Lipid regulation of exocytosis

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Exocytosis is the cellular pathway mediating release of biologically active molecules; late steps, including vesicle docking, priming, Ca^{2+} sensing/triggering and fusion, enable release and exert substantial regulation on the process, defining the ensuing physiological responses. Thus, regulation of release is as critical as the mechanism itself. As protein actions become clearer, functional/modulatory specifics demand detailed understanding to selectively target therapeutics.

Sea urchin eggs have proven an invaluable model to study molecular mechanisms underlying late steps of fast, Ca^{2+}-triggered exocytosis (Abbineni *et al*., 2013, 2014). Shearing eggs yields plasma membrane sheets with fully docked cortical vesicles (CV) that are 'locked' in the fusion-ready state – increasing $[Ca^{2+}]_{free}$ triggers fusion. As isolated CV also retain Ca^{2+} sensitivity/fusion competence, components in their membranes represent the minimal molecular machinery for docking, Ca^{2+} sensing/triggering and fusion. Thus, stage-specific, fusion-ready CV enable the tightest coupling of quantitative functional (end-point and kinetic fusion assays) and molecular (lipid and protein) analyses necessary to dissect molecular mechanisms underlying the Ca^{2+}-triggered release reaction (Coorssen *et al*., 2003; Szule *et al*., 2003; Churchward *et al*., 2005, 2008; Rogasevskaia & Coorssen, 2011; Abbineni *et al*., 2013).

There is ample evidence to support the concept that membrane merger proceeds *via* transient, high negative curvature lipidic intermediates, downstream of protein actions. Consistent with this, cholesterol contributes a critical local negative curvature that promotes formation of intermediates (Churchward *et al*., 2005). Lipids having negative curvature ≥ cholesterol can substitute in fusion but not fusion efficiency (Ca^{2+} sensitivity and kinetics; Churchward *et al*., 2008). Cholesterol- and sphingomyelin-enriched regions of the membrane regulate efficiency of the mechanism, apparently *via* spatial & functional organization of other critical lipids and proteins at the docking/fusion site (Rogasevskaia & Coorssen, 2006 & 2011; Churchward & Coorssen, 2009).

While immediate roles for phospholipase products have largely been excluded from the fusion step *per se*, upstream and direct regulatory/modulatory effects are likely (*i.e.* 'tuning' of local membrane composition) (Rogasevskaia & Coorssen, 2011; Rogasevskaia *et al*., 2012). Accordingly, phosphatidylethanolamine also has direct roles in the fusion mechanism, contributing critical local negative curvature and shaping fusion kinetics. Polyphosphoinositides play upstream roles in priming, with physiologically important modulatory effects on kinetics that: (i) suggest details of docking/fusion site composition; and (ii) confirm that fully-docked vesicles are subject to different priming states and can undergo depriming – this may be the last priming step to establish full fusion competence of docked vesicles. Moreover, in line with long-held ideas, phosphatidylserine appears to act as a Ca^{2+} sensor and/or effector in fast, triggered fusion. In ongoing studies, the effects of selective Phospholipase D (PLD) and phosphatidic acid inhibitors are most pronounced in intact eggs and in a docking assay. Thus, PLD is likely localised at vesicle docking sites and local PA modulates fusion, particularly kinetics, *via* effects on docking. Overall, lipid modulation may be a key target for regulating the release mechanism in different secretory cell types.

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