Unraveling the role of zinc in memory

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Dietary zinc deficiency has been associated with memory impairment but the mechanisms underlying this effect remain unclear. The divalent cation zinc is one of the most abundant trace elements in the body and plays myriad functional roles (1). Although the vast majority of zinc is tightly bound to proteins, a pool of zinc in the mammalian forebrain is selectively stored in and released from glutamatergic neurons. This chelatable zinc is sequestered in synaptic vesicles and coreleased with glutamate during neuronal activity. Synaptically released zinc has the potential to interact with and modulate many different synaptic targets, including glutamate receptors and voltage-gated channels (2). Zinc can also modulate synaptic plasticity. The ability of zinc to modulate both ion channels and synaptic plasticity predicts that it plays a key role in learning and memory. This prediction was previously tested using mice in which the zinc transporter-3 (ZnT3) had been genetically deleted. ZnT3 is essential for loading zinc into synaptic vesicles (3). Targeted deletion of this transporter ablates vesicular zinc uptake and synaptic release of zinc and causes zinc in the forebrain to fall to undetectable levels (4). However, despite lacking chelatable zinc, ZnT3 KO mice did not exhibit impairments in spatial learning, memory, or sensorimotor functions (5). These findings suggested that vesicular zinc (and hence, ZnT3) was not essential for cognitive function. However, recent studies have forced a rethinking of the role of zinc in cognition. In particular, detailed examination has now revealed that ZnT3 KO mice exhibit impaired fear memory (6) as well as accelerated aging related decline of spatial memory (7). Despite these advances, the functional role of synaptic zinc in learning and memory remains largely a mystery. In PNAS, Sindreu et al. (8) provide a compelling and thorough study demonstrating an essential role for ZnT3 and thus vesicular zinc in memory formation in mice. Furthermore, they show that zinc and ZnT3 regulate memory formation by acting through the Erk1/2-dependent signaling pathway.

In examining the role of zinc in memory, Sindreu et al. (8) focus on the Erk signaling pathway of the MAPK family because of its well established role in the regulation of synaptic plasticity, learning, and memory (9). Furthermore, zinc can activate Erk in neurons (10), raising the possibility that zinc could influence learning and memory by acting on the Erk signaling pathway. However, earlier studies examining the interaction between zinc and Erk had used exogenously applied zinc, making it unclear whether synaptically released zinc acted as an endogenous regulator of Erk. Therefore, in the first set of experiments, Sindreu et al. (8) used ZnT3 KO mice to explore the role of endogenous zinc in Erk signaling. They found that the phosphorylated form of pErk (pErk) was selectively reduced in synaptic membranes from hippocampal mossy fibers (MFs) of ZnT3 KO mice. These studies were performed in the MFs because this pathway expresses high levels of zinc and is enriched in pErk. Their findings suggest that vesicular zinc is required to maintain normal levels of Erk activation. However, the possibility that developmental compensation in the ZnT3 KO mice was responsible for the loss of pErk could not be excluded. To address this limitation, Sindreu et al. (8) demonstrated that ZnT3 KO mice exhibit normal expression of Erk protein, normal development of MFs, and normal density of MF boutons. The authors then show that acute injection of the zinc chelators diethyldithiocarbamate or N,N,N′,N′-tetrakis(2-pyridylmethyl)ethylenediamine (TPEN) decreased the levels of MF pErk in WT mice, reproducing the pErk phenotype of ZnT3 KO mice. Together, these studies elegantly show that endogenous zinc (and ZnT3) is necessary for Erk activation.

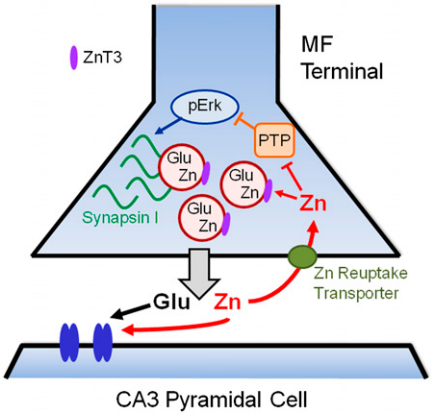

Fig. 1. ZnT3 and zinc in MF synaptic function. Synaptic vesicles in MF terminals contain both glutamate and zinc that is released upon MF activity. Glutamate acts on postsynaptic receptors on CA3 pyramidal cells. Zinc can modulate activity at some glutamate receptors and is recycled into the presynaptic terminal by reuptake transporters of the SLC39 family. Zinc is repackaged into synaptic vesicles through the action of ZnT3. Cytoplasmic zinc inhibits PTP, thereby preventing dephosphorylation of pErk and prolonging pErk activity. Among its actions, pErk phosphorylates synapsin I, thereby regulating synaptic plasticity. The absence of ZnT3 leads to a loss of zinc from the terminal, removal of zinc inhibition of tyrosine phosphatase, and a reduction of Erk signaling.

Is extracellular release of zinc necessary for modulation of pErk? A requirement for extracellular release of vesicular zinc would tie pErk activation to neuronal activity of the MFs. Sindreu et al. (8) examine this possibility by assaying MF pErk after toxin-induced blockade of vesicular exocytosis in vivo. They hypothesized that, if released zinc were necessary for pErk activation, blockade of vesicular exocytosis at MF synapses should reduce MF pErk. This is precisely what they observed. Furthermore, in ZnT3 KO mice in which zinc exocytosis is ablated, toxin block of exocytosis had little additional effect on MF pErk. These experiments demonstrate a critical role for zinc exocytosis and ZnT3 in the regulation of MF pErk.

What is the mechanism through which pErk is reduced in ZnT3 KO mice? Sindreu et al. (8) show in ZnT3 KO mice that activation of a host of other signaling molecules implicated in synaptic plasticity is unaffected, indicating that Erk signaling is special in its sensitivity to ZnT3. Importantly, they found that phosphorylation of the MAPK kinase MEK1/2 was intact in ZnT3 KO mice, despite a reduction of pErk in the same animals. As MEK1/2 is the immediate upstream kinase for Erk, this finding precludes changes upstream of Erk in ZnT3 KO mice. Furthermore, in an in vitro kinase assay, zinc failed to stimulate Erk2 activity. Together, these findings point to an indirect action of zinc on Erk. One possible indirect mechanism through which zinc could enhance pErk is by inhibiting its dephosphorylation by protein tyrosine phosphatases (PTPs). PTPs terminate Erk activity by dephosphorylation, and it is well established that zinc inhibits the activity of these PTPs (11). Sindreu et al. (8) report that

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dephosphorylation of Erk2 in vitro is faster in ZnT3 KO lysates and that addition of zinc slows dephosphorylation. These findings provide an elegant demonstration that endogenous zinc enhances Erk activation by inhibiting tyrosine phosphatases. In ZnT3 KO mice, the lack of zinc disinhibits these tyrosine phosphatases, thereby suppressing Erk activity (Fig. 1).

The ability of synaptically released zinc to inhibit PTPs and enhance Erk phosphorylation predicts that increased activity at MFs will lead to increased Erk activation. The Erk signaling pathway plays a crucial role in the regulation of activity-dependent changes in the strength of synaptic transmission (9). Erk signaling modulates synaptic plasticity through a variety of post- and presynaptic mechanisms. Stimulation of Erk in presynaptic terminals regulates synaptic plasticity through a mechanism involving phosphorylation of synapsin I (12). Furthermore, in behavioral studies Erk is necessary for the development of several forms of memory, including fear conditioning and spatial memory (9). Given that Erk-dependent synaptic plasticity is essential for cognitive processes, the ability of zinc and ZnT3 to regulate Erk activation predicts cognitive deficits in ZnT3 KO mice.

To determine if such cognitive deficits exist, Sindreu et al. (8) examine spatial working memory and contextual discrimination memory in ZnT3 KO mice. These forms of memory are dependent on intact hippocampal function. Whereas WT mice learned the behavioral tasks quickly, ZnT3 KO mice were severely impaired. In contrast, ZnT3 KO mice were not impaired in behavioral tasks that were not hippocampal-dependent. These important findings show the critical role of ZnT3 in hippocampal-dependent memory. However, it is possible that changes in behavioral performance of ZnT3 KO mice are caused by developmental alterations. Sindreu et al. (8) address this problem by demonstrating that TPEN infusion into CA3, but not CA1, of WT mice impaired contextual discrimination memory, reproducing the ZnT3 KO phenotype. These findings provide strong evidence that a loss of synaptic zinc in ZnT3 KO mice is responsible for the observed cognitive deficits. However, they do not reveal whether these cognitive deficits are produced through regulation of Erk signaling.

Zinc and ZnT3 regulate memory formation by acting through the Erk1/2-dependent signaling pathway.

To address this issue, Sindreu et al. (8) demonstrate that Erk activation in CA3 MFs is increased following contextual fear conditioning. As predicted by their findings in vitro, this increase in pErk is absent in ZnT3 KO mice as well as in WT mice that received a TPEN infusion into CA3. Together, these findings indicate that ZnT3 and zinc are required for Erk activation in vivo. The increase in Erk activity in CA3 occurred presynaptically as indicated by an increase in phosphorylation of synapsin I in WT, but not ZnT3 KO, mice after training. To further demonstrate a role for Erk signaling in contextual discrimination, Sindreu et al. (8) carry out two additional experiments. First, they show that pharmacological inhibition of MEK1/2 blocked the ability of WT mice to learn a contextual discrimination task. Second, they find that knockdown of MEK1 by injection into the dorsal dentate gyrus of a lentivirus expressing a dominant-negative form of MEK1 interfered with learning of the contextual discrimination task. Mice injected with a control lentiviral vector learned the task as usual. Because the only proven substrate of MEK1/2 is Erk (13), these findings together strongly argue that Erk signaling in granule cells is required for contextual discrimination.

Sindreu et al. (8) provide a compelling demonstration that zinc and zinc recycling are required for spatial working memory and contextual discrimination memory. Building on previous discoveries, they elegantly demonstrate that zinc affects these forms of learning by acting presynaptically in hippocampal MFs to inhibit PTPs, thereby enhancing pErk signaling. These findings are an important step in our efforts to understand the physiological roles of zinc in the brain. They also raise additional questions. For example, zinc regulates a number of postsynaptic targets, including NMDA (2) and kainate receptors (14). Results of this study (8) demonstrate that the effects of zinc on certain cognitive tasks can be explained by a presynaptic effect on Erk signaling. What, then, is the role of zinc on its other targets in cognition? Perhaps inhibition of postsynaptic NMDA or kainate receptors by zinc regulates other forms of learning. Additionally, results in this study indicate that the effects of zinc on Erk signaling are specific for the MF pathway. However, zinc is found throughout the forebrain and has been shown to affect other non–hippocampal-dependent forms of learning. To what extent do the findings reported in this study (8) generalize to other brain regions or forms of learning? Answering these questions will require multidisciplinary approaches similar to those reported by Sindreu et al. (8) and will be essential for further progress in understanding the functional role of zinc in the brain.

1. Frederickson CJ, Koh JY, Bush AI (2005) The neurobiology of zinc in health and disease. *Nat Rev Neurosci* 6: 449–462.
2. Paoletti P, Vergnano AM, Barbour B, Casado M (2009) Zinc at glutamatergic synapses. *Neuroscience* 158:126–136.
3. Palmiter RD, Cole TB, Quaife CJ, Findley SD (1996) ZnT-3, a putative transporter of zinc into synaptic vesicles. *Proc Natl Acad Sci USA* 93:14934–14939.
4. Cole TB, Wenzel HJ, Kafer KE, Schwartzkroin PA, Palmiter RD (1999) Elimination of zinc from synaptic vesicles in the intact mouse brain by disruption of the ZnT3 gene. *Proc Natl Acad Sci USA* 96:1716–1721.
5. Cole TB, Martyanova A, Palmiter RD (2001) Removing zinc from synaptic vesicles does not impair spatial learning, memory, or sensorimotor functions in the mouse. *Brain Res* 891:253–265.
6. Martel G, Hevi C, Friebely O, Baybutt T, Shumyatsky GP (2010) Zinc transporter 3 is involved in learned fear and extinction, but not in innate fear. *Learn Mem* 17: 582–590.
7. Adlard PA, Parncutt JM, Finkelstein DI, Bush AI (2010) Cognitive loss in zinc transporter-3 knockout mice: a phenocopy for the synaptic and memory deficits of Alzheimer's disease? *J Neurosci* 30: 1631–1636.
8. Sindreu C, Palmiter RD, Storm DR (2011) Zinc transporter ZnT-3 regulates presynaptic Erk1/2 signaling and hippocampus-dependent memory. *Proc Natl Acad Sci USA* 108:3366–3370.
9. Adams JP, Sweatt JD (2002) Molecular psychology: Roles for the ERK MAP kinase cascade in memory. *Annu Rev Pharmacol Toxicol* 42:135–163.
10. Besser L, et al. (2009) Synaptically released zinc triggers metabotropic signaling via a zinc-sensing receptor in the hippocampus. *J Neurosci* 29:2890–2901.
11. Haase H, Maret W (2003) Intracellular zinc fluctuations modulate protein tyrosine phosphatase activity in insulin/insulin-like growth factor-1 signaling. *Exp Cell Res* 291:289–298.
12. Kushner SA, et al. (2005) Modulation of presynaptic plasticity and learning by the H-ras/extracellular signal-regulated kinase/synapsin I signaling pathway. *J Neurosci* 25:9721–9734.
13. Wimmer R, Baccarini M (2010) Partner exchange: Protein-protein interactions in the Raf pathway. *Trends Biochem Sci* 35:660–668.
14. Mott DD, Benveniste M, Dingledine RJ (2008) pH-dependent inhibition of kainate receptors by zinc. *J Neurosci* 28:1659–1671.