ment of the photo-switchable ligand and the empirical demonstration that the modification leads to biologically relevant readout *ex vivo* and *in vivo*. Levitz *et al.*[1] confirmed the power of using tethered photo-switchable ligands to control cell surface protein activity and control neuronal excitability without directly modifying ion channels themselves. Because the authors exploited native proteins that were only slightly modified, these tools are optimized to perfectly mimic the proteins' natural coupling to the intracellular signal transduction pathways, as the modified GPCR should behave similarly to the endogenous GPCR in terms of its binding and signaling partners and intracellular trafficking. This concept is well illustrated by the success of the light-antagonized LimGluR2block in interfering with the function of the endogenous intracellular signaling pathway downstream of mGluR2. There is no doubt that such tools will help link precise molecular events to behavior and help propose better strategies for disease treatments given the diversity of GPCRs in the mammalian genome.

COMPETING FINANCIAL INTERESTS
The author declares no competing financial interests.

1. Levitz, J. *et al. Nat. Neurosci.* **16**, 507–516 (2013).
2. Coward, P. *et al. Proc. Natl. Acad. Sci. USA* **95**, 352–357 (1998).
3. Zhao, G.Q. *et al. Cell* **115**, 255–266 (2003).
4. Armbruster, B.N. *et al. Proc. Natl. Acad. Sci. USA* **104**, 5163–5168 (2007).
5. Rogan, S.C. & Roth, B.L. *Pharmacol. Rev.* **63**, 291–315 (2011).
6. Garner, A.R. *et al. Science* **335**, 1513–1516 (2012).
7. Tye, K.M. & Deisseroth, K. *Nat. Rev. Neurosci.* **13**, 251–266 (2012).
8. Airan, R.D. *et al. Nature* **458**, 1025–1029 (2009).
9. Volgraf, M. *et al. Nat. Chem. Biol.* **2**, 47–52 (2006).
10. Gorostiza, P. & Isacoff, E.Y. *Science* **322**, 395–399 (2008).
11. Wyart, C. *et al. Nature* **461**, 407–410 (2009).
12. Nicoletti, F. *et al. Neuropharmacology* **60**, 1017–1041 (2011).
13. Choi, S. & Lovinger, D.M. *J. Neurosci.* **16**, 36–45 (1996).
14. Tochitsky, I. *et al. Nat. Chem.* **4**, 105–111 (2012).
15. Sandoz, G. *et al. Neuron* **74**, 1005–1014 (2012).

mTORC2: actin on your memory

Sheena A Josselyn & Paul W Frankland

To be become long-lasting, short-term memories must be transformed into more permanent forms. mTORC2 has now been found to be crucial for the molecular reorganization of the cytoskeleton needed for memory consolidation.

How do you make a memory? The formation of a long-term memory is thought to involve at least two ingredients. First, it requires the strengthening of synaptic connections between neurons, a process that depends on protein synthesis. Second, it also requires changes in the physical structure of the synaptic connections, a process that depends on actin cytoskeleton rearrangement. Most studies have focused on the role of protein synthesis in memory formation; much less is known about actin polymerization and memory. Although protein synthesis and actin cytoskeleton rearrangement must be intimately intertwined, even less is known about how these necessary memory processes interact at the molecular level. A study by Huang *et al.*[1] in this issue of *Nature Neuroscience* reveals that mTORC2 (mammalian target of rapamycin complex 2) positively regulates the actin dynamics required for the formation of long-term memories (**Fig. 1**) and also suggests that actin polymerization is upstream of protein synthesis.

mTOR (mammalian target of rapamycin) is a serine-threonine protein kinase integral to signal transduction pathways that control cell growth and survival, translation, autophagy and cytoskeleton organization[2]. It serves as an intracellular sensor for energy metabolism, nutrient availability and stresses, regulating cellular and organism growth and metabolism to adapt to environmental changes. The best studied and understood function of mTOR is the regulation of protein translation. mTOR forms two distinct multiprotein complexes, which are distinguished by their accessory proteins. The mTOR complex 1 (mTORC1) contains the accessory protein raptor (regulatory-associated protein of mTOR) and is sensitive to the drug rapamycin. By contrast, mTORC2 contains rictor (rapamycin-insensitive companion of mTOR) and is resistant to acute rapamycin treatment[3–5]. Not only do these mTOR complexes differ in their sensitivity to rapamycin, but they also show functional differences. mTORC1 is well characterized and is known to positively regulate protein translation necessary for long-term synaptic plasticity and memory formation[6,7]. In contrast, relatively little is known about the overall function of mTORC2, let alone its role in synaptic plasticity and memory. Hints from other species and tissues suggest that mTORC2, through its defining component rictor, controls the actin cytoskeleton[5,8] and therefore might regulate some aspects of the structural plasticity required for memory formation. By targeting rictor, Huang *et al.*[1] identified an important function of this complex in regulating both long-term synaptic plasticity and long-term memory formation.

Because rictor is important during brain development, Huang *et al.*[1] developed a conditional knockout mouse in which rictor was deleted only from postnatal forebrain excitatory neurons. These rictor-deficient mice showed disrupted mTORC2, but intact mTORC1, activity. The authors first used these mice to examine the effects on synaptic plasticity of deleting mTORC2. Long-term potentiation (LTP) of excitatory synaptic responses is a commonly used electrophysiological correlate of memory formation. In the CA1 region of the hippocampus, weak tetanization (for example, one train at 100 Hz) induces early phase-LTP (E-LTP), which, as its name implies, decays over time. In contrast, stronger tetanization (for example, four trains at 100 Hz) induces late phase-LTP (L-LTP), a form of LTP that lasts for several hours. The conversion of E-LTP into L-LTP requires both protein synthesis and actin cytoskeletal rearrangement[9,10]. Actin is the main structural component of dendritic spines, and its rearrangement via polymerization is critical for L-LTP (but not E-LTP). Mice with a forebrain deletion of rictor showed normal basal synaptic transmission and E-LTP. However, this E-LTP could not be converted to L-LTP.

Of course, studies of LTP and synaptic physiology greatly inform our understanding of the mechanistic recipe for memory formation, but the real proof is in the pudding. In short, do these mTORC2-deficient mice show impaired long-term memory? Previous studies have shown that actin polymerization is required for the conversion of a weak short-term memory into long-term memory[10]. To examine mTORC2 in memory, the authors

Sheena A. Josselyn and Paul W. Frankland are in the Program in Neurosciences & Mental Health at The Hospital for Sick Children, and in the Departments of Psychology and Physiology and the Institute of Medical Science, University of Toronto, Toronto, Ontario, Canada.
e-mail: sheena.josselyn@sickkids.ca

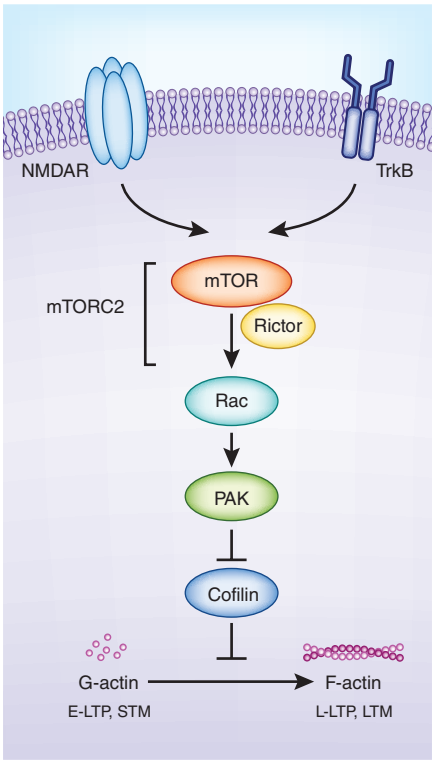

Figure 1 mTORC2 regulates actin dynamics to form long-term memories. The mTOR complex 2 (mTORC2) is composed of mTOR and rictor (rapamycin-insensitive companion of mTOR), among other accessory proteins. Several signaling pathways (for example, Ca^{2+} acting through NMDA-type glutamate receptors (NMDAR), or brain-derived neurotrophic factor (BDNF) acting through TrkB receptors) activate mTORC2, which in turn acts through Rac, PAK and cofilin to promote the actin polymerization required for the conversion of early LTP (E-LTP) into late LTP (L-LTP) and short-term memory (STM) into long-term memory (LTM).

tested rictor-deficient mice in several different tasks that differ in their training schedule and performance demands. Classical fear conditioning is a memory model in which a tone is paired with a shock in a specific context. Rictor-deficient mice showed normal memory for both the context and tone when tested 2 h after training, indicating that their short-term memory was intact. However, when tested 24 h after training, they showed impaired memory for both the context and tone. These behavioral results parallel the synaptic physiology data: similarly to the failure to convert E-LTP to L-LTP, these mice seemed unable to convert a short-term memory into a long-term memory. Consistent with this conclusion, Huang *et al.*[1] went on to examine spatial memory formation in rictor-deficient mice using the Morris water maze. In this model, mice learn to find a hidden platform submerged under the surface of an opaque liquid by using spatial cues around the room. Training takes place over several days, and therefore mice need to remember from one day to the next. Again, rictor-deficient mice were unable to form a stable spatial memory.

Many foundational studies of the molecular basis of long-term memory were conducted in invertebrates, including *Drosophila melanogaster*[11,12]. Although the results of fly studies have certainly informed rodent studies (and vice versa), these research streams have often existed in parallel universes. Notably, Huang *et al.*[1] unite these research traditions by comparing memory in both rodents and flies with genetically disrupted mTORC2 function. Seymour Benzer and his students pioneered the use of classical fear conditioning, in which an odor is paired with shock, to examine memory in flies[10]. In this model, massed training (training trials with no inter-trial rest periods) produces a short-term memory that is independent of protein synthesis, whereas spaced training (training trials with intervening rest periods) produces protein synthesis–dependent long-term memory[10]. In a striking parallel to the findings with mice, rictor-deficient flies showed intact short-term but impaired long-term memory, indicating that the function of rictor in memory is conserved.

To better understand the mechanism behind mTORC2's effect on memory, the authors turned to its downstream targets. Consistent with mTORC2 and rictor's role in actin polymerization, the authors found that rictor-deficient mice showed disrupted actin dynamics. The polymerization of free globular actin (G-actin) to form filamentous actin (F-actin) is thought to underlie the growth of dendritic spines necessary for memory formation[10]. Rictor-deficient mice showed a reduction in the ratio of F-actin to G-actin, as well as a reduction in expression of a number of upstream positive regulators of actin polymerization. These correlational data suggested that rictor is required for long-term memory formation by increasing the F-actin important for dendritic spine growth and remodeling.

To directly test this hypothesis, the authors manipulated actin itself. First they mimicked the effect of disrupting actin polymerization in otherwise normal wild-type mice using cytochalasin D, a drug that specifically disrupts actin polymerization. Like rictor-deficient slices, wild-type slices treated with cytochalasin D showed intact E-LTP but disrupted L-LTP. Is normalizing actin dynamics sufficient to rescue the L-LTP and long-term memory deficits in rictor-deficient mice? Jasplakinolide (JPK), a compound that promotes actin polymerization, restored both the F-actin to G-actin ratio and the ability to produce L-LTP in slices in rictor-deficient mice, without affecting these processes in slices from wild-type mice. Remarkably, infusion of JPK directly into the hippocampus similarly reversed the long-term memory deficits in rictor-deficient mice. Might increasing actin polymerization enhance synaptic plasticity and memory formation in wild-type mice? To examine this, the authors used weak training conditions that are normally sufficient to induce only E-LTP and short-lasting memory in wild-type mice and found that this drug enabled both L-LTP and long-term memory formation. Intriguingly, the L-LTP induced by weak training in the presence of JPK was blocked by application of anisomycin, a protein synthesis inhibitor, suggesting that actin polymerization is upstream of protein synthesis. Finally, systemic injection of a small molecule that increases mTORC2 activity (A-443654) not only facilitated L-LTP in the slice but also enhanced long-term memory formation in wild-type mice.

By both increasing and decreasing mTORC2 function, Huang *et al.*[1] provide compelling evidence that mTORC2, acting through actin polymerization, is important in formation of stable memories. They showed that this mechanism was conserved in both flies and mice. Taken together, these findings raise the question of whether targeting mTORC2 might similarly enhance memory and other cognitive functions in humans. Given that mTORC2 disruption is implicated in many human diseases associated with cognitive dysfunction[13–15], the present study offers a tantalizing prospect for future therapeutic avenues.

COMPETING FINANCIAL INTERESTS
The authors declare no competing financial interests.

1. Huang, W. *et al. Nat. Neurosci.* **16**, 441–448 (2013).
2. Hay, N. & Sonenberg, N. *Genes Dev.* **18**, 1926–1945 (2004).
3. Kim, D.H. *et al. Cell* **110**, 163–175 (2002).
4. Sarbassov, D.D. *et al. Curr. Biol.* **14**, 1296–1302 (2004).
5. Wullschleger, S., Loewith, R. & Hall, M.N. *Cell* **124**, 471–484 (2006).
6. Tang, S.J. *et al. Proc. Natl. Acad. Sci. USA* **99**, 467–472 (2002).
7. Stoica, L. *et al. Proc. Natl. Acad. Sci. USA* **108**, 3791–3796 (2011).
8. Guertin, D.A. & Sabatini, D.M. *Cancer Cell* **12**, 9–22 (2007).
9. Cingolani, L.A. & Goda, Y. *Nat. Rev. Neurosci.* **9**, 344–356 (2008).
10. Lamprecht, R. & LeDoux, J. *Nat. Rev. Neurosci.* **5**, 45–54 (2004).
11. Weiner, J. *Time, Love, Memory: a Great Biologist and His Quest for the Origins of Behavior* (Knopf, New York, 1999).
12. Dubnau, J. & Tully, T. *Annu. Rev. Neurosci.* **21**, 407–444 (1998).
13. Murata, H. *et al. J. Biol. Chem.* **286**, 7182–7189 (2011).
14. Ljungberg, M.C. *et al. Ann. Neurol.* **60**, 420–429 (2006).
15. Knobbe, C.B., Trampe-Kieslich, A. & Reifenberger, G. *Neuropathol. Appl. Neurobiol.* **31**, 486–490 (2005).