of GABAergic or glycinergic signaling slightly increased the burst frequency of the Math1 null, but the effect was relatively mild. Other neuromodulators known to be expressed in Math1-dependent brainstem lineages, such as acetylcholine, corticotropin-releasing hormone (CRH), and nitric oxide (Rose et al., 2009a) had no effect. Although overlap between Math1 lineages and serotonergic neurons has not been demonstrated, given serotonin's known role in respiration, its effects on this preparation may be interesting to examine in future studies. Notably however, in contrast to their negative findings with other neuromodulators, application of the glutamate reuptake inhibitor dihydrokainic acid (DHK) caused the Math1 null preparation to exhibit a striking increase in rhythmic frequency to wild-type levels. Furthermore, the rhythmic pattern was also rescued to a large extent in the Math1 null preparation upon application of DHK. Thus, the authors conclude that the respiratory defects observed in the Math1 null animals are due to decreased glutamatergic signaling.

The insights into brainstem development and function provided by these studies are tantalizing (Rose et al., 2009a, and Rose et al., 2009b). However, given the large numbers of cell types that express Math1 in the brainstem, the precise correspondence between the cells expressing this protein and their roles in conscious proprioception, interoception, and respiration remains to be clarified. For instance, with regards to respiration specifically, it is still not clear which Math1-dependent lineage (or lineages) is critical for maintaining proper activity within the preBötC. Although increasing glutamatergic activity in the Math1 null brainstem preparation rescued the rhythmic activity, the authors did not detect any obvious changes in glutamatergic innervation of the preBötC in Math1 null animals. Thus, it seems likely that other excitatory circuits that regulate the activity of the preBötC are themselves critically dependent on Math1 lineages. In this regard, the unique role of Math1 in the development of the pFRG/RTN will be particularly interesting to explore. Happily, given the availability of a conditionally null *Math1* allele, these authors have at hand precisely the right tool to address these questions. Hence, we can all eagerly await for Math1 to take its next breath.

REFERENCES

Ben-Arie, N., Bellen, H.J., Armstrong, D.L., McCall, A.E., Gordadze, P.R., Guo, Q., Matzuk, M.M., and Zoghbi, H.Y. (1997). Nature *390*, 169–172.

Bermingham, N.A., Hassan, B.A., Price, S.D., Vollrath, M.A., Ben-Arie, N., Eatock, R.A., Bellen, H.J., Lysakowski, A., and Zoghbi, H.Y. (1999). Science *284*, 1837–1841.

Machold, R., and Fishell, G. (2005). Neuron *48*, 17–24.

Rose, M.F., Ahmad, K.A., Thaller, C., and Zoghbi, H.Y. (2009a). Proc. Natl. Acad. Sci. USA, in press.

Rose, M.F., Ren, J., Ahmad, K.A., Chao, H.-T., Klisch, T.J., Flora, A., Greer, J.J., and Zoghbi, H.Y. (2009b). Neuron *64*, this issue, 341–354.

Wang, V.Y., Rose, M.F., and Zoghbi, H.Y. (2005). Neuron *48*, 31–43.

Complexins Living Up to Their Name—New Light on Their Role in Exocytosis

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Ca^{2+}-dependent exocytosis of synaptic vesicles is mediated by the SNARE proteins synaptobrevin/VAMP, SNAP-25, and syntaxin. SNARE function is controlled by conserved regulatory proteins, including the complexins. In a study by Xue et al. in this issue of *Neuron*, contradictory data from *Drosophila* and mouse complexin mutants have been resolved, revealing a complex pattern of facilitatory and inhibitory domains.

When an action potential arrives in a nerve terminal, voltage-gated calcium channels open and calcium enters, triggering exocytosis of synaptic vesicles. The protein machinery mediating fusion of the vesicle with the plasma membrane includes the SNARE proteins as core components. Upon membrane contact, the SNAREs interact and form membrane-bridging *trans*-complexes. These complexes progressively assemble toward the membrane anchors in the vesicle and plasma membrane, respectively, forming an extended bundle of four intertwined α helices. The energy released during assembly is thought to overcome the energy barrier for fusion (Rizo and Rosenmund, 2008). SNAREs form a superfamily of conserved proteins, and thus SNARE assembly between membranes destined to fuse appears to be a common mechanism for intracellular fusion reactions (Kloepper et al., 2007). Synaptic exocytosis, however, is one of the most specialized

fusion reactions, because it is tightly regulated both spatially and temporally. Specific proteins have been identified that are responsible for this regulation, particularly for Ca^{2+}-mediated triggering. These include the vesicular Ca^{2+}-sensor synaptotagmin, the active zone protein Munc-13, and a small family of proteins termed complexins whose function is controversially discussed. Using elegant approaches, Rosenmund and colleagues have now resolved some of the discrepancies surrounding these proteins (Xue et al., 2009 [this issue of *Neuron*]).

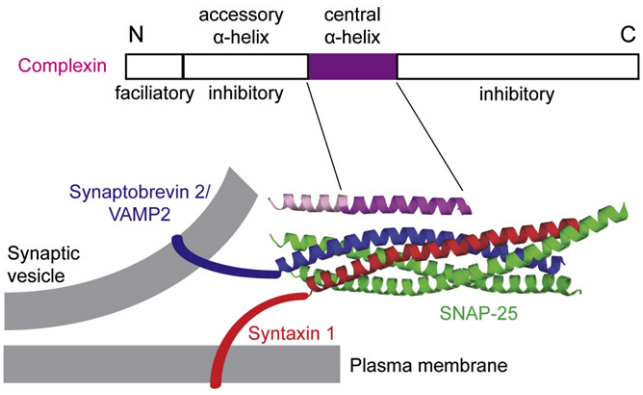

Figure 1. Domain Organization of Complexins
The effect of each domain on vesicle release is indicated. The bottom part shows the crystal structure of the central helix of complexin bound to the neuronal SNARE complex (structure model based on PDB ID: 1KIL [Chen et al., 2002]), depicted here as tight *trans*-complex.

Complexins are small cytoplasmic proteins of about 140 amino acids, with four members in mammals but only one member in *Drosophila* (for recent review see Brose, 2008). They were originally identified as interaction partners of the synaptic SNARE complex (McMahon et al., 1995), to which they bind with high affinity and fast kinetics. Structural analysis revealed that complexins possess a central α helix that binds in an antiparallel fashion to a groove in the central part of the helical SNARE bundle, formed by syntaxin 1 and synaptobrevin (Bracher et al., 2002; Chen et al., 2002) (see Figure 1). These findings were exciting, as they suggested that complexin may bind to the SNARE complex immediately before fusion, i.e., to a state in which the N-terminal part of the helical bundle has already formed, while the transmembrane anchors of syntaxin and synaptobrevin still reside in opposing membranes. This state is thought to be metastable and driven by synaptotagmin toward fusion upon calcium triggering.

The idea that complexins are late-acting regulatory proteins is supported by genetic evidence in both mouse and *Drosophila*, but so far it has been difficult to integrate the data into a coherent picture. In CNS neurons obtained from complexin knockout mice, both evoked and spontaneous release are reduced, with the Ca^{2+} sensitivity of evoked exocytosis being shifted toward higher concentrations (Reim et al., 2001; Xue et al., 2008). Thus, complexin appears to act as a facilitator of release, for instance, by pushing SNARE assembly toward completion. Rescue experiments showed that the facilitatory effect of complexin depends on the binding of the central helix to the SNARE complex (Xue et al., 2007). Studies involving complexin variants in which domains were chopped off showed that evoked release is facilitated by complexins' N terminus, whereas an accessory α helix N-terminally adjacent to the SNARE interacting region (see Figure 1) acts inhibitory (Maximov et al., 2009; Xue et al., 2007).

In contrast, a different picture was observed when the neuromuscular junction of *Drosophila* complexin null mutants was analyzed. Whereas—similar to the mouse knockouts—evoked release is diminished, spontaneous release is dramatically increased (Huntwork and Littleton, 2007). Intriguingly, the frequency of spontaneous release events is also increased in murine cortical neurons when complexins are knocked-down using small hairpin RNAs (Maximov et al., 2009). These data led to an alternative proposal for the function of complexin, namely that it acts as a SNARE clamp that prevents the SNARE machinery from progressing toward fusion, requiring Ca^{2+}-synaptotagmin to release the clamp. This view is supported by experiments in which cells are made to fuse by expressing "flipped" SNAREs on the surface of the plasma membrane. Here, the SNAREs act on the extracellular face of the plasma membrane, with none of the cofactors and regulators being available. Intriguingly, cell-cell fusion is inhibited when a membrane-anchored complexin is coexpressed, with synaptotagmin releasing the inhibition (Giraudo et al., 2006). Further experiments led to the suggestion that the accessory α helix of complexin may reach from the surface into the core of the SNARE complex and bind to the site that is normally occupied by the C terminus of synaptobrevin, thus preventing completion of assembly (Giraudo et al., 2009).

To shed light on the seemingly contradictory data, Xue et al. now studied the effects of introducing murine and fly complexins in either mouse or *Drosophila* synapses in cross-species rescue experiments (Xue et al., 2009). A complex picture of inhibitory and facilitatory effects has emerged that is different for each domain and varies between the species. The overall phenotype depends both on the strength of the individual effects of each of the species isoforms and on the type of synapse (fly or mouse) that appear to have different set points on which complexin acts. In comparison to the mouse variant, fly complexin appears to be primarily inhibitory. Consequently, expression of *Drosophila* complexin in wild-type murine synapses inhibits both spontaneous and evoked release, i.e., it dominates over the endogenous murine protein. Mutagenesis revealed that the inhibition is caused exclusively by the accessory helix and the C terminus. Furthermore, a facilitatory function of *Drosophila* complexins' N terminus was uncovered in elegant experiments using chimeric complexin constructs. A chimeric complexin with the N terminus derived from the fly protein and the rest from the mouse protein restores evoked release and leads to an increase of spontaneous release events exceeding that caused by the murine complexins' N terminus. The latter finding also shows that the impairment of evoked release in the fly null mutant represents

a true loss of function rather than a result of draining releasable vesicle pools via increased spontaneous release rates. Conversely, the facilitatory effects dominate when murine complexins are expressed in the *Drosophila* null mutant: spontaneous release is reduced but still higher than in wild-type flies. Evoked release, on the other hand, is increased even above the level observed in the wild-type.

What do these results tell us about the molecular function of complexins? Obviously, there is no easy answer, but this alone may teach us a lesson to be cautious with molecular models that are primarily derived from kinetic data obtained after perturbing synaptic proteins. Release kinetics only reports the final outcome of interfering with molecules in a complex multistep pathway. Thus, it needs to be borne in mind that the effect of proteins on the energy landscape of the fusion pathway (which defines the rate constants of each step [Sorensen, 2009]) may vary dependent on changes that are subtler than hitherto appreciated. For instance, the primed, release-ready state would represent an energy minimum, surrounded by maxima in the reverse (unpriming) and forward (fusion) direction. Depths and heights of energy minima and maxima are likely to be affected by many factors, such as membrane lipid composition, stability and number of *trans* SNARE complexes, local copy numbers of regulatory proteins, local membrane bending, and other factors. Proteins acting on SNAREs and membranes such as complexins contribute to the shape of this energy landscape. Furthermore, it is likely that minima and maxima are heterogeneous with respect to their molecular structure, creating a finer structure of the energy landscape. Thus, even a state that appears to be kinetically homogeneous may be represented by molecularly heterogeneous states. Probably, the fusion pathway is not represented by a deterministic linear sequence of steps but rather defined by an intricate network of different states. The removal of a single component from such complex network can result in release kinetics that cannot be easily explained by our still rudimentary knowledge of the underlying molecular structures and stoichiometries. Small differences in the state of this network in different species or synapses, caused, for example, by differences in affinities between homologous proteins, may thus explain why knock out of complexins leads to discrepant phenotypes although the release machinery is composed of conserved proteins.

In summary, the study by Rosenmund and colleagues reconciles seemingly contradictory findings on complexins in fly and mouse synapses by uncovering subtle changes with respect to the function of conserved domains. It documents that the molecular models of the primed state are still too simple for explaining release kinetics, and more work is needed to understand the molecular structures of the intermediate states along the fusion pathway.

REFERENCES

Bracher, A., Kadlec, J., Betz, H., and Weissenhorn, W. (2002). J. Biol. Chem. *277*, 26517–26523.

Brose, N. (2008). Traffic *9*, 1403–1413.

Chen, X., Tomchick, D.R., Kovrigin, E., Arac, D., Machius, M., Sudhof, T.C., and Rizo, J. (2002). Neuron *33*, 397–409.

Giraudo, C.G., Eng, W.S., Melia, T.J., and Rothman, J.E. (2006). Science *313*, 676–680.

Giraudo, C.G., Garcia-Diaz, A., Eng, W.S., Chen, Y., Hendrickson, W.A., Melia, T.J., and Rothman, J.E. (2009). Science *323*, 512–516.

Huntwork, S., and Littleton, J.T. (2007). Nat. Neurosci. *10*, 1235–1237.

Kloepper, T.H., Kienle, C.N., and Fasshauer, D. (2007). Mol. Biol. Cell *18*, 3463–3471.

Maximov, A., Tang, J., Yang, X., Pang, Z.P., and Sudhof, T.C. (2009). Science *323*, 516–521.

McMahon, H.T., Missler, M., Li, C., and Sudhof, T.C. (1995). Cell *83*, 111–119.

Reim, K., Mansour, M., Varoqueaux, F., McMahon, H.T., Sudhof, T.C., Brose, N., and Rosenmund, C. (2001). Cell *104*, 71–81.

Rizo, J., and Rosenmund, C. (2008). Nat. Struct. Mol. Biol. *15*, 665–674.

Sorensen, J.B. (2009). Annu. Rev. Cell. Dev. Biol. *25*, 513–537.

Xue, M., Reim, K., Chen, X., Chao, H.T., Deng, H., Rizo, J., Brose, N., and Rosenmund, C. (2007). Nat. Struct. Mol. Biol. *14*, 949–958.

Xue, M., Stradomska, A., Chen, H., Brose, N., Zhang, W., Rosenmund, C., and Reim, K. (2008). Proc. Natl. Acad. Sci. USA *105*, 7875–7880.

Xue, M., Lin, Y.Q., Pan, H., Reim, K., Deng, H., Bellen, H.J., and Rosenmund, C. (2009). Neuron *64*, this issue, 367–380.