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Review

The econobiology of pancreatic acinar cells granule inventory and the stealthy nano-machine behind it

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Dedicated to Prof. Raymond Coleman for his retirement. Raymond is one of the world's premier educators in histology and has been a truly distinguished Editor-in-Chief of the international 60-year-old Elsevier journal *Acta Histochemica*. Under his leadership *Acta* has truly thrived.

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ABSTRACT

The pancreatic gland secretes most of the enzymes and many other macromolecules needed for food digestion in the gastrointestinal tract. These molecules play an important role in digestion, host defense and lubrication. The secretion of pancreatic proteins ensures the availability of the correct mix of proteins when needed. This review describes model systems available for the study of the econobiology of secretory granule content. The secretory pancreatic molecules are stored in large dense-core secretory granules that may undergo either constitutive or evoked secretion, and constitute the granule inventory of the cell. It is proposed that the Golgi complex functions as a distribution center for secretory proteins in pancreatic acinar cells, packing the newly formed secretory molecules into maturing secretory granules, also known functionally as condensing vacuoles. Mathematical modelling brings forward a process underlying granule inventory maintenance at various physiological states of condensation and aggregation by homotypic fusion. These models suggest unique but simple mechanisms accountable for inventory buildup and size, as well as for the distribution of secretory molecules into different secretory pathways in pancreatic acinar cells.

Introduction

The 1974 Nobel Prize in Physiology and Medicine award was a major celebration for the pancreatic acinar cell scientists. George Emile Palade, Albert Claude and Christian de Duve were granted the prestigious award for their joint and independent innovations in correlating autoradiographic and histochemical approaches of transmission electron microscopy with cell fractionation followed by biochemical investigation, which together laid the foundations of modern molecular cell biology. The key result steps, considered correct to this day, establish the classical model of secretory granule formation, under which the newly synthesized secretory proteins, transported from the rough endoplasmic reticulum (RER) to the Golgi complex (GC), undergo post-translational modification and are packaged for secretion by condensation within membrane-bound granules—later realized to be of "unit" size. These unit granules may fuse with other granules to form larger granules that reside in the cytoplasm as granule inventory, to be secreted spontaneously or upon demand. The pancreatic acinar granule life-cycle has served steadily as a key model in the investigation of the secretory system.

Almost 40 years later (2013) the Nobel Prize in Physiology and Medicine was awarded to James E. Rothman, Randy W. Schekman and Thomas C. Südhof for their discoveries of nano-machinery regulating secretory granule traffic and life-cycle in almost all of our cells (reviewed in Bonifacino (2014); Brose (2014); Ray (2014)).

Cells use a wide array of nano-machines. The key nano-machine associated with secretion is the soluble N-ethylmaleimide-sensitive attachment protein receptor (SNARE), a protein assemble controlling an array of functions that lead to the exocytosis of granules (Hong et al., 2005; Rothman, 2014; Schekman and Südhof, 2014; Hammel and Meilijson, 2012; Jena, 2009, 2011; Lorentz et al., 2012). Like all eukaryotic cells, pancreatic acinar cells display

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an inventory of cytoplasmic secretory granules, which store zymogen proteins as well as active enzymes, assigned to be secreted. These granules are accordingly called zymogen granules. The key factor for decline in granule inventory involves fusion of the secretory granule with the plasma membrane (PM) by a mechanism of protein secretion that appears to be similar in all eukaryotic cells, in spite of evident granule size differences: neuro-secretory cells stockpile mainly small molecules, while hematopoietic and epithelial cells store proteins to be secreted in large secretory vesicles (protein molecular weight >10,000 Da in most cases), or granules, which characteristically have electron dense morphology. It has been well documented that granule growth is due to homotypic fusion (reviewed in: Hammel et al., 2010a). One of the authors (Hammel, 1980) was the first to document quantal size characteristics of mast cell secretory granules, based on cross-section areas measured by digitized planimetry, recorded in transmission electron micrographs (TEM) of sections of tissue-cultured mast cells. Raymond Coleman, a new Faculty member at the Technion that had just arrived from the UK, investigated in a nearby laboratory the morphology of pancreatic acinar cells (Finkelbrand et al., 1978).

"Quantal" means that each member of the granule inventory is composed of an integer number ($n = 1, 2, 3$) of unit granules ($G_n = n \times G_1$). This induces a multi-modal measured content distribution with equally spaced peaks (Hammel et al., 1983; Hammel et al., 2010a). The pancreatic acinar cell and the mast cell became the first choices for the investigation of granule growth, although additional cells were also investigated as comparison (reviewed in: Hammel et al., 2010a). The presence of these periodic modes of discrete steps was evident in further studies that applied a moving-bin technique to mouse, rat and human secretory cell data (Hammel et al., 1987). The establishment of the notion of quantal granule size was assisted by biochemical investigation (Hammel et al., 1988a,b; Mroz and Lechene, 1986), pulse and chase autoradiography (Hammel et al., 1998) and immunohistochemical approaches correlated with quantitative electron-microscopy (Kalina et al., 1988; Hammel et al., 2010a,b; Bialik et al., 2001; Shoichetman et al., 2001). The quantal model of granule size and content gained acceptance a few years later, after it was confirmed in different cells by other methodologies, e.g., the patch clamp technique (Alvarez de Toledo and Fernandez, 1990; Hartmann et al., 1995), intra-granule enzyme content estimation (Mroz and Lechene, 1986) and *in-vitro* biochemical studies correlated with granule morphology (Hammel et al., 1988a,b). The peritoneal mast cell served as an excellent electro-physiological cell model for follow-up of single granule degranulation by time-resolved patch-clamp capacitance measurements, disclosing that the plasma membrane increases in discrete steps (Alvarez de Toledo and Fernandez, 1990). In contrast, the fast regranulation of pancreatic acinar cells made these cells an excellent candidate for granule inventory assessment.

Granule inventory may be defined as the goods stocked in the cell cytoplasm to serve as the portion of the cell's "business' assets" that are ready or will be ready for secretion. Granule inventory is one of the most important resources that any cell contains, since the turnover of granule inventory characterize one of the primary sources of "cell revenue generation" to be used for communication with or processing of the cell environment. Maintaining high granule inventory for long periods is usually detrimental to "cell business" since storage, obsolescence and deterioration incur costs. However, possessing too low granule inventory is also harmful, because of the risk of providing insufficient supply to the environment, and the instability it induces on cell content composition. Granule inventory management seems the result of evolution-driven learnt forecasts and strategies which help minimize granule inventory costs by calibrating granule contents to reflect safety of inventory supply when needed. Such prospect requires granules to be distributed within the cell cytoplasm so as to pave the way for the regular cell duty. Accordingly, the mast cell granule inventory, as that of most hematopoietic cells, is nearly evenly distributed in the cell cytoplasm, while in the pancreatic acinar cell and in many goblet epithelial cells, it is stocked distal to the cell nucleus, near the acinus lumen which collects and transports the secreted content.

Pancreatic acinar granule content secretion has two main routes. At homeostatic state, content is secreted constitutively as a rare event. In contrast, following food consumption, it may be exocytosed by massive fast secretion of bursts of granules. Early literature on turn-over rates of granule content (chymotrypsinogen, trypsinogen, lipase, DNase and RNase) in pancreatic acinar cells documented fluctuation between slow basal secretion (granule half-life between 8 and 16 h; Christophe et al., 1973; Hammel et al., 1998; Adelson et al., 1995; Case, 1978; Schick et al., 1984; Poort and Poort, 1981; Jeraldo et al., 1996) and faster evoked secretion when acinar cells are activated by proteins (Case, 1978) or neurological stimuli (Case, 1978). Further studies (Hammel et al., 1988a,b) performed on tissue-cultured connective tissue-like mast cells (CTMC) found significant evidence for a marked difference between the two modes. Assuming that during 35 days some 400–600 mast cell granules are basally secreted, one granule is secreted every 1–2 h (Wingren et al., 1983; Enerbäck and Jarlstedt, 1975; Hammel et al. (1989, 2012)). In contrast, during immunological activation these same 500 granules will be exocytosed in less than half an hour (Lagunoff, 1972, 1973; Lagunoff and Chi, 1978; Lagunoff et al., 1983; Hammel et al., 1988a,b, 1989).

Morphological evidence for granule inventory buildup

The exocrine pancreas has been widely used as a model for stereological study of intracellular organelles associated with secretory content synthesis, intracellular transport and discharge. The two most common conditions for quantitative microscopy analysis are pancreas development during the rodent's prenatal life (Ermak and Rothman, 1980, 1981, 1983, 1986; Uchiyama and Watanabe, 1984a) and granule inventory buildup following significant degranulation (Aughsteen and Cope, 1987; Aughsteen et al., 1996; Hammel et al., 1993; Kern et al., 1985; Nevalainen, 1970; Weintraub et al., 1992). Secretory granule formation in pancreatic acinar cells is known to involve massive membrane flow. Morphometric analysis (Uchiyama and Watanabe, 1984a,b; Aughsteen and Cope, 1987; Aughsteen et al., 1996; Nevalainen, 1970) demonstrates noteworthy variations in the average volume of the cell, nucleus and cytoplasm, as well as in the volume, surface and numerical density of various cytoplasmic organelles during granule inventory buildup.

In the rat fetus, zymogen granules first appear at 17 days of gestation and occupy by 21 days—delivery day—the greater fraction of the cytoplasm of the acinar cell. No secretion of zymogen granules has been observed during fetal life (Ermak and Rothman, 1980, 1981, 1983, 1986; Uchiyama and Watanabe, 1984a). The observed linear rate of granule inventory buildup during fetal life (Fig. 1) suggests that the GC packs about 0.5 unit granules per minute, as compared to the three times higher rate observed during regranulation of mature acinar pancreatic cells. Electron micrographs of rodent pancreatic acinar cells are commonly taken at several time points following extensive degranulation (≈95%) induced by pilocarpine injection (Nevalainen, 1970; Lew et al., 1994), in order to investigate volume changes in granule inventory. Newly formed granules appear 2–4 h following activation. Beyond this point, increased autophagosomal activity is observed when pancreatic cells demonstrate a complete loss of granules (Lew et al., 1994), coupled with GC restructuring. In both parotid (Cope, 1983; Cope and Williams, 1973a,b, 1980; Williams and Cope, 1981) and pancreatic acinar cells (Lew et al., 1994) the regranulation span can be differentiated into