The role of dynamin in fusion pore closure and endocytosis in pancreatic acinar cells

A. Banerjee and P. Thorn, School of Biomedical Sciences University of Queensland , Brisbane, QLD 4067, Australia.

Introduction: In previous work on mouse pancreatic acinar cells that secrete precursor digestive enzymes, we have shown that the end of exocytosis and beginning of endocytosis is marked by the closure of the fusion pore (Larina *et al.*, 2007). Here we show that the mechanism that closes the fusion pore is regulated by dynamin, a GTPase implicated in vesicle dynamics in other cells. We suggest that the closure of fusion pore is the limiting step in secretion that differentiates the two distinct irreversible processes - exocytosis and endocytosis - and following fusion pore closure the vesicle is committed to be internalized.

Methods: CD-1 mice were killed according to the approved ethical procedures of The University of Queensland. The pancreas was excised and collagenase-digested to produce fragments of pancreatic tissue. The tissue was suspended in extracellular buffer at 37°C and bathed in 800 μM extracellular dye Sulforhodamine B (SRB). The cells were stimulated with 600 nM Acetylcholine (ACh) which was limited at 2 min by applying the cholinergic antagonist, atropine (6 μM). Cells were then incubated in the extracellular buffer with or without the dynamin inhibitor Dyngo* (50 μM) for between 0 to 50 minutes. The cells were then centrifuged and resuspended in an extracellular buffer with the extracellular dye HPTS in place of SRB (800 μM). Endocytic objects were therefore labelled with SRB only and granules still undergoing exocytosis at the time of centrifugation were labelled with HPTS. The cells were then imaged using 2-photon microscopy with an excitation of 800 nm.

Results: Control cells (absence of Dyngo) had vesicles that still contained the first dye, SRB as the dye was unable to escape when the cells were resuspended due to the closure of the fusion pore. Control cells also showed vesicles with the second dye, HPTS indicating these vesicles are still exocytotic and in continuity with the extracellular environment. Using MetaMorph® imaging software, 2D surface area of vesicles in a particular cell was quantified as those which contain SRB (red) and that contain HPTS (blue) and expressed as a percentage of internalisation: endocytosis = red surface area/ (red + blue) surface area. The area of SRB-positive vesicles significantly increased over time 0 min - 18.9%, 5 min - 30.7%, 10 min - 35.9%, 20 min - 47.1%, 30 min - 53.2%, 50 min - 63%. In Dyngo-treated cells significantly fewer vesicles contained SRB,indicating the fusion pore had not closed and the dye was able to leave the vesicle upon resuspension in the HPTS. At 30 minutes there was a significant decrease in the area of SRB-positive vesicles (53.2±2.1%,compared with 7±1.5%, N=3 (number of mice) *student t-test:* $p<0.0001$).

Conclusion: The rate of endocytosis in pancreatic acinar cells appears to be quite slow, with internalisation continuing over the entire 50 minute time frame of our experiments. This is in contrast to other tissues such as frog neuromuscular junction, in which fast endocytosis occurs over 20 seconds and slow endocytosis occurs in 5 minutes (Royle & lagnado, 2003). Application of a dynamin inhibitor significantly reduced the surface area of SRB-positive vesicles indicating the importance of dynamin in fusion pore closure. Our results support the idea that dynamin plays a critical role at the transition from exocytosis to endocytosis.

Larina O, Bhat P, Pickett JA, Launikonis BS, Shah A, Kruger WA, Edwardson JM, Thorn P.(2007) Dynamic regulation of the large exocytotic fusion pore in pancreatic acinar cells. *Molecular Biology of the Cell* **18:** 3502-11.

Royle S, Lagnado L. (2003) Endocytosis at the synaptic terminal. *Journal of Physiology* **553:** 345-355.

---

*Dyngo acts on a dynamin GTPase domain allosteric site. It was developed through a joint venture between the Children's Medical Research Institute (CMRI) and the University of Newcastle. It is marketed by Biolink (www.bio-link.com).