Different strokes for different synaptotagmins

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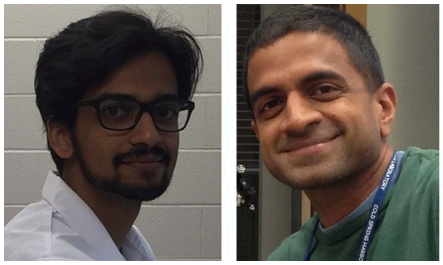
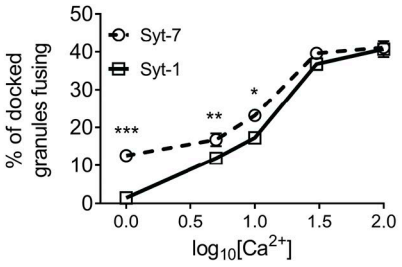
JGP study shows how synaptotagmins 1 and 7 affect chromaffin cell granule fusion.





Tejeshwar C. Rao (left), Arun Anantharam (right), and colleagues detail many differences in the behavior of secretory granules bearing Syt-1 and Syt-7, including how granule fusion is affected by rising intracellular calcium (see graph). PHOTOS COURTESY OF THE AUTHORS.

Exocytosis is a highly regulated process aimed at producing appropriate and proportional secretory responses to various stimuli. For example, in response to elevated intracellular calcium (Ca^{2+}), the chromaffin cells of the adrenal medulla secrete the neurotransmitter epinephrine (also known as adrenaline) into the bloodstream, along with several other neuropeptides and neurotransmitters. In their paper published this month in JGP, Rao et al. explore how different synaptotagmin isoforms help regulate secretory activity by chromaffin cells (1).

Secretion from chromaffin cells is conducted by a complex molecular machine, of which synaptotagmins are just one component. Synaptotagmins located on secretory granules have one end of the protein inserted into the granule lumen, and the other, containing a Ca^{2+}-binding domain, exposed to the cell cytoplasm. When Ca^{2+} flows into the cell through plasma membrane voltage-dependent Ca^{2+} channels, it binds to the synaptotagmin cytoplasmic domain, which helps drive granule fusion with the plasma membrane. Synaptotagmins were thus thought to simply be Ca^{2+} sensors that promote the random fusion of individual secretory granules with the plasma membrane. However, scientists are increasingly recognizing that synaptotagmins may instead direct some of the more complex features of granule secretion.

"I'm interested in the question of heterogeneity: what makes granules different from one another?" says Arun Anantharam, Assistant Professor at the University of Michigan. A 2014 study by Anantharam's group indicated that the two synaptotagmin isoforms expressed in chromaffin cells, synaptotagmin-1 (Syt-1) and Syt-7, are located on distinct populations of chromaffin secretory granules (2). This dovetails with other data showing differences among synaptotagmins. For example, Syt-7 has a much higher affinity for Ca^{2+} than does Syt-1 in vitro (3). These two isoforms are also thought to be involved in release of different populations of chromaffin secretory granules in PC12 cells (4, 5) and cultured neurons (6).

Anantharam's graduate student Tejeshwar Rao wanted to tease out more details about how Syt-1 and Syt-7 affect the behavior of their respective granules. Rao expressed fluorescently tagged Syt proteins in bovine chromaffin cells and used TIRF microscopy to observe granule fusion. Consistent with the in vitro data on the isoforms' Ca^{2+} sensitivity (3, 4), Rao et al. observed that Syt-7 granules began fusing at lower levels of cytoplasmic Ca^{2+} than did Syt-1 granules. At higher cellular Ca^{2+} levels, however, Syt-1 granules began fusing more rapidly and with higher probability than Syt-7 granules.

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The different granule populations also exhibited other behavioral differences. For example, whereas fusing Syt-7 granules were usually visible near the membrane before Ca^{2+} stimulation, Syt-1 granules often seemed to pop in from outside the field of view after Ca^{2+} levels increased. This implies Syt-1 granules may be more mobile, and may reside in a different space in the cell from Syt-7 granules. Furthermore, the researchers detected differences in the spatial distribution of granule fusion events between the two granule populations.

"Using special software we wrote, we found that Syt-7 granules have a tendency to fuse in clusters, whereas Syt-1 fusion sites are much more dispersed, almost random, on the chromaffin cell plasma membrane," notes Anantharam.

It is possible that chromaffin granules target molecularly and spatially distinct sites on the plasma membrane for fusion. For example, Syt-1 and Syt-7 could interact with different types of plasma membrane Ca^{2+} channels, which may be heterogeneously distributed across the membrane. Interestingly, Rao et al. observed that the fusion kinetics of Syt-1 and Syt-7 granules were differently sensitive to ω-conotoxin, a drug that blocks N/P/Q-type Ca^{2+} channels.

Collectively, these data suggest that the molecular identity of chromaffin granules may profoundly impact their fusion behavior. Anantharam is now keen to explore whether Syt-1 and Syt-7 granule populations might carry different contents and how synaptotagmins affect the physiology of chromaffin cells in situ in the adrenal medulla.

1. Rao, T.C., et al. 2017. *J. Gen. Physiol.* 149. http://dx.doi.org/10.1085/jgp.201711757
2. Rao, T.C., et al. 2014. *Mol. Biol. Cell.* 25:2416–2427.
3. Bhalla, A., et al. 2005. *Mol. Biol. Cell.* 16:4755–4764.
4. Sugita, S., et al. 2002. *EMBO J.* 21:270–280.
5. Zhang, Z., et al. 2011. *Mol. Biol. Cell.* 22:2324–2336.
6. Luo, F., et al. 2015. *J. Neurosci.* 35:11024–11033.

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