Syt I, as a major component of synaptic vesicles, is thought to interact with plasma membrane residues of the t-SNAREs syntaxin 1 and SNAP-25, and may regulate Ca^{2+}-dependent fusion by promoting SNARE assembly and fusion (5, 6, 21). Syntotagmins undergo Ca^{2+}-dependent homo- and heterooligomerization, which may potentially aid SNARE fusion by bringing opposing membrane and SNARE complexes in close proximity (3, 10, 22). Ca^{2+}-dependent fusion can be reconstituted in vitro by the addition of the cytoplasmic domain of syt I to an assay that measures SNARE-dependent fusion of liposomes (26). Strikingly, not only does the syt I cytoplasmic domain make the reaction Ca^{2+}-dependent, it also stimulates the rate of vesicle fusion, consistent with a role for syntotagmins in promoting the fusion reaction. Genetic experiments show that expression of mutant syt I in mice, Caenorhabditis elegans, or Drosophila melanogaster disrupts exocytosis of the rapidly releasable pool of synaptic vesicles (12, 15, 17, 29). Similarly, exocytosis of a release-ready pool of dense core vesicles is perturbed in chromaffin cells isolated from syt I-deficient mice (27). In all cases, significant Ca^{2+}-dependent exocytosis remains, but the rate of residual exocytosis is slower, and may reflect the utilization of other syt isoforms.

A widely used model system to study syt function is the rat pheochromocytoma PC12 cell line. These cells have a population of dense core granules that contain catecholamines, and express multiple syt isoforms including I, III, IV, VII, and IX (9, 16, 28). Syt I and IX are thought to be expressed at relatively high levels, whereas expression of the other isoforms is low and somewhat variable depending on the PC12 cell line. Evidence from mechanically perforated PC12 cells show that both syt III and syt VII C2A domains inhibit exocytosis (25). Syt IX may also play a significant role in dense-core vesicle exocytosis as antibodies to the syt IX C2A domain inhibits exocytosis in permeabilized PC12 cells (11), and downregulation of syt IX by interfering RNA (RNAi) impairs dense-core granule exocytosis (9). The function of syt I in PC12 cells is controversial because earlier studies indicate that it may be dispensable. Naturally occurring variant PC12 cell lines, which have low levels of syt I expression, still undergo significant Ca^{2+}-dependent exocytosis (9, 20). Furthermore, knockdown of syt I by transient RNAi expression has no impact on
exocytosis (9). In contrast to these findings, antibodies to syt I or isolated cytoplasmic domains of syt I block exocytosis of dense-core granule content (8, 11, 25).

The article by Moore et al. (Ref. 16, see p. C270 of this issue) revisits the question whether syt I expression is important for exocytosis of dense core granules in PC12 cells. The approach taken in this study was to generate stable PC12 cell lines that homogenously expressed short hairpin RNA (shRNA) to block expression of syt I by RNAi. The previous analysis by Fukuda (9) generated syt I knockdowns using transient transfection, which affected knockdown in only a fraction of the cells. The cell line used by Moore et al. has no detectable levels of Syt I and there is no effect on the expression of syts III, VII, or IX or of the SNAREs synaptobrevin 2, syntaxin 1, or SNAP-25. The knockdown cells show a normal rise in Ca\(^{2+}\) in response to KCl-mediated depolarization. With the use of amperometry, a sensitive tool that measures catecholamine release from individual cells by quantifying changes in voltage across a carbon-fiber electrode, the authors observed that the number of exocytic events and the amplitude of these events are significantly decreased. Furthermore, other parameters that measure catecholamine release (peak amplitude, rate of rise, quantal content, half width, and falling phase) are all significantly reduced in cells expressing syt I shRNA, consistent with changes in fusion pore opening. The decrease in exocytic events is rescued if cDNA encoding human syt I, which differs in 4/19 nucleotides encoded by the shRNA target region, is coexpressed in the syt I knockdown cells. Finally, the authors observe that the stimulated release of catecholamines, as measured by HPLC analysis, is also significantly reduced in syt I shRNA-expressing cells.

Besides prompting a reexamination of the role of syt I in PC12 cells, the study by Moore et al. (16) suggests an approach whereby stable cell lines could be made lacking each of the known syts expressed in PC12 cells. The systematic down-regulation of individual syts, coupled with careful amperometric analysis, would allow for a more defined analysis of syt function in dense-core secretory granule exocytosis. While other studies (9, 28) have used a similar approach, the use of stable cell lines may increase the likelihood of success. Furthermore, by reintroducing expression of the downregulated protein, or mutant versions of the protein, one can define the functional domains of syts in the absence of an endogenous pool of the wild-type protein. Such an approach has been successfully used in Drosophila (29). The work by Moore et al. is consistent with previous studies in that syt function is likely to be somewhat redundant, as residual Ca\(^{2+}\)-dependent exocytosis is always observed when individual syts are knocked down or inhibited by biochemical approaches. In the case of PC12 cells, syt IX is likely to be functional in the absence of syt I expression (9, 11). Silencing the expression of multiple syts may allow for a clearer picture of the involvement or redundancy of function of various syt proteins.

What is the purpose of having multiple syts expressed in one cell type? Beyond a simple mechanism that allows for redundancy, different syts may allow the cell to refine the cellular response to extracellular stimuli or regulate exocytosis of different populations of secretory vesicles. The C2 domains of syt VII bind divalent cations (Ba\(^{2+}\), Ca\(^{2+}\), Sr\(^{2+}\)) with a higher affinity than those of syt I or IX (28). While an increase in syt VII expression increases the metal sensitivity of exocytosis, a decrease in endogenous syt VII expression by RNAi results in a decrease in metal sensitivity (28). These results indicate that syt VII may modulate that metal requirement for exocytosis. The rates of syt dissociation from membranes may also have functional significance. There are three classes of syts that are characterized by distinct rates of dissociation from membranes when Ca\(^{2+}\) is chelated (13). Consistent with a role in fast synchronous release, syt I C2 domains release quickly, whereas syt VII C2 domains dissociate slowly (13). It is possible that syt members that are slow to release Ca\(^{2+}\) may account for the asynchronous slow release that occurs after the initial Ca\(^{2+}\) burst has subsided because they are slow to release Ca\(^{2+}\) . Localization of syts to different intracellular compartments may allow syts to regulate multiple exocytic events. For example, syt VII is not only localized to secretory granules, but also to secretory lysosomes, which undergo Ca\(^{2+}\)-regulated fusion in response to various extracellular stimuli, including cell damage (1). Finally, beyond their role in regulating exocytosis, syts also play an important role in the endocytic recovery of secretory vesicle content (18, 19). Thus syts are multifunctional proteins that may play divergent roles in multiple trafficking steps and pathways, and the use of RNAi, combined with techniques such as amperometry, allow for the function of syts to be carefully dissected and explored in a range of cell types.

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