Exocytosis: The Chromaffin Cell As a Model System

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ABSTRACT: Neurons and neuroendocrine cells release transmitters and hormones by exocytosis of secrctory vesicles or granules. Among the cell models that have provided insight into the molecular machinery underlying the successive steps of exocytosis, adrenal chromaffin cells have taken a prominent place. Thus, most of the molecular players that orchestrate the formation, targeting, docking, and fusion of secrctory granules have been identified in chromaffin cells. By offering the opportunity to combine the use of recent biophysical techniques allowing single-vesicle resolution and specific biochemical modifications in the protein machinery involved in exocytosis, chromaffn cells remain a powerful model to address new and still open questions in the field of secretion.

KEYWORDS: exocytosis; chromaffin cells; model systems

INTRODUCTION

Exocytosis, the final step in neurosecretion and neurotransmitter release, has been the topic of intense investigation for decades. The adrenal chromaffin cell has provided and continues to provide profound insights into the secretory pathway. As demonstrated at this conference, it is amenable to the use of diverse and novel techniques that greatly increase the understanding of the complex events underlying exocytosis. Thus, new questions and hypotheses about the regulation of exocytosis are continuously emerging, keeping “exocytosis in chromaffin cells” among the most active fields of research.

Several stages have been recognized in exocytosis. After recruitment and translocation, the secretory granules enter a step of docking, which is followed by the fusion process itself. Docking is not a sufficient condition for fusion, and some

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Ann. N.Y. Acad. Sci. 971: 178–183 (2002). © 2002 New York Academy of Sciences.

biochemical events, described as priming reactions, are likely to occur, rendering the granules competent for fusion. Details of the molecular mechanisms for each of these steps gained by electrical approaches, such as the patch-clamp technique and amperometry, or by biochemical analysis of proteins participating in granule fusion *in vitro* or in permeabilized cell systems have been described. Agreement is growing regarding the identities of the key proteins that form the core of the exocytotic machinery, although considerable work is still needed to determine how these proteins act in concert and to define their molecular partners in promoting membrane fusion.

SECOND MESSENGERS REGULATE GRANULE CONTENT

Several second messengers have been implicated in the trigger and modulation of calcium-evoked exocytosis. In chromaffin cells, the effects of cAMP on secretion have been studied over many years, and many investigators have proposed that the cAMP/protein kinase A (PKA) system modulates catecholamine secretion by increasing the number of granules released with a Ca^{2+} stimulus. Ricardo Borges (Tenerife, Spain) presented data offering an alternative view, using amperometry with carbon microelectrodes to measure catecholamine release upon fusion of individual granules. He found that cAMP slows the kinetics of release of catecholamine, but also increases the amount of catecholamine released during individual secretory events. The latter effect may be due to compound granule fusion before exocytosis. Nitric oxide, in contrast, speeds the kinetics of catecholamine release without altering the amount of catecholamine released during an individual secretory event. Thus, chromaffin cells may be able to produce rapid and reversible modifications of their secretory performance through transient variations in second messengers controlling both the size and the kinetics of the released quantum.

THE ACTIN CYTOSKELETON

In chromaffin cells, secretory granules are present in at least two compartments, the release-ready granule pool and the reserve pool comprising the majority of granules. It has been postulated that actin filaments form a cortical network that separates and acts as a barrier between these two pools, subjecting the traffic of granules from the reserve to the docking sites to fine regulation. Experimental evidence provided by Trifaro and his colleagues[1] has suggested that the dynamics of the actin cortex controls the size of the release-ready pool and thereby the initial rates of exocytosis. Under physiological conditions, the mechanism controlling actin depolymerization in secretagogue-stimulated cells involves the calcium-dependent activation of scinderin with consequent actin severing in the subplasmalemmal region (Lejen *et al.*, this volume).

However, the actin cytoskeleton not only may be a barrier for motion, but also may facilitate granule movement. The exchange of granules between pools requires calcium and ATP, suggesting the activation and function of a molecular motor. In a recent report, the motion of green fluorescent protein (GFP)-labeled secretory granules in the subplasmalemmal region in PC12 cells was described by evanescent-field fluorescence microscopy.[2] Interestingly, the mobility of granules was diminished not

only when actin filaments were stabilized, but also when the actin cortex was completely degraded. Kumakura *et al.*[3] suggested previously that actin-myosin interactions might be essential for exocytosis in chromaffin cells. In good agreement, Rosé (this volume) presented recent results indicating that myosin V is likely to be the molecular motor involved in the transport of chromaffin granules to the sites of exocytosis. In line with these findings, Sasakawa (this volume) presented data obtained by amperometry and analysis of track motion of chromaffin granules, indicating that the inhibition of the actin-myosin interactions abolished the recruitment of chromaffin granules to the release sites, probably by reducing the population of rapidly translocating granules. Thus, the actin cortex, classically viewed as a barrier that hinders the movements of granules to the plasma membrane, is likely also to provide tracks along which granules are pulled to sites of exocytosis.

RAB3 AND RIM

Henry and colleagues (this volume) presented evidence that Rab3a, a low molecular GTPase on the chromaffin granule membrane, specifically regulates the rapid phase of exocytosis, probably indicating an action on the readily releasable pool of granules. A possible effector for Rab3a, RIM, strongly enhances secretion (Bittner, this volume). However, because the Rab3a-binding and secretion-enhancing domains are separate and unique, it is unlikely that binding to Rab3a is necessary for the action of RIM in chromaffin cell exocytosis.

DOCKING AND PRIMING

Reasonable agreement now exists about the essential role of the SNARE complex in late stages of the exocytotic pathway. It has been postulated that these proteins present on secretory granules (v-SNARE: synaptobrevin) and on the plasma membrane (t-SNARE: SNAP-25 and syntaxin) to form an initial loose complex, and it is the final zippering into a tight complex that provides energy to pull membranes into close proximity and render them fusion competent.[4,5] Biochemical events, termed priming, that probably precede trans-SNARE interactions are essential for calcium-triggered membrane fusion, and a number of ATP-dependent priming reactions have been described.[6] One important role for ATP in priming is the maintenance of phosphatidylinositol (PtdIns)-4,5-P_2 through the action of lipid kinases on PtdIns and-PtdIns-4-P. (See presentation by Holz and Axelrod, this volume.) Martin (this volume) presented data showing that CAPS, a protein required for the calcium-triggered exocytosis of docked dense core granules, but not synaptic vesicles, is likely to be an important effector for the function of PtdIns-4,5-P_2 in PC12 cells. When expressed as a GFP-labeled protein, CAPS is directly targeted to the plasma membrane and to dense core granules through a targeting signal at the C-terminus domain, suggesting that it may function by interacting with both membranes involved in fusion. Other recently described priming factors include munc-13[7] and NSF.[8] Thus, a key future issue will be to determine how these priming factors act in concert to promote the fusion competence of docked granules. Another important issue is the extent to which the fusion pathways for large dense core granules and synaptic ves-

icles differ, since recent genetic studies performed on *Drosophila* indicate that CAPS is required not only for dense-core granule, but also for synaptic vesicle release at glutamatergic synapses.[9]

THE LATE FUSION STEP

An important challenge concerns the mechanisms underlying the final fusion event. A powerful technique, carbon fiber amperometry, was introduced to biology over a decade ago by Wightman *et al.*[10] It allows high-time resolution of single secretory granule release events. By analyzing electrical currents reflecting the oxidation of released catecholamine during individual exocytotic events, it is possible to model the dynamics of the opening of the final fusion pore and the release of granule contents. A theme that emerges (also see below) is that membrane fusion and the release of granule contents are distinct events that can be controlled individually. For example, hypertonic solution suppresses the explosive release of catecholamine, although fusion still occurs (see Troyer and Wightman, this volume).

With amperometry, Burgoyne (this volume) further defined the role of cysteine string protein (Csp) and munc-18 as molecular partners for syntaxin involved in the control of fusion pore dynamics. Using a similar approach, Vitale (this volume) described that expression of phospholipase D1 mutants with reduced catalytic activity specifically increases the rising phase of amperometric spikes, highlighting the role of lipids in fusion pore expansion.

The combination of amperometry and cell-attached capacitance measurements revealed that calcium may shift the mode of exocytosis in chromaffin cells from full fusion to reversible fusion, leading to kiss-and-run events.[11,12] These findings raise critical questions that will certainly be the focus of future work. For example, what determines the transition between reversible and full fusion? What is the nature of the proteins that allow rapid pore closure? Can cells control the amount of hormones and transmitters released per granule? The use of biophysical approaches under physiological conditions will be key to our understanding of exocytotic fusion pores and the function of specific proteins at different stages of fusion pore formation, expansion, and closure.

NEW TECHNOLOGIES TO STUDY EXOCYTOSIS

Dramatic methodological advances in the field of exocytosis have been achieved by the ability to visualize vesicle dynamics in living cells, especially with GFP-labeled proteins. Among the earliest studies were those of Levitan and his colleagues[13] who demonstrated that proAtrial Natriuretic Peptide (ANP) fused to GFP is sorted to secretory granules in PC12 cells and is released upon stimulation. Mobile and immobile pools of granules can be distinguished in the nerve terminal of differentiated PC12. Importantly, the mobile pool preferentially undergoes exocytosis, and release can be increased by increasing the fraction of mobile granules (Levitan, this volume). Fluorescent measurements of GFP fused to ANP reveal that Ca^{2+} induces alkalinization of the granule interior, even when secretion is inhibited.

A promising approach to monitor exocytosis optically with high spatial and temporal resolution is total internal reflection fluorescent microscopy (TIRFM). Within a very thin section (100–300 nm) illuminated by an evanescent field near the underside of cells investigators have access to the three-dimensional motion of granules in close proximity of the plasma membrane.[14,15] Using TIRFM in chromaffin cells, Holz (this volume) presented data indicating that granule motion (granules labeled with proANP-GFP) is severely limited in the immediate vicinity of the plasma membrane. There is a 100-fold decrease in the short-term diffusion coefficient of granules as granules move a distance equivalent to their granule diameter (300 nm) closer to the plasma membrane. SNARE proteins are not responsible for the restricted motion of granules upon approach to the plasma membrane. However, lack of functional SNARES reduces the time that some granules spend immediately adjacent to the plasma membrane, consistent with continuous and reversible formation of trans-SNARE complexes. TIRFM is also being used in a novel manner to monitor single granule exocytosis in a broken cell system that permits free access to the secretory machinery (Wiegand and colleagues, this volume).

Atomic force microscopy (ATM), by probing the outer surface of living cells in a time-dependent manner and with a z resolution in the nanometer range, provides another promising high-resolution imaging approach. ATM has brought additional support for transient vesicle fusion, revealing prolonged fusion without total incorporation of the vesicle membrane into the plasma membrane (Cho, this volume).

Different laboratories have used transient transfection techniques to study the roles of specific proteins in the secretory pathway in chromaffin cells since the techniques were introduced several years ago. The techniques, however, suffer from low transfection efficiencies. The recent use of Semliki forest virus greatly improves transfection efficiency, permitting the future application of biochemical techniques to investigate the effects of transiently expressed protein on the secretory pathway. The dynamics of chromaffin granule maturation are being investigated using Semliki forest virus infection (Greaves *et al*., this volume).

Finally, computational methods should facilitate the analysis of the vast amounts of data available about the secretory pathway in chromaffin cells. Synthesizing electrophysiological, biochemical, biophysical, imaging, and genetic data collected on chromaffin cells at a bioinformatic level has the potential to increase our understanding of the fine tuning of the exocytotic process.

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