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Characterization of SNARE interactions from the early endosomal compartment

The early endosomal compartment is a central relay station of intracellular trafficking. Material internalized from the plasma membrane is first directed to the early endosomes and then accordingly is recycled back to the plasma membrane or targeted to late endosomes and lysosomes for degradation. These organelles also receive input from the Golgi, serving as an intermediate station of secretory granules. Therefore, the membranes of the compartment should possess all the necessary molecules mediating fusion with various target membranes. The SNARE proteins constitute main components of the cellular fusion machinery. They are present in both of two opposing membranes and by their assembly in four helical bundles, they bring the membranes to fuse. Various SNARE isoforms have been identified within the early endosomal compartment, such as the early endosomal SNAREs, the exocytic and the late endosomal ones. It has been recently shown that the early endosomal SNAREs (syntaxin 6, syntaxin 13, vtla and vamp4), and not the exocytic or late endosomal ones, mediate the homotypic fusion of early endosomes (Brandhorst et al., 2006). In the present study, I investigated the possibility that SNAREs associate only with their cognate partners on the early endosomal membrane to ensure the specificity of their function.

I performed SNARE immunoprecipitation experiments using postnuclear supernatant from PC12 cells. This fraction is rich in early endosomes and free of nuclei and a significant fraction of the plasma membrane. I specifically immunoprecipitated the exocytic and early endosomal SNAREs and analyzed each precipitant for the existence of the other SNAREs. When I preincubated the PNS sample with an ATP-regenerating system, I observed associations between the exocytic SNAREs syntaxin 1, SNAP-25 and synaptobrevin. Depletion of ATP from my experimental system revealed numerous interactions, and, interestingly, some were detected between non-cognate partners. For example SNAP-25 was seen to interact with vamp4 or syntaxin 6, and synaptobrevin was shown to pull down the early endosomal SNAREs syntaxin 6 and 13. The strongest of these interactions prevailed when PNS was pre-incubated with the NSF-inhibitor NEM.

The immunoprecipitations were also performed in a PC12 fraction highly enriched in early endosomes, where I observed similar promiscuous SNARE interactions. Using a rat brain fraction enriched in synaptic vesicles to perform the same SNARE immunoprecipitations, I recorded associations between the exocytic proteins and an interaction between syntaxin 6 and syntaxin 1, when ATP was present. ATP depletion resulted in the detection of more non-cognate associations between early endosomal and exocytic SNAREs. In order to check the availability and possibility of endogenous SNAREs to interact with given SNAREs, epitope tagged endobrevin, vamp4 and synaptobrevin were exogenously added to PNS. Immunoprecipitation of the tagged proteins indicated that all three recombinant proteins were able to associate with exocytic and early endosomal SNAREs.

Summarizing, I conclude that the exocytic and early endosomal SNAREs can associate rather promiscuously in vivo. Their interactions seem to depend, at least to some level, on the enzymatic activity of NSF, a fact that suggests their biological significance: they could be part of fully-assembled SNARE complexes, sensitive to NSF uncoiling. Such interactions were verified in both a pure early endosomal fraction but also in a neuronal preparation enriched in synaptic vesicles. Promiscuity of SNARE associations was also demonstrated when all three recombinant SNAREs (a late endosomal, an early endosomal and a neuronal) interacted with the same early endosomal and exocytic endogenous SANRE isoforms.

Therefore, how is the functional separation of each set of SNAREs regulated when the proteins can easily interact with non-cognate partners? Regulatory proteins, specific for different fusion events, may recruit the SNAREs involved in the same process and specifically orient them to meet their cognate partners on the opposing membrane. A spatial organization of the members of each SNARE set to membrane microdomains may also explain the selective exclusion of non-cognate SNAREs to a fusion event, even though the
interactions between members of functionally separated SNARE sets do occur (possibly at
the borders of such microdomains). The actual mechanisms mediating the spatial and
temporal specificity of these intracellular fusion events remain to be addressed.