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Identification of new structural elements within '*porosomes*' of the exocrine pancreas: A detailed study using high-resolution electron microscopy

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ABSTRACT

In the past two decades, great progress has been made in our knowledge of how cells secrete. This progress has been possible primarily due to discovery of the '*porosome*', the universal secretory portals at the plasma membrane in cells. Porosomes are permanent cup-shaped lipoprotein structures at the cell plasma membrane, where membrane-bounded secretory vesicles temporarily dock and fuse to expel all or part of their contents during cellular secretion. Porosomes have been found in neurons, in neuroendocrine cells, as well as in the exocrine pancreas. Furthermore, porosomes have been isolated, functionally reconstituted, and their composition determined. Although, the neuronal porosome has been exhaustively investigated, the detailed morphology of porosomes in the exocrine pancreas *in situ* remains to be further explored. The current study was carried out to determine the detailed morphology of the porosome in rat exocrine pancreas using high-resolution electron microscopy. Results from our study, demonstrate for the first time the presence of tethers or cables (which could be t-SNAREs) associated at the base of porosomes. Furthermore, for the first time our studies demonstrate the docking of a single secretory vesicle at the base of more than one porosome complex. Detailed spoke-like elements lining the porosome cup are also demonstrated for the first time in our study, providing a better understanding of the molecular architecture and physiology of this important cellular organelle.

1. Introduction

Secretion is a very important process occurring in all cells and is involved in the physiology of neurotransmission, and for the release of hormones and digestive enzymes. A number of diseases are known to result from defects in cell secretion. The area of cell secretion has been intensely investigated for over half a century. Until recently, it was commonly accepted that the final step in secretion is the total incorporation of secretory vesicle membrane into the cell plasma membrane leading to the release of intravesicular contents by diffusion, and the compensatory retrieval of excess membrane by endocytosis at a later time (Ichikawa, 1965; Ceccarelli et al., 1972; Dreifuss, 1975; Saras et al., 1981; Ryan et al., 1996; Valentijn et al., 1999; Zenisek et al., 2002; Heidelberger, 2001; Sudhof, 1995; Fischer von Mollard et al., 1994; Walch-Solimena et al., 1995). Studies within the past 20 years have finally revealed a completely different molecular mechanism of secretion and membrane fusion in cells (Schneider et al., 1997; Cho et al., 2002a,c,e, 2004; Jena, 2002, 2004, 2005, 2007, 2008, 2009a,b, 2010; Jena et al., 1997, 2003; Jeremic et al., 2003, 2004a,b, 2005, 2006; Clary et al., 1990; Söllner et al., 1993; Rothman and Söllner, 1997; Weber et al., 1988; Craciun, 2004; Jeftinija, 2006; Leabu, 2006; Anderson, 2006a,b). Monck and Fernandez (1996) suggested the existence of fusion pore at the cell plasma membrane, which became continuous with the secretory vesicle membrane after stimulation of secretion. However, the question persisted as to how partially empty secretory vesicles are generated following the secretory process in cells. Then, in the mid 1990s AFM studies confirmed for the first time the existence of the permanent structures at the cell plasma membrane, where secretory vesicles transiently dock and fuse to expel their contents (Schneider et al., 1997; Cho et al., 2002a,c,e). These studies were performed using atomic force microscopy (AFM) at nanometer resolution and in real time (Jena et al., 2003). Using electron microscopy (EM) and AFM, studies further revealed the detailed structure of the secretory machinery, and was named '*porosome*'. It became rapidly clear from this discovery (Schneider et al., 1997; Cho et al., 2002a,c,e, 2004; Jena et al., 2003; Jeremic et al., 2003, 2004a,b; Jena, 2004; Jeftinija, 2006; Leabu, 2006) how partially empty vesicles are generated following cell secretion, and that contrary to accepted belief how the mechanism of cell secretion is quite different and a highly regulated process. In brief, these landmark discoveries combined with other studies (Cho et al., 2002b,c; Jeremic et al., 2003, 2004a,b, 2005; Jeftinija, 2006; Leabu, 2006; Jena et al., 1997; Abu-Hamdah et al., 2004; Kelly et al., 2004; Taraska

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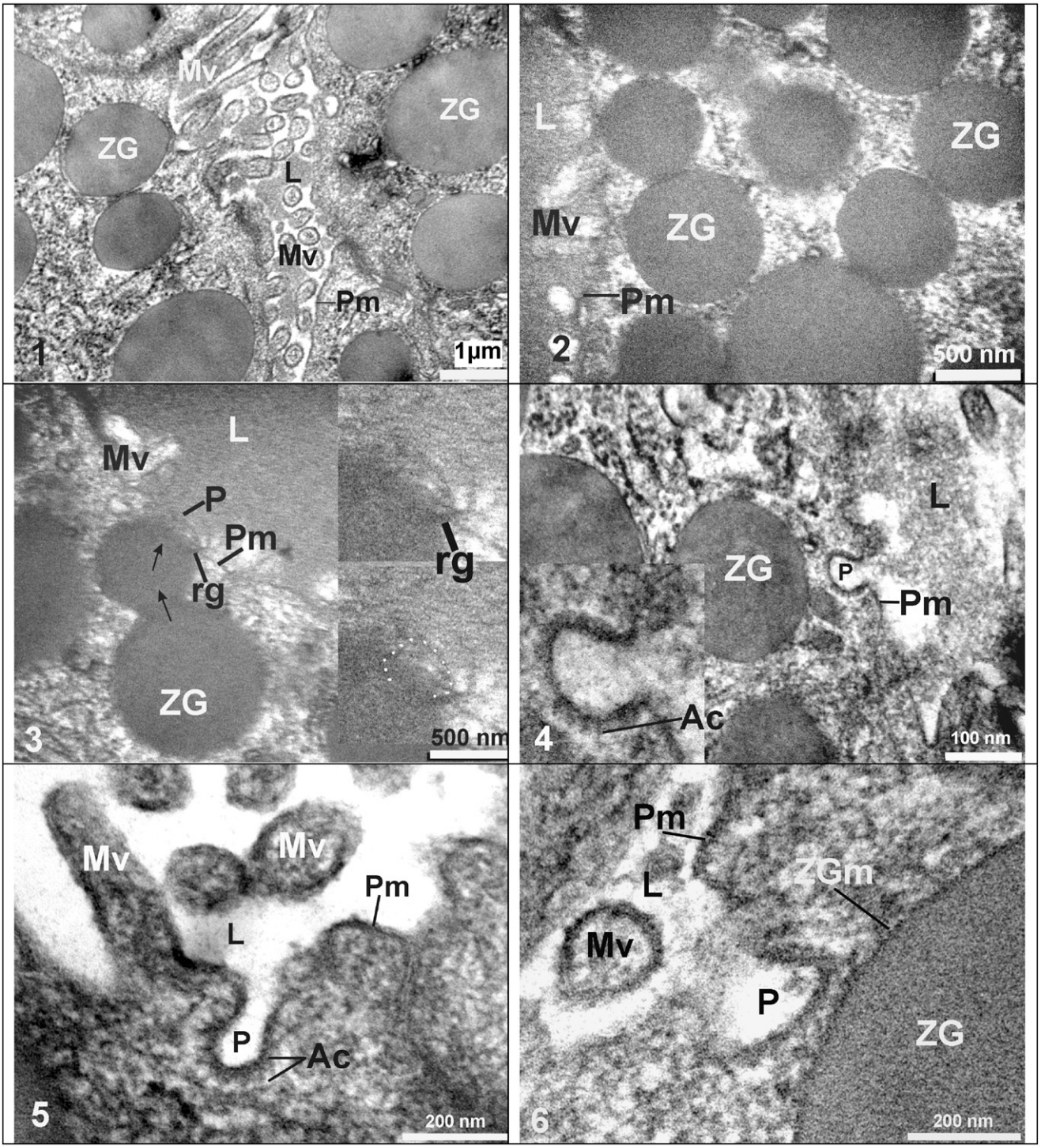

Figs. 1–6. **Fig. 1** Many zymogen granules (ZGs) are agglomerated near apical plasma membrane (Pm) of exocrine pancreatic cells that delimit the lumen (L) of acinus. **Fig. 2** Interconnected zymogen granules (ZGs) found near the plasma membrane (Pm) that delimit the lumen (L) of acinus (Mv – microvilli). **Fig. 3** A zymogen granule (ZG) found near plasma membrane (Pm) is in contact with a porosome (P) to deliver their content in lumen (L); it has small diameter after partially delivery of their content. **Figs. 4–6** Different aspects of porosomes (P) found near membrane of zymogen granule (ZGm). Anchoring cables (Ac) of porosomes are visible (Pm – plasma membrane; L – lumen).

et al., 2003; Wang et al., 2012) demonstrate that membrane-bound secretory vesicles dock and transiently fuse at the base of specialized plasma membrane structures called porosomes, to discharge vesicular contents. Contrary to what was previously suggested, there is no incorporation of the vesicle membrane at the cell plasma membrane.

In the exocrine pancreas, porosome at the plasma membrane is 100–180 nm macromolecular cup-shaped lipoprotein baskets, where membrane-bound secretory vesicles called zymogen granules (ZGs) dock and transiently fuse to release vesicular content. Studies on the lipid-free immuno-isolated supramolecular porosome complex are found to measure 125–150 nm oval-shaped structure with three concentric rings and 10 spokes that originate from the small inner ring and traverse the larger two outer rings (Cho et al., 2002c; Jeremic et al., 2003; Jeftinija, 2006; Leabu, 2006). The central inner ring has a diameter of 25–30 nm, with a 20–25 nm