and still arrives at the same place on the adaptive road.

References

1. Aminetzach, Y.T., Srouji, J.R., Kong, C.Y., and Hoekstra, H.E. (2009). Convergent evolution of novel protein function in shrew and lizard venom. Curr. Biol. *19*, 1925–1931.
2. Roelants, K., Fry, B.G., Norman, J.A., Clynen, E., Schoofs, L., and Bossuyt, F. (2010). Identical skin toxins by convergent molecular adaptation in frogs. Curr. Biol. *20*, 125–130.
3. Losos, J.B. (2009). Lizards in an Evolutionary Tree (Berkeley, CA: University of California Press).
4. Larusso, N.D., Ruttenberg, B.E., Singh, A.K., and Oakley, T.H. (2008). Type II opsins: Evolutionary origin by internal domain duplication. J. Mol. Evol. *66*, 417–423.
5. Bennici, A. (2003). The convergent evolution in plants. Riv. Biol. *96*, 485–489.
6. Bowie, J.H., and Tyler, M.J. (2006). Bioactive peptides from Australian amphibians: caerulein and other neuropeptides. In The Handbook of Peptides, A.J. Kastin, ed. (Amsterdam: Academic Press), pp. 283–289.
7. Fry, B.G., Roelants, K., Champagne, D.E., Scheib, H., Tyndall, J.D.A., King, G.F., Nevalainen, T.J., Norman, J.A., Lewis, R.J., Norton, R.S., *et al.* (2009). The toxicogenomic multiverse: convergent recruitment of proteins into animal venoms. Annu. Rev. Genomics Hum. Genet. *10*, 483–511.
8. Feldman, C.R., Brodie, E.D., Jr., Brodie, E.D., III, and Pfrender, M.E. (2009). The evolutionary origins of beneficial alleles during the repeated adaptation of garter snakes to deadly prey. Proc. Natl. Acad. Sci. USA *106*, 13415–13420.
9. ffrench-Constant, R.H., Daborn, P.J., and Goff, G.L. (2004). The genetics and genomics of insecticide resistance. Trends Genet. *20*, 163–170.

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Lipid Kinases: Charging PtdIns(4,5)P_2 Synthesis

Phosphatidylinositol (4,5) bisphosphate is a lipid second messenger that controls diverse cellular processes. Phosphatidylinositolphosphate-5-kinases synthesise this lipid at the plasma membrane, although it is not clear how the localisation of these kinases is controlled. A recent study suggests that the intrinsic surface charge of the plasma membrane may be an important factor.

Nullin Divecha

Different permutations of phosphorylation of the 3, 4 or 5 position of the inositol head group of phosphatidylinositol generates seven different phosphoinositides that form the basis of a ubiquitous membrane-associated signalling system [1]. There are over eighty isoforms of phosphoinositide kinases, phosphatases and phospholipases that modulate the level of phosphoinositides to regulate processes such as membrane trafficking, cell survival, proliferation and migration. Phosphoinositides carry out their functions by regulating the activity of proteins that harbour specific phosphoinositide-interacting modules (such as the PH, FYVE, FERM, and PHOX domains) [2]. Phosphatidylinositol (4,5) bisphosphate (PtdIns(4,5)P_2) is one of the busiest phosphoinositides, being the substrate for receptor-activated phospholipase C (PLC) and phosphatidylinositol-3 kinase, as well as having its own unique signalling functions in plasma membrane trafficking and actin polymerisation [1]. Furthermore, polarised synthesis of PtdIns(4,5)P_2 is important for the maintenance of epithelial cell morphology [3], podosome function, and membrane ruffling [4] and for the first asymmetric cleavage after fertilisation in *Caenorhabditis elegans* [5]. How localised synthesis of PtdIns(4,5)P_2 is achieved is far from clear. A new study into the role of phosphoinositides in phagocytosis by Grinstein and colleagues [6] has now revealed that the plasma membrane localisation of PIP5K is dependent on the electrostatic interactions between PIP5K and the negative surface of the plasma membrane.

Phagocytosis is the major mechanism by which apoptotic bodies and foreign particles such as bacteria are eliminated from the organism [7]. Opsonisation of the bacterial surface promotes phagocytosis, leading to the interaction of the bacteria with specific cell-surface receptors on macrophages. The ensuing pseudopod formation extends the macrophage plasma membrane around the bacterium, eventually leading to the isolation of the bacterium in an intracellular membrane-bounded vacuole (the phagosome). The phagosome matures over time, becoming acidic and rich in hydrolases and anti-microbial agents capable of degrading bacteria. In a series of elegant studies the Grinstein laboratory has previously demonstrated exquisite spatial and temporal changes in phosphoinositides as the phagosome forms and matures (for a review, see [8]). Initially, upon cell surface binding of the particle, there is an increase in PtdIns(4,5)P_2 [9] and then PtdIns(3,4,5)P_3 at the base of the particle, which is maintained as the pseudopods traverse around the particle. The increased PtdIns(4,5)P_2 accumulation probably helps to drive actin polymerisation, which is required for pseudopod extension. At the point of engulfment and sealing, the PtdIns(4,5)P_2 at the base of the phagosome — and only at the base — dramatically decreases, leading to actin depolymerisation and sealing and internalisation of the phagosome (Figure 1).

Interventions that compromise either PtdIns(4,5)P_2 synthesis or its degradation also compromise phagocytosis [10,11]. PIP5K isoforms (α, β and γ) phosphorylate PtdIns4*P* on the 5′ position and are responsible for the synthesis of the majority of cellular PtdIns(4,5)P_2 [1]. During phagocytosis, PIP5Kγ is required for clustering receptors that interact with bacteria, while PIP5Kα regulates actin polymerisation and bacterial internalisation. PIP5Kα localises to the forming phagosome [12] and, together with PIP5Kγ, probably increases PtdIns(4,5)P_2 in the pseudopods. But how is the decrease in PtdIns(4,5)P_2 at the base of the phagosome achieved? Increased PtdIns(3,4,5)P_3 synthesis is critical as it can activate PLC-γ, which hydrolyses PtdIns(4,5)P_2. However, although PIP5Kα is targeted to the phagocytic cup at early stages, it is lost just before phagosome internalisation [11], suggesting that reducing PtdIns(4,5)P_2 synthesis may also be important in

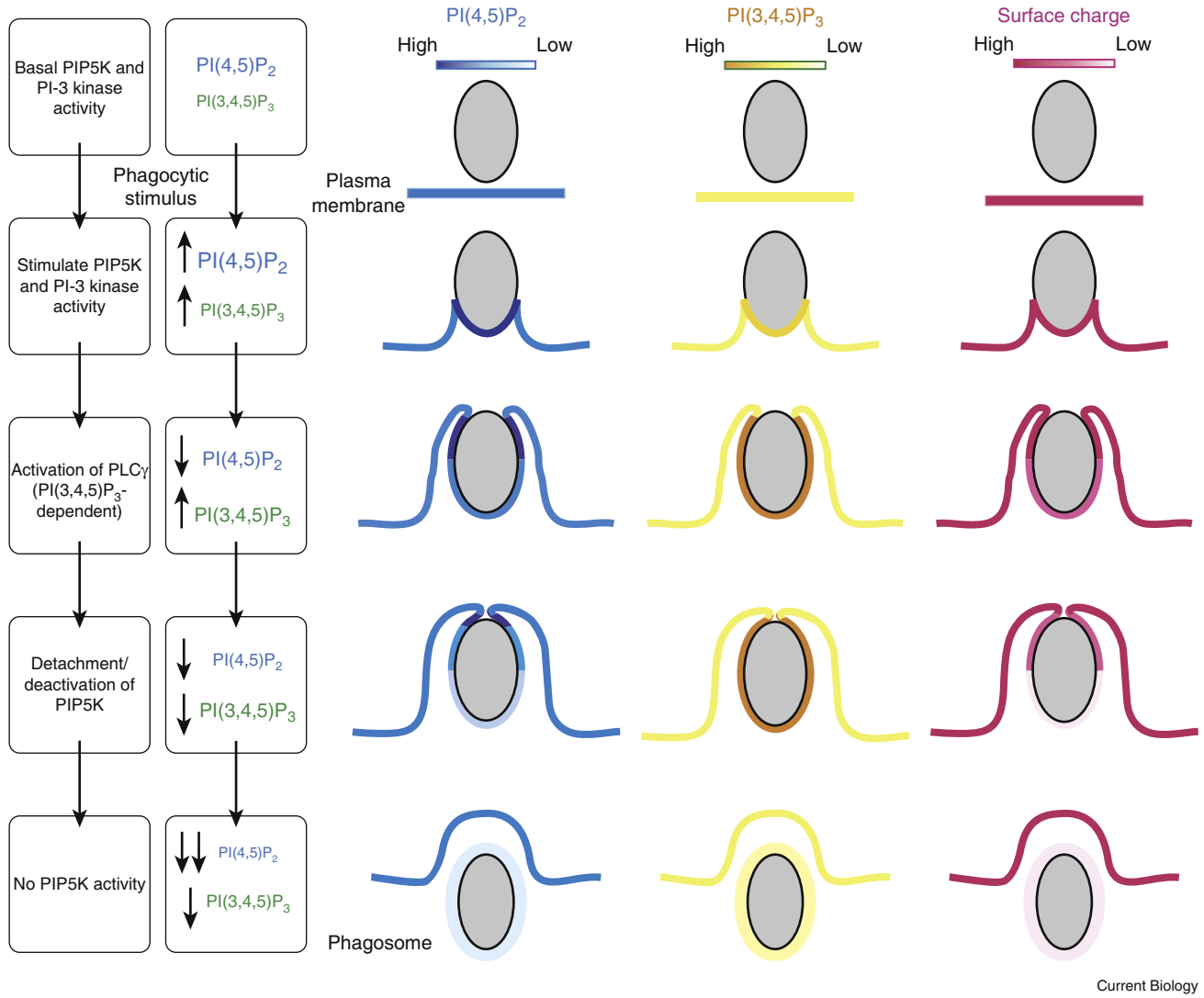

Figure 1. PtdIns(4,5)P_2 and PtdIns(3,4,5)P_3 signalling during phagocytosis.

The figure shows the temporal and spatial aspects of PtdIns(4,5)P_2 (blue) and PtdIns(3,4,5)P_3 (yellow) signalling upon binding of an opsonised particle to the cell surface. As the pseudopods engulf the particle, the PtdIns(4,5)P_2 levels in the phagocytic cup dramatically decrease and are not present once the particle has been internalised (i.e. phagosome formation). The drop in PtdIns(4,5)P_2 coincides with the sharp drop in surface charge (pink). (Reproduced with permission from [6].)

addition to increasing its hydrolysis. So how is PIP5K specifically lost from the phagocytic membrane but not from the bulk of the plasma membrane, even though the two are contiguous? In the new study, Grinstein and colleagues [6] suggest that changes in the surface charge of the two membranes may control PIP5K localisation.

On the basis of the crystal structure of PIP4Kβ [13], a closely related lipid kinase, the authors suggested that, similar to PIP4Kβ, PIP5K forms a flat dimer with a highly positively charged face that could interact electrostatically with the negative surface of the plasma membrane. The charge of the inner surface of the plasma membrane is negative as a consequence of polarised accumulation of negatively charged lipids such as phosphatidylserine and phosphoinositides. So does PIP5K interact with the membrane in an electrostatic manner? Using lipid dot blots the authors show that PIP5K can interact with a number of negatively charged phospholipids, including PtdIns(4,5)P_2, PtdIns(3,4,5)P_3, phosphatidic acid and phosphatidylserine, with the strongest interaction being with its substrate PtdIns4*P*. PtdIns4*P* is also a key determinant for plasma membrane association of PIP5K [14]. Using liposome-binding assays the authors demonstrate that the interactions of PIP5K with PtdIns4*P* are additive, to some extent, with the putative electrostatic interactions with phosphatidylserine and PtdIns(4,5)P_2.

Using a number of tricks *in vivo* either to deplete PtdIns(4,5)P_2 levels or to decrease the surface negativity of the plasma membrane while maintaining PtdIns(4,5)P_2 levels, the authors conclude that the plasma membrane localisation of PIP5K is sensitive to the negative charge of the membrane. These data led the authors to postulate that, for membrane association of PIP5K, both the interaction with its substrate PtdIns4*P* and the electrostatic interaction with the

negatively charged membrane are required, with neither alone being sufficient. Coincidence signalling between an electrostatic interaction and another tethering mechanism enables low-affinity interactions to be easily regulated to control membrane localisation. For example, the protein MARCKS (myristoylated alanine-rich C kinase substrate) is targeted to the membrane by a myristoylation group in combination with a cluster of positively charged amino acids that interact electrostatically with the plasma membrane. Phosphorylation of serine residues within the basic cluster, or interaction with calmodulin, decreases the electrostatic interaction, leading to dissociation of MARCKS from the membrane [15].

So why is the mechanism behind the membrane association of PIP5K so interesting from the point of view of phagocytosis? Using fluorescent probes that are sensitive to membrane charge [16], Grinstein and colleagues [6] show that, during phagocytosis, the surface charge at the base of the phagocytic cup becomes less negative right at the point of engulfment prior to sealing, probably as a consequence of PtdIns(4,5)P_2 removal because phosphatidylserine is still present in the maturing phagosome. So, a reduction in the negativity of the membrane appears to be the switch to induce PIP5K removal from the phagocytic cup. Tethering PIP5K on the membrane during phagocytosis is sufficient to maintain PtdIns(4,5)P_2 levels at the base of the phagosome and attenuate actin depolymerisation and phagosome engulfment, demonstrating the importance of the loss of PIP5K [6].

So do the authors make a convincing case for PIP5Ks being regulated by an electrostatic switch? While the idea is attractive, the experiments are not entirely convincing. First and foremost, PIP5K makes PtdIns(4,5)P_2 and likely has an intrinsic affinity for this lipid. The electrostatic interaction with liposomes is not adequately addressed and the analysis should have included PtdIns(3,4)P_2 and PtdIns(3,5)P_2, which carry the same charge density as PtdIns(4,5)P_2. The use of mutants of PIP5K that are unable to interact with PtdIns4*P* [14] could have more convincingly demonstrated that the electrostatic interactions are independent of the PtdIns4*P*-binding site, a prerequisite for the coincidence signalling concept. Furthermore, the large flat positive surface of PIP5K is proposed to be created through dimerisation. However, no attempt to demonstrate dimerisation of PIP5K was made. Finally, to demonstrate electrostatic dependency of the PIP5K membrane interaction, the authors reverse the positive charge of some residues by mutation. However, they do not mutate residues identified in the putative structure that contribute to the flat positive surface of PIP5K but instead mutate other residues in the activation loop that are involved in PtdIns4*P* interaction and catalysis [14].

Despite these shortcomings, the study presents a new framework for the explanation of PIP5K membrane localisation. Notably, while PtdIns4*P* is present on endomembranes as well as the plasma membrane [17], PIP5K is found only on the plasma membrane, suggesting that the interaction with PtdIns4*P* is not sufficient for the targeting of PIP5K to the endomembranes. Furthermore, PtdIns(4,5)P_2 is required for efficient coating and generation of endocytic vesicles; however, PIP5K is rarely found to associate with vesicles once internalised. Interestingly, overexpression of PIP5K often leads to an accumulation of endocytic vesicles that are coated with PIP5K, PtdIns(4,5)P_2 and actin and appear to 'jam up' the endocytic trafficking system (our unpublished data and [18]). Thus, PtdIns(4,5)P_2 probably has to be removed for proper vesicle trafficking once the vesicle is formed and prevention of PIP5K from associating with the vesicle may be important for this. It should be noted that, according to the idea of electrostatic regulation of PIP5K localisation, an initial loss of PtdIns(4,5)P_2 should induce PIP5K delocalisation and precipitate a drop in PtdIns(4,5)P_2 levels. On the flip side, once PtdIns(4,5)P_2 synthesis is initiated, this cooperative signalling will strongly induce further PtdIns(4,5)P_2 synthesis which must be controlled. The ability of PtdIns(4,5)P_2 to inhibit PIP5K activity may be an important facet in self-regulation of its synthesis. PIP5Kα is also highly phosphorylated [1] and enzyme activation correlates with dephosphorylation, perhaps suggesting that the electrostatic interaction and therefore membrane interaction can be manipulated in a similar manner to that of MARCKS.

Finally, PIP5K localisation is regulated by small G proteins of the Rho family [1], which themselves are regulated by the surface charge of the plasma membrane [19]. Rac is also recruited and activated in the phagocytic cup, but its activity is dramatically decreased prior to engulfment and sealing. Further studies should define how these events are coordinated and whether the special negative surface charge properties of the plasma membrane really can dictate PtdIns(4,5)P_2 synthesis.

References

1. van den Bout, I., and Divecha, N. (2009). PIP5K-driven PtdIns(4,5)P_2 synthesis: regulation and cellular functions. J. Cell Sci. *122*, 3837–3850.
2. DiPaolo, G., and DeCamilli, P. (2006). Phosphoinositides in cell regulation and membrane dynamics. Nature *443*, 651–657.
3. Martin-Belmonte, F., Gassama, A., Datta, A., Yu, W., Rescher, U., Gerke, V., and Mostov, K. (2007). PTEN-mediated apical segregation of phosphoinositides controls epithelial morphogenesis through Cdc42. Cell *128*, 383–397.
4. Doughman, R.L., Firestone, A.J., Wojtasiak, M.L., Bunce, M.W., and Anderson, R.A. (2003). Membrane ruffling requires coordination between type Ialpha phosphatidylinositol phosphate kinase and Rac signaling. J. Biol. Chem. *278*, 23036–23045.
5. Panbianco, C., Weinkove, D., Zanin, E., Jones, D., Divecha, N., Gotta, M., and Ahringer, J. (2008). A casein kinase 1 and PAR proteins regulate asymmetry of a PIP(2) synthesis enzyme for asymmetric spindle positioning. Dev. Cell *15*, 198–208.
6. Fairn, G.D., Ogata, K., Botelho, R.J., Stahl, P.D., Anderson, R.A., De, C.P., Meyer, T., Wodak, S., and Grinstein, S. (2009). An electrostatic switch displaces phosphatidylinositol phosphate kinases from the membrane during phagocytosis. J. Cell Biol. *187*, 701–714.
7. Yeung, T., and Grinstein, S. (2007). Lipid signaling and the modulation of surface charge during phagocytosis. Immunol. Rev. *219*, 17–36.
8. Yeung, T., Ozdamar, B., Paroutis, P., and Grinstein, S. (2006). Lipid metabolism and dynamics during phagocytosis. Curr. Opin. Cell Biol. *18*, 429–437.
9. Botelho, R.J., Teruel, M., Dierckman, R., Anderson, R., Wells, A., York, J.D., Meyer, T., and Grinstein, S. (2000). Localized biphasic changes in phosphatidylinositol-4,5-bisphosphate at sites of phagocytosis. J. Cell Biol. *151*, 1353–1368.
10. Scott, C.C., Dobson, W., Botelho, R.J., Coady-Osberg, N., Chavrier, P., Knecht, D.A., Heath, C., Stahl, P., and Grinstein, S. (2005). Phosphatidylinositol-4,5-bisphosphate hydrolysis directs actin remodeling during phagocytosis. J. Cell Biol. *169*, 139–149.
11. Coppolino, M.G., Dierckman, R., Loijens, J., Collins, R.F., Pouladi, M., Jongstra-Bilen, J., Schreiber, A.D., Trimble, W.S., Anderson, R., and Grinstein, S. (2002). Inhibition of phosphatidylinositol-4-phosphate 5-kinase Ialpha impairs localized actin remodeling and suppresses phagocytosis. J. Biol. Chem. *277*, 43849–43857.
12. Mao, Y.S., Yamaga, M., Zhu, X., Wei, Y., Sun, H.Q., Wang, J., Yun, M., Wang, Y., Di Paolo, G., Bennett, M., *et al*. (2009). Essential and unique roles of PIP5K-gamma and -alpha in Fcgamma receptor-mediated phagocytosis. J. Cell Biol. *184*, 281–296.

13. Rao, V.D., Misra, S., Boronenkov, I.V., Anderson, R.A., and Hurley, J.H. (1998). Structure of type IIbeta phosphatidylinositol phosphate kinase: a protein kinase fold flattened for interfacial phosphorylation. Cell *94*, 829–839.
14. Kunz, J., Fuelling, A., Kolbe, L., and Anderson, R.A. (2002). Stereo-specific substrate recognition by phosphatidylinositol phosphate kinases is swapped by changing a single amino acid residue. J. Biol. Chem. *277*, 5611–5619.
15. McLaughlin, S., and Aderem, A. (1995). The myristoyl-electrostatic switch: a modulator of reversible protein-membrane interactions. Trends Biochem. Sci. *20*, 272–276.
16. Yeung, T., Terebiznik, M., Yu, L., Silvius, J., Abidi, W.M., Philips, M., Levine, T., Kapus, A., and Grinstein, S. (2006). Receptor activation alters inner surface potential during phagocytosis. Science *313*, 347–351.
17. Balla, A., Kim, Y.J., Varnai, P., Szentpetery, Z., Knight, Z., Shokat, K.M., and Balla, T. (2008). Maintenance of hormone-sensitive phosphoinositide pools in the plasma membrane requires phosphatidylinositol 4-kinase IIIalpha. Mol. Biol. Cell *19*, 711–721.
18. Brown, F.D., Rozelle, A.L., Yin, H.L., Balla, T., and Donaldson, J.G. (2001). Phosphatidylinositol 4,5-bisphosphate and Arf6-regulated membrane traffic. J. Cell Biol. *154*, 1007–1017.
19. Heo, W.D., Inoue, T., Park, W.S., Kim, M.L., Park, B.O., Wandless, T.J., and Meyer, T. (2006). PI(3,4,5)P_3 and PI(4,5)P_2 lipids target proteins with polybasic clusters to the plasma membrane. Science *314*, 1458–1461.

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Dendritic Spines: The Stuff That Memories Are Made Of?

Two new studies explore structural changes of nerve cells as a potential mechanism for memory formation by studying synaptic reorganization associated with motor learning.

Sonja B. Hofer[1] and Tobias Bonhoeffer[2]

One of the most impressive abilities of the mammalian brain is its capacity to constantly learn new skills, integrate new experiences and form long-lasting memories. How the brain accomplishes this, in view of the vast amount of information we are faced with every day, has been the subject of intensive study for many decades. Two new studies [1,2], together with work published earlier last year [3], now provide strong evidence in support of a structural basis for information storage in cortical circuits.

It is generally believed that changes in the synaptic connections between neurons play a major role in learning and memory formation. While short-term memory might rely mainly on the strengthening and weakening of pre-existing synapses [4,5], long-term storage of information is thought to require structural reorganization of neuronal networks, the formation of new synapses and the loss of existing connections [6]. The first evidence for the importance of functional and structural synaptic plasticity for learning and memory came from seminal studies in the sea slug *Aplysia*. These showed that simple learning processes, like habituation or sensitization, are based on the weakening or strengthening of synaptic connections [7]. Importantly, long-term effects were accompanied by structural changes such as an increase in the number of synapses [6].

Might similar mechanisms of structural reorganization of synapses also account for more complex forms of learning and memory formation in the mammalian brain? Because of recent advances in genetic labelling techniques and imaging methods, it is now possible to repeatedly image fluorescently labelled structures such as axonal boutons and dendritic spines — the pre- and postsynaptic components of excitatory synapses — in the intact cortex of living animals, either through a chronically implanted cranial window or by repeated thinning of the skull [8,9]. The dynamics of synapses thus can be followed while the animal experiences altered sensory input or learns a behavioural task, and structural reorganization can be correlated with changes in neuronal activity and behaviour. Studies using this method demonstrated that a subset of synapses remains highly dynamic even in adult cortex, and boutons and spines appear and disappear continuously [9–14].

These ongoing structural changes have been hypothesized to endow cortical networks with the capacity to translate novel experiences into anatomical traces. And indeed several studies have recently confirmed that altered sensory experience induced by whisker removal, eye closure or retinal lesions causes synaptic and even axonal reorganization in the cortex of rodents and primates throughout life [3,8,15–18]. The two new studies [1,2] have now pursued this idea further by studying spine dynamics during different forms of learning and experience, providing further evidence for structural changes as a possible basis for long-term memory.

Yang *et al.* [1] looked at the effect of two different plasticity paradigms — a novel somatosensory experience and a form of motor learning — on the generation, maintenance and elimination of dendritic spines in mouse cortex. The animals were either presented with an enriched environment that provided new somatosensory input or they learned to maintain their posture on a rotating rod (rotarod) that was continuously accelerated. The structural changes were remarkably similar for both paradigms: after two days new spines were generated in somatosensory or motor cortex, respectively, of which a small percentage persisted over the next weeks. The percentage of new spines that remained stable increased with longer training or exposure periods. Some animals that had learned the motor task were reassessed three months after the initial training. They still mastered the skill of running on the rotarod better than naïve mice. Interestingly, while there was no additional spine growth when repeating the original protocol, a slightly different training paradigm, running backwards on the accelerating rod, resulted in the formation of new spines. In other words, new learning induced new spines, but recall of previously learned skills did not.

The other study, by Xu *et al.* [2], reinforces the idea that motor learning causes lasting structural changes