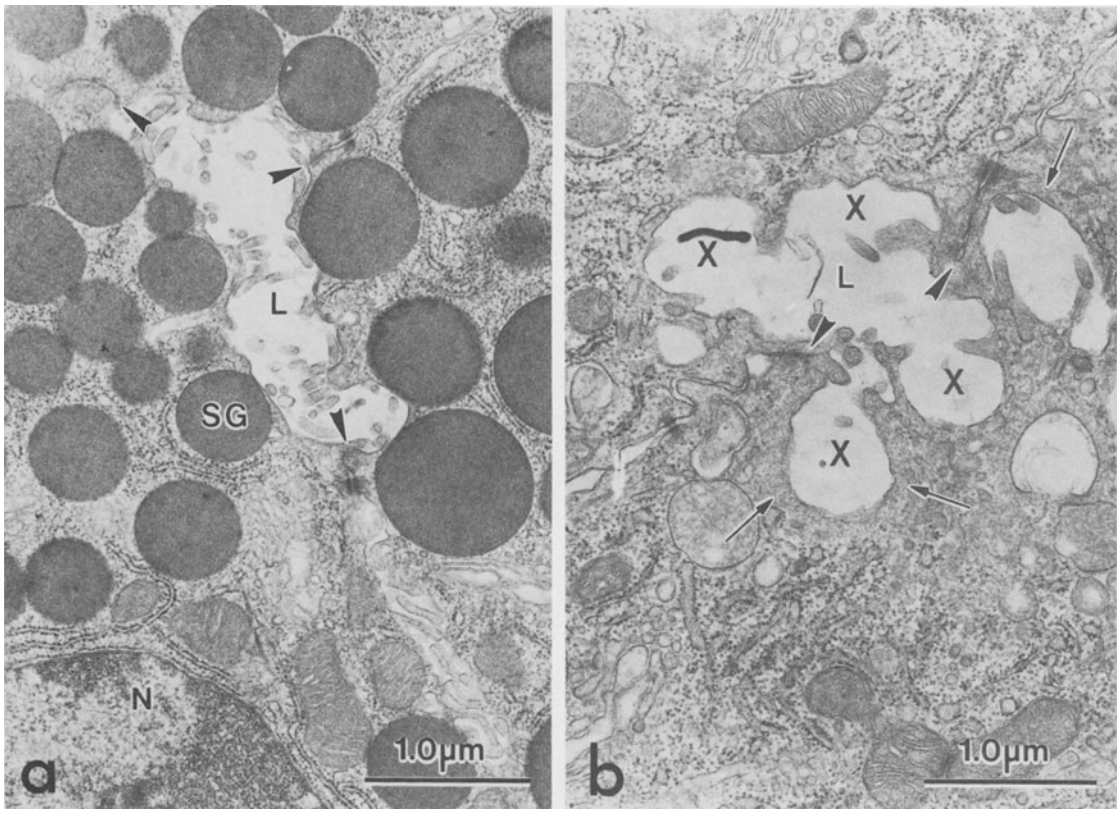

Fig. 1-7. Exocytosis in rat parotid acinar cells. **a**: The apical cytoplasm of unstimulated cells is filled with secretory granules (SG). N = nucleus; L = lumen. **b**: Stimulation with the β-adrenergic agonist isoproterenol causes exocytosis of all of the stored secretory granules. The lumen (L) is enlarged due to fusion with the granule membranes (X). A network of fine filaments (arrows) can be discerned beneath portions of the luminal membrane. Tight junctions (arrowheads). (From [41]; reproduced with permission of Lea & Febiger, Philadelphia.)

tent with this hypothesis. Recent observations of exocytosis of the giant granules of beige mouse mast cells indicate that fusion actually precedes the swelling of the granules [116]. Influx of extracellular molecules through the fusion pore may cause the subsequent swelling of the granule; enlargement and stabilization of the pore may depend upon granule swelling.

Following release of the granule content, the inserted membrane is retrieved from the cell surface by endocytosis. Small vesicles, frequently of the coated variety, but also some smooth-surfaced vesicles, pinch off of the granule membrane and fuse with either the Golgi saccules, immature granules, or lysosomes [82–85, 117, 118]. Fusion of the endocytic vesicles with Golgi saccules or immature granules suggests reutilization of the membrane in a subsequent round of granule formation. In contrast, fusion with lysosomes suggests that a portion of the membrane may be degraded and that new membrane must enter the cycle, probably at the level of the Golgi apparatus. Whether all secretory cells employ similar mechanisms for membrane recycling and the proportion of recycled versus new membrane contributing to granule formation remain to be established.

7. Regulation of the secretory process

Secretion by the pancreas and salivary glands is regulated physiologically by the autonomic nervous system and by circulating hormones. The

latter have a much greater influence in regulating pancreatic secretion than salivary secretion. The neurotransmitters and hormones known to affect protein, water, and electrolyte secretion by the salivary glands and pancreas are listed in Table 1–3. It should be noted that wide variations in the pattern of innervation and in the response to those transmitters and hormones exist between species, and even among the different salivary glands within one species. The neurohormonal regulation of secretion has been an area of intense research activity, and significant advances have been made in the last few years. Several excellent reviews have been published on this topic [119–124].

The binding of a neurotransmitter released from a nerve terminal or a hormone to a specific receptor protein on the cell surface initiates the secretory response. The secretagogue receptors on pancreatic and salivary gland cells generally are of two classes, those whose effects are mediated by cyclic AMP and those that elevate intracellular calcium. The rapid increase in the concentration of these *second messengers* results in the activation of other intracellular enzymes and begins the cascade of reactions leading to exocytosis or secretion of fluid and electrolytes. Generation of elevated second-messenger levels is dependent upon the coupling of the receptor to a membrane enzyme, either adenylate cyclase or phospholipase C, through a guanine nucleotide binding protein, called a *G protein* [125]. In the case of receptors that elevate cyclic AMP, the G protein activates adenylate cyclase, which catalyzes the formation of cyclic AMP from ATP. Cyclic AMP subsequently activates the enzyme cyclic-AMP-dependent protein kinase, which phosphorylates other cellular proteins that presumably are involved in the secretory response. Several proteins undergo phosphorylative modification in response to receptor stimulation [126–129], but their identity and function are, for the most part, unknown. Recently, the phosphorylation of two rat parotid secretory granule membrane proteins after β-adrenergic stimulation was reported [130], but the role of these proteins in exocytosis has not been determined. Although

Table 1-3. Neurotransmitters and hormones regulating salivary and pancreatic secretion

Gland	Neurotransmitter or Hormone	Receptor	Intracellular Messenger	Process Regulated
Salivary	Norepinephrine	β-adrenergic α-adrenergic	Cyclic AMP Ca^{2+}	Exocytosis Fluid and electrolyte secretion
	Acetylcholine	Muscarinic cholinergic	Ca^{2+}	Fluid and electrolyte secretion, exocytosis
	Vasoactive intestinal peptide	VIP	Cyclic AMP	Exocytosis
	Substance P	Substance P	Ca^{2+}	Fluid and electrolyte secretion
Pancreas	Acetylcholine	Muscarinic cholinergic	Ca^{2+}	Exocytosis, fluid and electrolyte secretion
	Cholecystokinin Gastrin	CCK	Ca^{2+}	Exocytosis, fluid and electrolyte secretion
	Secretin	Secretin	Cyclic AMP	Fluid and electrolyte secretion
	Vasoactive intestinal peptide	VIP	Cyclic AMP	Exocytosis
	Bombesin	Bombesin	Ca^{2+}	Exocytosis
	Physalaemin Substance P	Physalaemin	Ca^{2+}	Exocytosis

cyclic-AMP-dependent protein kinase is widely distributed within these cells [131], the specificity of protein phosphorylation apparently results from its association with organelles and membranes, as well as stimulation-induced redistribution [132] and/or turnover of the enzyme subunits [133].

The G protein that is coupled to receptors elevating intracellular calcium activates phospholipase C, which hydrolyzes the membrane phospholipid, phosphatidylinositol-4,5-bisphosphate, to diacylglycerol and inositol-1,4,5-trisphosphate. Inositol-1,4,5-trisphosphate interacts with a receptor on an intracellular calcium storage compartment, believed to be the endoplasmic reticulum (possibly a specialized region of the ER) or a specific calcium-storage organelle [134], causing the release of calcium to the cytoplasm. The initial calcium release may be localized to sites near the cell membrane [135, 136]. A subsequent increase in cell-membrane permeability to calcium also occurs, resulting in the influx of extracellular calcium and a generalized elevation of cytoplasmic calcium concentrations. Diacylglycerol activates a calcium-dependent, membrane-associated protein kinase, protein kinase C [137]. Again, it is presumed that the phosphorylative modification of specific proteins by protein kinase C, as well as other calcium-activated protein kinases (e.g., calcium-calmodulin-dependent protein kinase) [138–140] is related to the secretory response observed after stimulation of this class of receptors.

8. Concluding remarks

This brief overview of protein secretion by the salivary glands and pancreas has focused on processes common to almost all secretory cells. These common features have enabled investigators from virtually all disciplines, using diverse technical approaches and a multitude of experimental systems, to contribute to our understanding of this universal property of eukaryotic cells. The increasing application of molecular biology techniques and the further development and refinement of in-vitro model systems promise to reveal, for many aspects of the secretory process, the molecular mechanisms underlying the problems that today remain unsolved.

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