A novel tool to visualize the cell secretory pathway. Focus on “A fluorimetry-based ssYFP secretion assay to monitor vasopressin-induced exocytosis in LLC-PK₁ cells expressing aquaporin-2”

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A NUMBER OF FLUORESCENCE-BASED techniques have been developed and used to visualize the trafficking and exocytosis of vesicles in living cells and intact tissues. Several of these techniques are based on the addition of exogenous fluorescent compounds that label the secretory vesicles through endocytosis or by acidotropic dye trapping. For example, FM dyes (Molecular Probes/Invitrogen) have been used for the past 15 years to study the dynamics of vesicle trafficking in a variety of cell types (1). These styryl dyes are essentially nonfluorescent when in solution but brighten markedly when inserted in membranes. After vesicle preloading by stimulated exocytosis-endocytosis, the FM dyes are released subsequent to a stimulus by exocytosis, which can be readily documented as a decrease in fluorescence. In acidotropic dye trapping, the acidotropic fluorophores, such as quinacrine, quickly penetrate cell membranes because a fraction of the molecules is uncharged at physiological pH, and then accumulate in acidic cell organelles where they are “trapped” after becoming protonated and are no longer membrane permeable. The dyes are rapidly released on exocytosis, as has been used to document secretion of ATP secretory vesicles (2) and renin granules in the kidney (5). Unfortunately, most of these dyes are not specific for secretory vesicles as they also label the lysosomes of the degradation pathway and even cell nuclei (5), nor do they directly label the secreted, cell-specific vesicle cargo itself, like neurotransmitters, insulin, and renin.

The use of genetically encoded fluorescent proteins like green fluorescent protein (GFP) has allowed specific targeting of fluorescent markers to the secretory pathway (3). Fluorescent proteins can be targeted to the secretory vesicles by creating molecular fusion constructs of the fluorescent protein with 1) a luminal cargo peptide, 2) a signal peptide specific for the secretory pathway (which is cleaved away from the fluorescent protein during processing), or 3) a vesicle membrane protein. Each of these three approaches offers specific advantages, and each provides distinct information.

Nunes et al. (4) report a novel fluorimetry-based bulk secretion assay to monitor vasopressin-induced exocytosis in LLC-PK₁ cells expressing aquaporin-2 (AQP2). This newly developed fluorescence assay measures exocytosis of post-trans-Golgi network (TGN) vesicles that are clearly distinguished from endocytic vesicles. The principle behind the assay is the following: cells are transfected with a plasmid coding for a soluble enhanced yellow fluorescent protein (EYFP) downstream from a signal peptide resulting in expression of a secreted soluble (ss) YFP (ssYFP). Upon expression, ssYFP molecules are translocated into the lumen of the endoplasmic reticulum. From there, they transit the Golgi and then passively enter and label secretory vesicles as they are released from the TGN. The EYFP content is secreted during vesicle exocytosis, and ssYFP fluorescence in the extracellular medium can be assayed using a fluorimeter. The use of YFP helps to overcome technical problems associated with tissue autofluorescence that are often present when using GFP. Using the expression of this construct as a tool, the authors were able to demonstrate that vasopressin stimulation increases ssYFP secretion only in cells expressing AQP2, not in native cells without AQP2 (4). This result supports the previously debated view that an exocytotic response occurred on vasopressin stimulation and that this is dependent on the expression of AQP2 within the cell.

In addition to enabling the study of the detailed molecular mechanism of vasopressin-induced AQP2 exocytosis as described by Nunes et al. (4), this novel assay has great potential for use in other cell types, for the study of the SNARE-complex and the exocytotic machinery. Also, and perhaps even more important, this assay may be developed further to visualize the secretion of various proteins and signaling molecules in real-time in vivo.

REFERENCES