Meeting report

Regulated secretion

Colin Jamora

Address: Department of Biology, University of California, San Diego, USA. E-mail: CJAMORA@BIOMAIL.UCSD.EDU

Published: 27 April 2000

Genome ***Biology*** 2000, **1(1)**:reports402.1–402.4

The electronic version of this article is the complete one and can be found online at http://genomebiology.com/2000/1/1/reports/402

A report from the minisymposium on regulated secretion at the 39th Annual Meeting of the American Society for Cell Biology, Washington DC, December 11–15, 1999

Despite the apparent differences in cellular architecture and behavior of different cell types and of cells from organisms separated by long evolutionary times, it is increasingly apparent that the core mechanisms and many of the components are common and can be usefully studied in a wide variety of cell types. Intracellular protein transport is one area in which studying a variety of cell types has provided many more insights than could have come from studies of a single cell type or organism. Proteins traveling through the secretory pathways of cells are processed and targeted to a variety of cellular destinations. This delivery system can be constitutive, or cargo can be stored within a regulated secretory vesicle or granule until an external stimulus causes it to fuse with the cell surface. The minisymposium on regulated secretion at the American Society of Cell Biology meeting concentrated on the two distinguishing features of the secretory pathway: namely the process by which specialized secretory vesicles are formed and cargo specifically sorted into them; and the molecular mechanism by which such vesicles rapidly fuse with the plasma membrane upon receipt of a signal. Although neuronal cells have traditionally been the predominant model for studying regulated secretion, Chris Kaiser (Massachusetts Institute of Technology) presented evidence that the yeast *Saccharomyces cerevisiae* can serve as a model organism for investigating regulated sorting of cargo. By altering the nutrients in the growth media of yeast cells, some proteins can be redirected from the vacuolar membrane to the plasma membrane. For example, in low nitrogen conditions, an amino acid permease, GAP1, is thus redirected, and using genetics several candidate proteins were identified that can modulate the differential localization of GAP1. As a result, complex assemblies of proteins are emerging as mechanisms by which the spatial and temporal coordination of cargo selection and concentration into a vesicle is achieved. The use of a differential targeting system in yeast has therefore provided a useful paradigm for understanding regulated secretion in other cells, such as neurons, which are not so tractable experimentally.

The formation of vesicles, however, is not solely reliant upon proteins to reconfigure a portion of the 'flat' membrane into a spherical structure. Use of permeabilized PC12 phaeochromocytoma cells (a model for neuroendocrine cells) provided a means of investigating the mechanism by which dense core granules containing polypeptides and hormones are generated in these cells (Dennis Shields, Albert Einstein College of Medicine): the generation of phosphatidic acid (PA) within the membrane bilayer was found to be required for the release of secretory vesicles from the trans-Golgi network. Shields has previously reported that the small GTPase protein ARF-1 could activate Golgi-localized phospholipase D to stimulate vesicle budding from the trans-Golgi network *in vitro*, and the new work presented at the meeting demonstrated that the generation of PA from the hydrolysis of phosphatidylcholine stimulated vesicle budding. Addition of reagents that increase PA levels can also increase the amount of budding vesicles, whereas inhibiting PA synthesis by transphosphatidylation can quantitatively block secretion and was observed to cause the Golgi to fragment. Of particular interest was the model that the ability of PA to promote vesicle budding was due to its regulatory control of the synthesis of phosphatidylinositol bisphosphate (PIP_2, a known precursor of intracellular signaling molecules). PA has traditionally been thought to be required for the generation of a local negative charge on the planar membrane, thereby causing a bud to form. Now, in addition to their established roles in signal transduction, phosphoinositides have taken center stage in affecting bud formation by altering the lipid composition of cellular membranes.

Once regulated secretory vesicles are formed and cargo is packaged into them, what allows them to respond to an

external signal so as to fuse rapidly with the plasma membrane? One way they respond is through the activity of proteins such as synaptotagmin, the putative calcium sensor involved in Ca^{2+}-dependent exocytosis of synaptic vesicles. The domains of synaptotagmin responsible for binding to phospholipids and proteins have been mapped, but determining its interacting partners has proven to be a difficult task (Shuzo Sugita, University of Texas Southwestern Medical Center). The problem lies in the fact that identification of interacting proteins *in vitro* does not necessarily reflect a functional interaction *in vivo*.

Another way of regulating the release of stored cargo is to control the protein–protein interactions of the components of the fusion apparatus, such as the SNARE proteins. The SNARE hypothesis for membrane fusion postulates that some of the SNARE proteins must act *in trans*: a v-SNARE on the vesicle must interact with a t-SNARE on the plasma membrane to form the 'core complex' and mediate fusion. Linda Robinson (University of Wisconsin) reported that most of the SNARE proteins in PC12 cells are in a '*cis*' complex at the plasma membrane: the v- and t-SNARES that had paired were both originally found on the plasma membrane. Interestingly, ATP hydrolysis was found to disassemble many SNARE complexes, and upon Ca^{2+} influx at the presynaptic cell, different sets of complexes could form. Hydrolysis of ATP was originally thought to be required for membrane fusion, which was facilitated by the disassembly of this core complex. This work provides further support for evidence from yeast that hydrolysis of ATP is instead required for the 'docking' step (the close apposition of the vesicle with its target membrane) prior to the actual mixing of bilayers and release of the vesicle's luminal contents.

One protein that is a component of a complex critical for synaptic membrane fusion is syntaxin 1. In its resting state this protein adopts a 'closed' conformation and is incapable of forming a competent fusion complex. During exocytosis, syntaxin 1 associates with munc 18-1 (a neuronal sec 1 homolog) to form a transitory complex (Irina Dulubova, University of Texas Southwestern Medical Center). This transitory complex leads to a significant conformational change in syntaxin to the 'open' conformation and the concomitant formation of the core complex.

Thus, it appears that there are several layers of complex formation in order to achieve the proper fusion of regulated secretory vesicles with their target membranes, whether in yeast, endocrine cells or neurons. Details such as these on the molecular mechanisms governing regulated secretion are beginning to be unraveled, and many of the key components have been identified. As the data presented at the meeting bore out, many of the models used to describe the multiple steps of regulated secretion are too simplistic or require refinement. In addition to identifying other regulatory molecules, the next step will be to integrate these multiple steps into a cohesive unit. For example, coordinating the replenishment of synaptic vesicles once those docked at the presynaptic terminus have fused requires communication among vesicles at the plasma membrane and the storage granules as well as the early secretory pathway that generates these vesicles in the neuron. This may be an avenue in which genomics and proteomics can be used to complement biochemical techniques by allowing the study of many genes or proteins simultaneously in order to understand a multifactorial and complex process.