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Article Addendum

New insights into the control of secretion

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Vesicular secretion is a fundamental process in the body with vesicle fusion releasing vesicle contents to the outside. This process called exocytosis is usually thought of as leading to an all-or-none release of content; regulation of secretory output dependent on regulating the numbers of fused vesicles. However, it is well established that the fusion pore that forms when the vesicle membrane fuses with the cell membrane is dynamic. More recent evidence indicates the dynamic opening and closing, and the size of the fusion pore, are limiting factors to the release of vesicle content. What remains unclear is whether these fusion pore behaviors are under cellular control and therefore relevant to cell physiology.

Accumulating evidence over the last two years points to myosin 2 as one regulator of fusion pore behavior. This is interesting since myosin 2 activity is in turn controlled by kinases and phosphatases, well known to be under cellular control. We conclude that fusion pore behavior is likely a genuine control point for vesicle content release. This leads to a model for secretion with secretory output controlled not only by the numbers of vesicles fused but also by the regulation of the behavior of individual vesicles.

Vesicular secretion, exocytosis, is fundamental to normal body function and health. It is the key process in neurotransmission, endocrine, paracrine, or autocrine signaling, and protein secretion from cells. As such it plays a pivotal role in almost every aspect of animal. Furthermore, secretory dysfunction is central in many diseases, such as type 2 diabetes and pancreatitis[1-3] and the mechanisms of secretory control the target for many drugs. While some of the core molecular components regulating secretion have been identified[4,5] it is still largely unknown how these are orchestrated to control secretion.

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Graded Secretion as a New Model for Secretory Control

Neurotransmitters, hormones and peptides are packed inside secretory vesicles and classical models for secretion propose that these vesicles fuse with the cell membrane and then collapse releasing their entire hormone content (Fig. 1A). This is therefore an all-or-none model of release and in terms of neurotransmission is the corollary of the classical descriptions of quantal neurotransmitter release.[6] However, there is evidence that after vesicle fusion the fusion pore can open and close over time.[7-10] While this behavior can still eventuate in vesicle collapse this does not necessarily happen. In the neuronal cell field, the mechanisms of subsequent vesicle recovery are hotly contested,[11,12] but in endocrine cells mounting evidence shows whole vesicles can be recaptured back into the cell in a processes termed cavicapture[13] (Fig. 1B). Significantly these recaptured vesicles can contain residual quantities of peptide hormones[13-15] suggesting that fusion pore dynamics is a limiting factor in the release of peptides. Some papers now suggest that fusion pore dynamics might specifically regulate the loss of low molecular weight (<200 Da) neurotransmitters.[14,16,17] In this new model, we here term graded secretion, the dynamics and size of the fusion pore lead to partial release of vesicle content (Fig. 1B). The differences between the models are fundamental to our understanding of secretory control. In the all-or-none model secretory output is adjusted by changing the numbers of vesicles fusing. In contrast, the new graded model places regulation of vesicle behavior as central to controlling secretory output.

Possible Regulators of Post-Fusion Vesicle Behavior

It could be argued that these complex post-fusion vesicle behaviors are essentially random, inherent in the nature of the protein and lipid interactions that underlie vesicle fusion and fission. So if fusion pore dynamics really were a control point for vesicle content release then we would expect to see regulatory mechanisms. Gathering evidence now supports this idea of cellular control. It has been shown, in some cell types, that complexin II, Munc18, dynamin and cysteine string proteins can affect pore dynamics[19] although it is not clear whether these are regulatory factors or necessary, static components in a macromolecular pore complex. More direct evidence for second messenger control shows that calcium,[20] and protein kinase C[21] can affect fusion pore opening possibly acting on calcium-sensitive targets like synaptotagmin.[22]

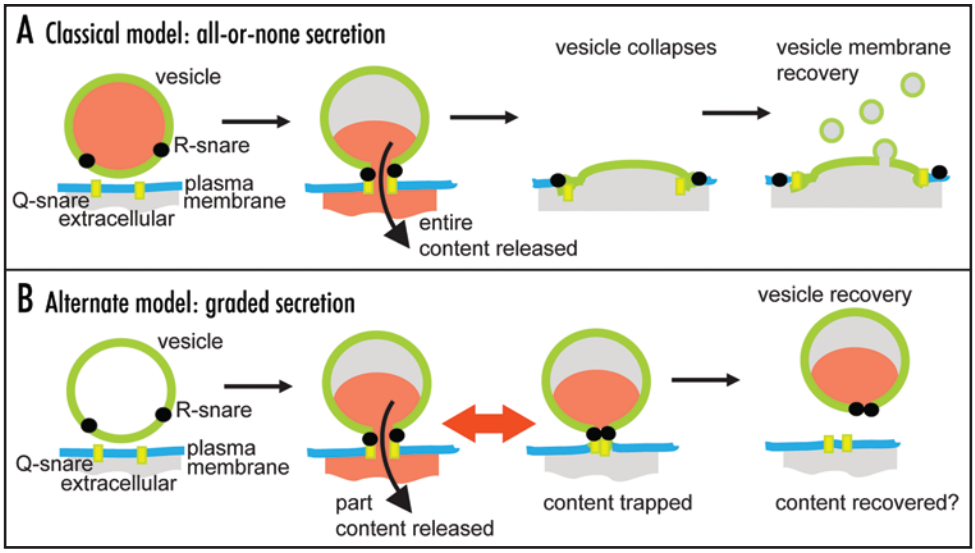

Figure 1. Models for secretory control. (A) in the classical model the entire vesicle content is released, the vesicle collapses and membrane is recovered. (B) in the new model, fusion pore opening and closing regulates content release then either the entire or part of the vesicle membrane is recovered via an unknown mechanism. In some cases it has been shown that part vesicle content can be recovered.

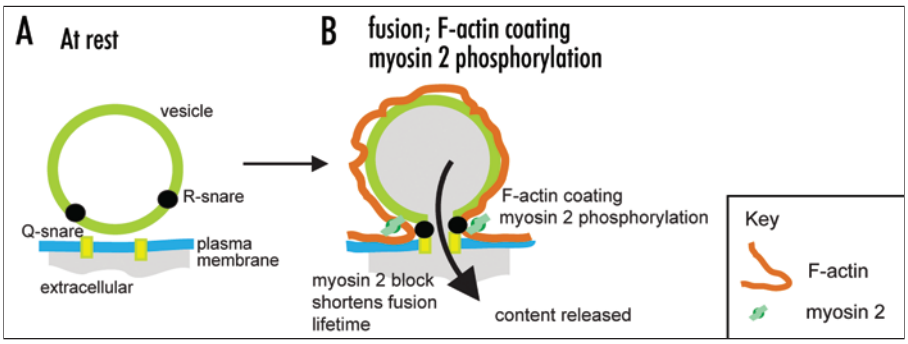

Figure 2. Myosin 2 action in secretion. F-actin coating and myosin 2 phosphorylation appear after fusion. Together we hypothesize these act to maintain the structural integrity of the vesicle and to keep the fusion pore open to allow content loss.

Further, a growing number of recent reports, from a wide range of cell types, are reaching a consensus that F-actin and myosin 2 are dynamic regulators of complex vesicle behavior. Work shows that actin polymerization is triggered immediately after vesicle fusion forming an F-actin network around the vesicle[23-28] that keeps the fusion pore open[27] and stabilizes the vesicle shape.[23,24,29,30] In the last year two reports show that myosin 2 phosphorylation directly regulates fusion pore opening.[31,32]

Myosin 2 Maintains an Open Fusion Pore

Bhat and Thorn (2009) adds to this body of evidence showing that in epithelial cells myosin 2 effects are specific to post-fusion vesicle dynamics. The time course of myosin 2 phosphorylation and the localization of the myosin 2A isoform are consistent with an action at the secretory vesicle. Imaging experiments identify the opening of the fusion pore as at least one target of myosin 2 action with both the direct myosin 2 inhibitor (-)-blebbistatin and an inhibitor of myosin light chain kinase (the likely regulatory kinase) ML-9, causing a closure of the fusion pore. This work then leads us to conclude myosin 2 acts, probably with F-actin, to keep the fusion pore open (Fig. 2). Given that myosin light chain kinase is calcium dependent, it supports the idea that fusion pore dynamics are under direct cellular control.

Concluding Remarks

We conclude that for many cell types the regulation of the post-fusion behavior of secretory vesicles is important in the control of secretory output. It is now suggested that dysfunction of this behavior, in type 2 diabetics, may lead to premature closure of the fusion pore and decrease vesicle content release, leading to the insufficient insulin secretion often seen in the disease.[33,34] Given the potential importance for our understanding of secretory control in health and disease further work is needed to unravel the complexities of these processes.

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