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Fatty Acids Add Grease to Exocytosis

In this issue of *Chemistry & Biology*, Rickman and Davletov suggest a novel mechanism for biological lipids to regulate synaptic transmission in the brain. Physiologically relevant concentrations of arachidonic acid help to dissociate a protein complex involved in exocytosis.

Considerable progress has been made in recent years to characterize the gene cascades that orchestrate synaptic transmission in the miniscule subcellular specializations between nerve cells, called synapses. One central molecular principle that has emerged is the zippering up of so called SNARE-domains in proteins resident in the synaptic plasma membrane and the synaptic vesicles that contain neurotransmitters [1]. This is believed to drive exocytosis of the transmitter vesicles by generating a fusion pore in the lipid bilayers. Now the focus is shifting toward the mechanisms that set up and regulate these complexes and especially to a few other genes found to be essential for synaptic transmission, such as the *sec1/munc18* gene (SM-genes; for a review, see [2]), Munc18-1. SM-proteins bind syntaxins, a class of the SNARE proteins, and inhibit SNARE complex formation in vitro [3]. SM-genes are invariably important for membrane fusion processes in many species and intracellular trafficking routes, but an uncomfortable lack of consensus exists as to how these genes act, especially in relation to the SNARE genes. The interaction studies in vitro suggest that Munc18-1 inhibits exocytosis by reducing syntaxin availability, but some in vivo studies suggest the opposite [4, 5]. What the molecular functions of Munc18-1 are has been "the million dollar question" in this research field [6].

Rickman and Davletov now report that biological lipids, especially the fatty acid arachidonic acid, allow SNARE complexes to assemble in the presence of Munc18-1, while they do not under standard in vitro conditions (without lipids) [7]. This assembly in the presence of Munc18-1 can also be achieved by addition of phospholipase A2 (PLA2) to synaptic membranes. PLA2 is abundant in brain and hydrolyzes membrane phospholipids to release unsaturated fatty acids such as arachidonic acid. PLA2 is known to be regulated by many physiological stimuli like Ca^{2+}-influx [8]. This direct effect of arachidonic acid on the syntaxin1/Munc18 complex suggests a novel mechanism in the assembly of SNARE complexes and also sheds new light on the apparent discrepancies between observations in vitro and in vivo concerning *munc18* function. The negative effect of Munc18-1 on syntaxin availability may not be such an obstacle to form SNARE complexes in vivo as previously thought.

Traditionally, the main focus in synapse research has been on genes and proteins. The findings by Rickman and Davletov add to a number of recent discoveries that underline the important role of lipids (for a review, see [9]). It is becoming clear that many proteins implicated in exocytosis and endocytosis bind lipids, such as diacylglycerol, phosphatidylinositol phosphates, and cholesterol, and that in some cases these lipids have profound effects on the activity of the proteins. Munc18-1 and syntaxin are probably the first proteins involved in secretion identified to be directly influenced by arachidonic acid. It remains to be determined whether these proteins (or their dimers) actually interact directly with the fatty acid and whether other biological lipids like the ones mentioned above may also affect the Munc18-syntaxin interaction and SNARE complex formation.

Two recent findings indicated that phosphatidylinositol-4,5-bisphosphate ($PI_{4,5}P_2$) levels are particularly important for synaptic vesicle trafficking (for instance by regulating the releasable vesicle pool size) and that defined microdomains enriched in this lipid exist in secretory cells [10–12]. Cellular Munc18-1 levels are known to regulate this releasable pool as well [4]. Since arachidonic acid is two enzymatic steps away from $PI_{4,5}P_2$, it is conceivable that the effect of arachidonic acid on the munc18-syntaxin interaction is one of the mechanisms underlying this regulation.

Lipid modifying enzymes also influence secretion (for a review, see [9]). This suggests that the presence of lipids is not merely a background requirement for proper protein function, but that their levels are probably regulated locally and that this is a biological mechanism to regulate secretion. Phosphoinositide kinases and phospholipases A2, C, and D have now all been firmly established as such regulators, but often without identifying the underlying molecular mechanisms. Clearly, the weakening of the munc18-syntaxin interaction is now a prime candidate principle to explain such regulation.

Rickman and Davletov provide clear reasons to argue that arachidonic acid acts as a regulator of SNARE complexes and vesicle fusion by allowing SNARE complex formation in the presence of Munc18-1. It remains to be determined how important Munc18-syntaxin dissociation actually is for secretion. At least four arguments challenge this. First, syntaxin is far more abundant than Munc18-1 in nerve terminals, and the distribution of the two proteins may only partially overlap. No other proteins are known to prevent the free syntaxin molecules from engaging in SNARE complexes, and only few SNARE complexes are expected to be required for exocytosis. Second, replacement of syntaxin in nematodes (UNC64) by a syntaxin mutant with no apparent affinity for UNC18 produced animals with (almost) normal transmission [11]. Third, mutations in the yeast SM-protein Sly-1 that abolish its interaction with its cognate syntaxin are fully functional in vivo [13]. Fourth, several proposed functions of (m)unc18, for instance docking, do not necessarily require syntaxin [4, 5]. This debate underlines the need for new studies that address the interactions between presynaptic proteins

like Munc18-1 and syntaxin, and the sequence of events taking place prior to exocytosis. Clearly, the presence and regulation of lipids cannot be neglected.

Therefore, a main future challenge will be to try to test the biological relevance of lipid actions like the arachidonic acid effect in functional nerve terminals. What are their local concentrations in nerve terminals, and are these levels regulated and how and when? Clearly, the new million dollar question is how to study causal relationships between local lipid metabolism in the nerve terminal and exocytosis. This is a tremendous future challenge because current methodology is definitely incapable of addressing such specific questions. But undoubtedly, the physiological regulation of local lipid levels and their spatial organization are crucial new directions for neuroscience and cell biology, more broadly. And by addressing the new million dollar question, we may also obtain answers for the previous one.

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Selected Reading

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