doi:10.1038/nature11850

NEUROSCIENCE

Memory and the single molecule

Sustained activity of the brain-specific enzyme PKM-ζ is thought to underlie the maintenance of long-term memories. Studies in PKM-ζ-deficient mice, however, cast the importance of this protein into question.

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Memory traces are thought to be formed by the strengthening of synaptic connections between particular collections of neurons. A major goal of neuroscience research is to identify the molecular machinery that sustains these strengthened connections once they are formed. Many molecules that were once thought to have starring roles in memory have since been relegated to supporting, albeit still important, roles. Two papers published on *Nature*'s website today (Volk *et al.*[1] and Lee *et al.*[2]) challenge the star status of yet another memory molecule — protein kinase M-ζ.

The human genome encodes more than 500 protein kinase enzymes, and many of these are known to mediate memory formation. Protein kinase M-ζ (PKM-ζ) is special in that it is proposed to maintain the enhanced synaptic strength associated with memory formation — unlike most other kinases, which are involved in synaptic strengthening per se. Moreover, in contrast to other kinases, PKM-ζ is always active, a perfect feature for a molecule that retains memory. It has therefore been considered truly deserving of 'memory molecule' billing.

One of the first hints of the involvement of PKM-ζ in sustaining synaptic strength came from a study[3] in which the function of the enzyme was disrupted after induction of long-term potentiation (LTP, a form of synaptic strengthening that is often used as a cellular proxy for memory formation) in the brain's hippocampus region. This investigation found that both an inhibitory form of PKM-ζ and a pharmacological inhibitor of this protein called ZIP reversed previously established LTP. (ZIP stands for zeta inhibitory peptide and is a 13-amino-acid sequence thought to mimic the natural substrate that turns PKM-ζ off.)

This finding received considerable attention, but the true red-carpet moment for PKM-ζ came when another paper[4] showed that intrahippocampal microinjections of ZIP — but not of a scrambled ZIP peptide that did not affect PKM-ζ and so was used as a control — reversed not only established LTP but also an established memory in rats. That disrupting the function of a molecule could erase a memory created a great buzz and sparked numerous sequels. Indeed, microinjection of ZIP into many different brain regions was subsequently shown to disrupt established spatial, fear, appetitive, habit and sensorimotor memories in rodents and sensitization memory in the sea slug *Aplysia*[5].

Using different strategies to genetically delete PKM-ζ, Volk *et al.* and Lee *et al.* now challenge the conclusions of these previous studies. They question both the specificity of ZIP and the importance of PKM-ζ in maintaining LTP and memory. Volk and colleagues created a line of mice in which *Pkm-ζ* and a closely related gene, *Pkc-ζ*, were deleted throughout the animal in embryonic development. Despite having no PKM-ζ, these mice seemed normal, and their hippocampal LTP was indistinguishable both in magnitude and duration from LTP in normal mice.

> ***"Asking whether PKM-ζ could really be 'the' memory molecule is probably not the right question."***

Because absence of a gene since early development may result in compensation for its function by other genes, the authors also created a line of mice in which PKM-ζ could be deleted specifically in excitatory forebrain neurons in adult animals. These mice similarly showed normal LTP. The authors went on to perform a clever experiment in which they examined the effects of ZIP on maintenance of LTP in mice that entirely lacked PKM-ζ. Remarkably, ZIP (and scrambled ZIP), at doses used in previous experiments, disrupted established LTP not only in normal mice, but also in PKM-ζ-deficient mice. This result indicates that ZIP disrupts established LTP by PKM-ζ-independent mechanisms and brings into question the role of PKM-ζ in the maintenance of LTP.

Lee *et al.* also created mice that lacked PKM-ζ from early in development and, using an exhaustive battery of behavioural tests, probed their memory. The mice could form persistent memories in the various tests — an observation that is independently reported by Volk and co-workers. Lee *et al.* also examined the effects of ZIP on existing memories. For this, they microinjected ZIP into the brain of PKM-ζ knockout mice after formation of a place memory. As a striking counterpart to the LTP results of Volk *et al.*, ZIP erased memory in mice lacking PKM-ζ. Although the specificity of ZIP (and of scrambled ZIP) has been debated[6,7] and the jury is still out on the precise molecular targets of these peptides, these data convincingly show that ZIP disrupts both established LTP and memory independently of PKM-ζ.

These results, however, do not entirely exclude the possibility that PKM-ζ is a key player in memory maintenance. They do not account for two previous studies in which genetic tools were used to manipulate PKM-ζ function following learning. One of these studies[8] found that acute expression of a mouse PKM-ζ transgene in the fruitfly *Drosophila* 30 minutes after training enhanced odour memory measured one or four days after training. The second study[9] showed that microinjecting a lentivirus expressing PKM-ζ into the brain of rats six days after training enhanced a taste-aversion memory. It also found that disrupting PKM-ζ function by inducing expression of an inhibitory form of the enzyme days after training was sufficient to erase an established taste-aversion memory.

How might these findings[1,2,8,9] be reconciled? Hundreds of molecules are probably involved in the formation and maintenance of memory, and within this 'mnemome' there may be a fair degree of redundancy and degeneracy — whereby related molecules compensate for each others' deficiency[10]. Such mechanisms could make up for long-term deficiency of a single molecule, allowing formation of stable memories. Acute, post-training manipulations may reduce the likelihood of this type of compensation but might not eliminate it.

So asking whether PKM-ζ could really be 'the' memory molecule is probably not the right question, as it fails to capture the complexity of the interactions between the molecules within the mnemome. Given evolution's penchant for redundancy, it seems unlikely that any single molecule will play this part solo. Rather, the question should be one of degree — is a molecule a lead player (and therefore irreplaceable) or does it have more of a supporting role? The casting debate on PKM-ζ will, no doubt, continue. ■

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1. Volk, L. J., Bachman, J. L., Johnson, R., Yu, Y. & Huganir, R. L. *Nature* http://dx.doi.org/10.1038/nature11802 (2013).
2. Lee, A. M. *et al. Nature* http://dx.doi.org/10.1038/nature11803 (2013).
3. Ling, D. S. F. *et al. Nature Neurosci.* **5,** 295–296 (2002).
4. Pastalkova, E. *et al. Science* **313,** 1141–1144 (2006).
5. Sacktor, T. C. *Nature Rev. Neurosci.* **12,** 9–15 (2011).
6. Wu-Zhang, A. X., Schramm, C. L., Nabavi, S., Malinow, R. & Newton, A. C. *J. Biol. Chem.* **287,** 12879–12885 (2012).
7. Yao, Y. *et al. Neuropharmacology* **64,** 37–44 (2013).
8. Drier, E. A. *et al. Nature Neurosci.* **5,** 316–324 (2002).
9. Shema, R. *et al. Science* **331,** 1207–1210 (2011).
10. Sanes, J. R. & Lichtman, J. W. *Nature Neurosci.* **2,** 597–604 (1999).