The Li et al. findings have important implications for the understanding of how human diets can shape disease susceptibility. Inflammatory bowel disease (IBD) is characterized by dysregulated immune responses to intestinal bacteria and is thought to be triggered in genetically susceptible individuals by environmental factors. It is strongly associated with a "Western" lifestyle, suggesting that diet may play a role in promoting the initiation or progression of the disease. In fact, epidemiological studies indicate that a diet low in fruit and vegetable intake is one risk factor for IBD (D'Souza et al., 2008). The findings of Li et al. suggest a mechanistic basis for this epidemiological link and offer the prospect of using AhR ligands as "nutriceuticals" to boost intestinal immunity.

REFERENCES

Cash, H.L., Whitham, C.V., Behrendt, C.L., and Hooper, L.V. (2006). Science *313*, 1126–1130.

Chen, Y., Chou, K., Fuchs, E., Havran, W.L., and Boismenu, R. (2002). Proc. Natl. Acad. Sci. USA *99*, 14338–14343.

D'Souza, S., Levy, E., Mack, D., Israel, D., Lambrette, P., Ghadirian, P., Deslandres, C., Morgan, K., Seidman, E.G., and Amre, D.K. (2008). Inflamm. Bowel Dis. *14*, 367–373.

Fernandez-Salguero, P., Pineau, T., Hilbert, D.M., McPhail, T., Lee, S.S., Kimura, S., Nebert, D.W., Rudikoff, S., Ward, J.M., and Gonzalez, F.J. (1995). Science *268*, 722–726.

Ismail, A.S., Severson, K.M., Vaishnava, S., Behrendt, C.L., Yu, X., Benjamin, J.L., Ruhn, K.A., Hou, B., DeFranco, A.L., Yarovinsky, F., and Hooper, L.V. (2011). Proc. Natl. Acad. Sci. USA *108*, 8743–8748.

Ito, S., Chen, C., Satoh, J., Yim, S., and Gonzalez, F.J. (2007). J. Clin. Invest. *117*, 1940–1950.

Kadow, S., Jux, B., Zahner, S.P., Wingerath, B., Chmill, S., Clausen, B.E., Hengstler, J., and Esser, C. (2011). J. Immunol. *187*, 3104–3110.

Li, Y., Innocentin, S., Withers, D.R., Roberts, N.A., Gallagher, A.R., Grigorieva, E.F., Wilhelm, C., and Veldhoen, M. (2011). Cell *147*, this issue, 629–640.

Quintana, F.J., Basso, A.S., Iglesias, A.H., Korn, T., Farez, M.F., Bettelli, E., Caccamo, M., Oukka, M., and Weiner, H.L. (2008). Nature *453*, 65–71.

Veldhoen, M., Hirota, K., Westendorf, A.M., Buer, J., Dumoutier, L., Renauld, J.-C., and Stockinger, B. (2008). Nature *453*, 106–109.

Dueling Ca^{2+} Sensors in Neurotransmitter Release

Mingshan Xue,[1,2] Nikolaos Giagtzoglou,[3] and Hugo J. Bellen[3,*]
[1]Neurobiology Section, Division of Biological Sciences
[2]Center for Neural Circuits and Behavior
University of California, San Diego, La Jolla, CA 92093, USA
[3]Program in Developmental Biology, Department of Molecular and Human Genetics and The Howard Hughes Medical Institute, Baylor College of Medicine, Houston, TX 77030, USA
*Correspondence: hbellen@bcm.edu
DOI 10.1016/j.cell.2011.10.007

Ca^{2+}-triggered neurotransmitter release is characterized by two kinetically distinct components: a fast synchronous phase and a slow asynchronous phase. Yao et al. (2011) now report that double C2 domain (Doc2) proteins function as high-affinity Ca^{2+} sensors to specifically regulate the asynchronous component of neurotransmitter release.

Chemical synaptic transmission in the nervous system results from the fusion of synaptic vesicles with the presynaptic plasma membrane, which causes release of the neurotransmitter stored within. Vesicle fusion can either be spontaneous or driven by action potentials. The latter, known as evoked release, is the primary means of neuronal communication. When an action potential invades presynaptic terminals, the elevated intracellular Ca^{2+} entering through voltage-gated calcium channels triggers what is known as synchronous neurotransmitter release, which is then followed by a phase of vesicle fusion known as asynchronous release. Although spontaneous release occurs in the absence of action potentials, it also depends, in part, on Ca^{2+}. How does the release apparatus detect the Ca^{2+} signal and translate it into vesicle fusion? What are the Ca^{2+} sensors for the different modes of release? In this issue, Yao et al. (2011) examine the function of double C2 domain (Doc2) proteins in vesicle fusion both in vitro and in vivo and provide evidence that Doc2 acts as a high-affinity Ca^{2+} sensor specifically for asynchronous release.

Ca^{2+}-evoked synchronous release and asynchronous release exhibit different properties. Synchronous release only occurs during and immediately following an action potential, whereas asynchronous release occurs over a longer period of time following the termination of an action potential. The distinct properties of synchronous and asynchronous release most likely reflect the existence of at least

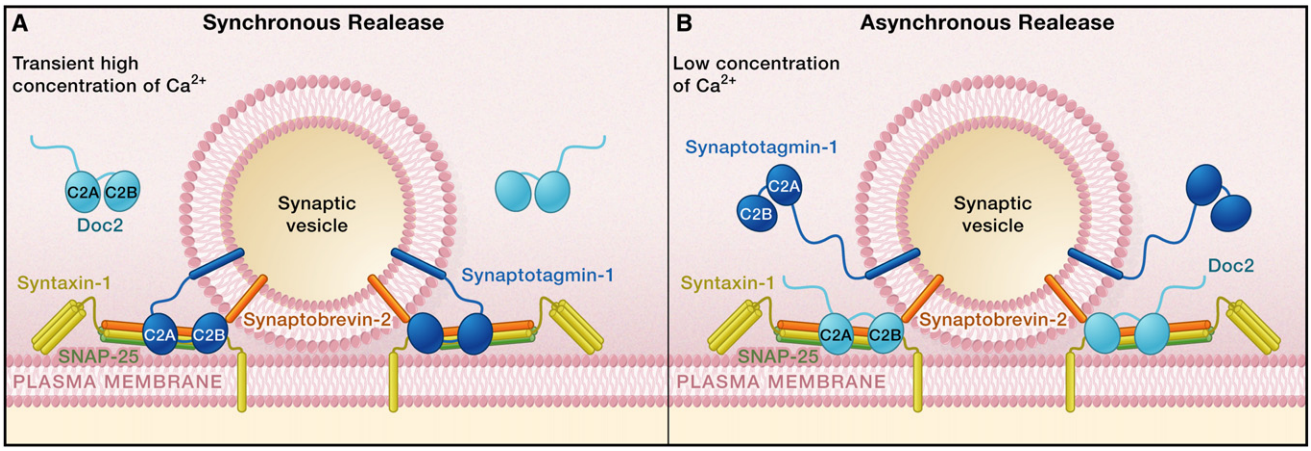

Figure 1. Ca^{2+} Sensors Synaptotagmin I and Doc2 Trigger Evoked Neurotransmitter Release

(A) Action potentials open voltage-gated calcium channels, resulting in a brief and high-concentration influx of Ca^{2+}. The C2A and C2B domains of synaptotagmin I bind to Ca^{2+} and interact with phospholipid membranes and SNAREs to trigger synchronous neurotransmitter release.

(B) Following the termination of action potentials, intracellular Ca^{2+} concentration decays to a much lower level. Doc2 binds to this residual Ca^{2+} and triggers asynchronous release, possibly through a mechanism similar to that of synaptotagmin I.

two different Ca^{2+} sensors that couple the Ca^{2+} signals to the vesicle fusion machinery with different kinetics and Ca^{2+} sensitivities (Goda and Stevens, 1994). A fast and low-affinity sensor triggers synchronous release in response to the localized, high concentration of Ca^{2+} that only briefly exists around the voltage-gated calcium channels during the action potential. This so-called Ca^{2+} microdomain, or nanodomains, quickly collapses due to diffusion and Ca^{2+} buffering after the calcium channels close, resulting in a much lower concentration of Ca^{2+}. A slow and perhaps high-affinity sensor continues to trigger asynchronous release in response to this residual Ca^{2+} signal. For the past two decades, numerous studies have demonstrated that synaptotagmin I is a Ca^{2+} sensor for synchronous release, functioning through its Ca^{2+}-dependent interaction with phospholipid membranes and soluble N-ethylmaleimide-sensitive factor attachment protein receptors (SNAREs) (Figure 1) (Rizo and Rosenmund, 2008). Other members of the synaptotagmin family have naturally become the prime candidates for the Ca^{2+} sensors of asynchronous release, but so far, no evidence supports this hypothesis.

Among a large number of Ca^{2+}-binding proteins is the Doc2 family (Doc2α, Doc2β, and Doc2γ), which contains two C2 domains (C2A and C2B) that are similar to those found in synaptotagmin I. The Doc2 proteins interact with phospholipids and some synaptic proteins involved in vesicle fusion, such as SNAREs, Munc18, and Munc13 (Friedrich et al., 2010). A recent study shows that Doc2 can stimulate SNARE-mediated fusion of reconstituted liposomes in a Ca^{2+}-dependent manner and that *Doc2α/β* double-knockout mice exhibit reduce spontaneous release (Groffen et al., 2010). When two Ca^{2+} ligands (aspartic acid residues) in the C2A domain are substituted with asparagines to resemble a dominant-active Ca^{2+}-bound state, this mutant Doc2 concurrently enhances spontaneous release. These results led to the proposal that Doc2 is a high-affinity Ca^{2+} sensor for spontaneous release (Groffen et al., 2010). Yao et al. (2011) now confirm the ability of Doc2 to stimulate SNARE-mediated membrane fusion. They further show that Doc2 binds to and dissociates from Ca^{2+} and phospholipid membranes more slowly than synaptotagmin I. Thus, the biochemical properties of Doc2 make it a good candidate for the Ca^{2+} sensor of asynchronous release.

Yao et al. test the in vivo function of Doc2 in neurotransmitter release by suppressing Doc2α expression using a short hairpin RNA (shRNA) in cultured mouse hippocampal excitatory neurons. To specifically assess the impact on asynchronous release, they take advantage of knockout neurons that lack *synaptotagmin I*, which display a specific deficit in synchronous release (Geppert et al., 1994), and find that suppressing Doc2α expression reduces asynchronous release. In wild-type neurons, knockdown of Doc2α selectively decreases asynchronous release without affecting synchronous release. This result is also confirmed in the Doc2α knockout neurons. Conversely, overexpression of wild-type Doc2 proteins in neurons lacking synaptotagmin I and in wild-type neurons causes a specific increase in asynchronous release. Yao et al. also mutated two residues involved in Ca^{2+} binding in each C2 domain to asparagines, and overexpression of this gain-of-function mutant in neurons lacking synaptotagmin I enhances asynchronous release more than overexpression of the wild-type protein. Thus, Doc2 levels bidirectionally regulate Ca^{2+}-evoked asynchronous release (Figure 1).

Is Doc2 a bona fide Ca^{2+} sensor for spontaneous release, evoked asynchronous release, or both? If so, loss-of-function mutations of the Ca^{2+}-binding residues in the Doc2 C2 domains should abolish its Ca^{2+} sensor function for spontaneous and asynchronous release. A recent study tested this prediction by mutating three residues that are important for Ca^{2+} binding in each C2 domain to

alanines to abolish Ca^{2+} binding. Unexpectedly, this mutant Doc2 fully rescues the decrease in spontaneous release induced by shRNA knockdown of Doc2 proteins in cultured mouse cortical inhibitory neurons, challenging the legitimacy of Doc2 as the Ca^{2+} sensor for spontaneous release (Pang et al., 2011). What is the effect of this mutant Doc2 in evoked asynchronous release? Unfortunately, Yao et al. do not report this. They also do not report how knockdown and overexpression of Doc2 affect the Ca^{2+} sensitivity of asynchronous release, another important parameter for assessing the Ca^{2+} sensor function.

Groffen et al. (2010), Pang et al. (2011), and Yao et al. (2011) all agree that Doc2 does not affect evoked synchronous release. Groffen et al. (2010) and Pang et al. (2011), however, claim that Doc2 is not involved in asynchronous release either. What could account for this discrepancy? The three studies were based on different experimental approaches. Yao et al. (2011) use both knockdown and knockout approaches, and the results are consistent with each other, providing substantial strength to the data. In Pang et al. (2011), the knockdown efficiency is measured from the entire neuronal culture, but inhibitory neurons constitute only a small fraction of the neuronal population. Hence, it is not obvious if the Doc2 proteins were sufficiently suppressed in inhibitory neurons. Although spontaneous release is reduced in these neurons, asynchronous release may have a different sensitivity to Doc2 reduction. For example, shRNA-mediated knockdown of complexins affects excitatory neurons, but not inhibitory neurons (Maximov et al., 2009), whereas a full genetic knockout has the same effects on both neuronal types (Xue et al., 2008). Alternatively, other Ca^{2+} sensors may compensate for the loss of Doc2 proteins in cortical neurons assayed by Pang et al. (2011). It is also possible that Doc2 is the Ca^{2+} sensor for asynchronous release in excitatory neurons, but not inhibitory neurons. Finally, it is not obvious why Groffen et al. (2010) did not observe a defect in asynchronous release.

The study by Yao et al. is important because it provides a promising candidate for the Ca^{2+} sensor of asynchronous release in many cell types and raises interesting questions about their mechanism of action. It also raises an issue with respect to their counterparts in invertebrates, given that Doc2 proteins are not evolutionarily conserved in many species (Craxton, 2010). Future work may test whether rabphilin, a conserved C2 domain-containing protein that shares a high degree of homology with Doc2 proteins, subserves this role in invertebrates.

REFERENCES

Craxton, M. (2010). BMC Genomics *11*, 37.

Friedrich, R., Yeheskel, A., and Ashery, U. (2010). Mol. Neurobiol. *41*, 42–51.

Geppert, M., Goda, Y., Hammer, R.E., Li, C., Rosahl, T.W., Stevens, C.F., and Südhof, T.C. (1994). Cell *79*, 717–727.

Goda, Y., and Stevens, C.F. (1994). Proc. Natl. Acad. Sci. USA *91*, 12942–12946.

Groffen, A.J., Martens, S., Díez Arazola, R., Cornelisse, L.N., Lozovaya, N., de Jong, A.P., Goriounova, N.A., Habets, R.L., Takai, Y., Borst, J.G., et al. (2010). Science *327*, 1614–1618.

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

Pang, Z.P., Bacaj, T., Yang, X., Zhou, P., Xu, W., and Südhof, T.C. (2011). Neuron *70*, 244–251.

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

Yao, J., Gaffaney, J.D., Kwon, S.E., and Chapman, E.R. (2011). Cell *147*, this issue, 666–677.

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

Two Routes for Remembering the Past

Wendy Suzuki[1,*] and Yuji Naya[1]

[1]Center for Neural Science, New York University, New York, NY 10003, USA

*Correspondence: ws21@nyu.edu

DOI 10.1016/j.cell.2011.10.005

Which brain circuits underlie retrieval of distant memories? Goshen et al. (2011) use a powerful optogenetic-based approach to reveal the critical contribution of the hippocampus to remote memory retrieval. In so doing, they provide new evidence toward resolving a long-standing debate in cognitive neuroscience.

The French psychologist T. Ribot was the first to note that there was something different about recent and remote memories (Ribot, 1881). Specifically, memory loss following brain injury tended to affect the remembrance of recent memories more than memories of the distant past. His observation suggested the possibility that memories might be reorganized over time. Findings from humans and animal models confirmed this idea, showing that damage to the hippocampus caused temporally graded memory deficits such that recall of information learned just before the time of hippocampal damage was severely impaired, whereas information learned in the remote past was remembered normally. This phenomenon,