

MIT Open Access Articles

Synaptic Growth: Dancing with Adducin

The MIT Faculty has made this article openly available. **Please share** how this access benefits you. Your story matters.

Citation: Stevens, Robin J., and J. Troy Littleton. "Synaptic Growth: Dancing with Adducin." *Current Biology* 21, no. 10 (May 2011): R402–R405. © 2011 Elsevier Ltd.

As Published: <http://dx.doi.org/10.1016/j.cub.2011.04.020>

Publisher: Elsevier

Persistent URL: <http://hdl.handle.net/1721.1/92025>

Version: Final published version: final published article, as it appeared in a journal, conference proceedings, or other formally published context

Terms of Use: Article is made available in accordance with the publisher's policy and may be subject to US copyright law. Please refer to the publisher's site for terms of use.



pharmacological inhibitors of transcription and translation during constant light [10]. However, circadian rhythm in PRX sulphonylation in constant light is blocked by inhibition of the proteasome, establishing the necessity of proteasomal function to rhythmicity [4]. In the dark, however, transcription ceases. Under these conditions, proteasomal inhibition failed to block rhythmic PRX sulphonylation, indicating that proteasomal degradation was necessary for rhythmicity only under conditions in which protein synthesis persisted. However, inhibitors of other post-translational modifications had similar effects on the period of PRX sulphonylation as they did in the light [9]. This argues that the transcription/translation feedback loop (TTFL) and the post-translational feedback loop (PTFL) are normally tightly coupled under physiologically relevant conditions. However, in the abnormal and stressful condition of extended dark, encountered perhaps when *O. tauri* cells are carried deeply into the water column, the transcription/translation feedback loop is absent due to the cessation in transcription. The cessation of transcription is presumably a survival mechanism to endure a period of energy starvation. Nonetheless, the persistence of the post-translational rhythm in PRX sulfonation suggest that there is still a survival value associated with rhythmicity, presumably in coordinating metabolism in these near-dormant conditions [4].

PRX proteins are widely distributed among taxa. Apparently rhythms in PRX sulphonylation are similarly widespread, because PRX proteins exhibit a robust circadian rhythm in PRX sulphonylation in human red blood cells [12]. This is a striking result, because human red blood cells lack nuclei and so are incapable of transcription. Of course, the demonstration that the cyanobacterial KaiA, KaiB, and KaiC proteins together with ATP are sufficient to reconstitute a robust temperature-compensated *in vitro* rhythm in KaiC phosphorylation had already established that rhythmicity was possible without transcription and translation [13], but now this has been extended to two eukaryotes of quite distinct lineages. Interestingly, 50 years ago it was observed that circadian rhythms in photosynthesis persist in enucleated

Acetabularia major and *A. crenulata* [14] and almost 40 years ago a rhythm in respiration was reported in dormant onion seeds [15]. Obviously, these multiple observations of circadian rhythmicity without *de novo* transcription in cyanobacteria, *Acetabularia*, *O. tauri*, onions, and humans fully refute the general necessity of transcription for circadian clock function.

Are there two circadian clocks present in most cells, one based on transcription/translation feedback loops and one based on transcription/translation-independent mechanisms (Figure 1)? Certainly there are multiple examples of circadian rhythmicity in genotypes in which the known transcription/translation feedback loop mechanism is disrupted [16]. Although it seems premature to claim the ubiquity of these two types of clocks, it nonetheless seems likely that the exploration of the interaction between these two clock mechanisms is likely to offer important insights. The implications of these two types of clocks for the evolution of circadian rhythmicity are profound.

References

1. Dunlap, J.C. (1999). Molecular bases for circadian clocks. *Cell* 96, 271–290.
2. Zhang, E.E., and Kay, S.A. (2010). Clocks not winding down: unravelling circadian networks. *Nat. Rev. Mol. Cell Biol.* 11, 764–776.
3. Gallego, M., and Virshup, D.M. (2007). Post-translational modifications regulate the ticking of the circadian clock. *Nat. Rev. Mol. Cell Biol.* 8, 139–148.
4. van Ooijen, G., Dixon, L.E., Troein, C., and Millar, A.J. (2011). Proteasome function is required for biological timing throughout

- the twenty-four hour cycle. *Curr. Biol.* 21, 869–875.
5. Courties, C., Vaquer, A., Troussellier, M., Lautier, J., Chretiennot-Dinet, M.J., Neveux, J., Machado, C., and Claustre, H. (1994). Smallest eukaryotic organism. *Nature* 370, 255.
6. Corellou, F., Schwartz, C., Motta, J.-P., Djouani-Tahri, E.B., Sanchez, F., and Bouget, F.-Y. (2009). Clocks in the green lineage: Comparative functional analysis of the circadian architecture of the picoeukaryote *Ostreococcus*. *Plant Cell* 21, 3436–3449.
7. McClung, C.R. (2006). Plant circadian rhythms. *Plant Cell* 18, 792–803.
8. Troein, C., Locke, J.C.W., Turner, M.S., and Millar, A.J. (2009). Weather and seasons together demand complex biological clocks. *Curr. Biol.* 19, 1961–1964.
9. Troein, C., Corellou, F., Dixon, L.E., van Ooijen, G., O'Neill, J.S., Bouget, F.-Y., and Millar, A.J. (2011). Multiple light inputs to a simple clock circuit allow complex biological rhythms. *Plant J.* 66, 375–385.
10. O'Neill, J.S., van Ooijen, G., Dixon, L.E., Troein, C., Corellou, F., Bouget, F.Y., Reddy, A.B., and Millar, A.J. (2011). Circadian rhythms persist without transcription in a eukaryote. *Nature* 469, 554–558.
11. Hall, A., Karplus, P.A., and Poole, L.B. (2009). Typical 2-Cys peroxiredoxins – structures, mechanisms and functions. *FEBS J.* 276, 2469–2477.
12. O'Neill, J.S., and Reddy, A.B. (2011). Circadian clocks in human red blood cells. *Nature* 469, 498–503.
13. Tomita, J., Nakajima, M., Kondo, T., and Iwasaki, H. (2005). No transcription-translation feedback in circadian rhythm of KaiC phosphorylation. *Science* 307, 251–253.
14. Sweeney, B.M., and Haxo, F.T. (1961). Persistence of a photosynthetic rhythm in enucleated *Acetabularia*. *Science* 134, 1361–1363.
15. Bryant, T.R. (1972). Gas exchange in dry seeds: circadian rhythmicity in the absence of DNA replication, transcription, and translation. *Science* 178, 634–636.
16. Lakin-Thomas, P.L. (2006). Transcriptional feedback oscillators: Maybe, maybe not. *J. Biol. Rhythms* 21, 83–92.

Department of Biological Sciences, Dartmouth College, Hanover, NH 03755, USA.
E-mail: C.Robertson.McClung@Dartmouth.Edu

DOI: 10.1016/j.cub.2011.04.024

Synaptic Growth: Dancing with Adducin

Manipulations of the actin-capping protein adducin in *Drosophila* and mammalian neurons provide new insights into the mechanisms linking structural changes to synaptic plasticity and learning. Adducin regulates synaptic remodeling, providing a molecular switch that controls synaptic growth versus disassembly during plasticity.

Robin J. Stevens and J. Troy Littleton

Developing neural circuits are often highly plastic and not only form new synaptic contacts, but also eliminate unnecessary or redundant synapses. Once the brain has matured, extensive remodeling of circuits is rare, but connections between neurons can be

modified in an activity-dependent fashion as well as in response to injury or disease [1]. Alterations of synaptic connections are hypothesized to underlie learning and memory and can occur through several mechanisms. The strength of a synapse can be increased or decreased by changing the properties of presynaptic release or

by altering the postsynaptic response to neurotransmitters. The addition or removal of contacts between neurons can also modify the strength of a connection or the wiring of a circuit. Remodeling of the underlying actin cytoskeleton plays a role in altering structural features of connectivity, including synapse formation and retraction. In a recent issue of *Neuron*, two papers by Pielage *et al.* [2] and Bednarek and Caroni [3] examined how adducin, a regulator of the actin cytoskeleton, controls synaptic stability and improves memory upon environmental enrichment.

Actin has the ability to alter synaptic function and plasticity through a variety of mechanisms. Presynaptically, actin plays a role in controlling the recruitment of synaptic vesicles from the reserve pool to the recycling pool [4]. Actin also modulates the recovery of synaptic vesicles via endocytosis after neurotransmitter release [5]. Postsynaptically, actin can affect the insertion or removal of AMPA receptors during long-term potentiation (LTP) or long-term depression (LTD) [6,7]. Dendritic spine formation and retraction are also dependent on structural rearrangements of the actin cytoskeleton. Therefore, regulation of actin polymerization, as well as of the interactions between actin and other cytoskeletal and structural proteins, is important for a variety of processes that potentially contribute to learning and memory. Adducin controls actin polymerization by capping the fast-growing ends of actin filaments and promoting the interaction of actin with the cytoskeletal protein spectrin [8,9]. As such, adducin is capable of integrating actin fibers into other cellular structures, such as the spectrin network, by recruiting spectrin to the ends of actin filaments. Loss of other actin-capping proteins has been shown to promote the growth of actin-rich filopodia in culture [10,11], while overexpression of the actin-binding domain of β 1-spectrin in dendritic spines can stabilize actin filaments by inhibiting depolymerization, reducing the morphological plasticity of spine heads [12]. Regulation of adducin activity could therefore alter both the stability and the morphology of synapses.

Mammalian genomes encode three closely related adducin proteins termed α -, β - and γ -adducin. These

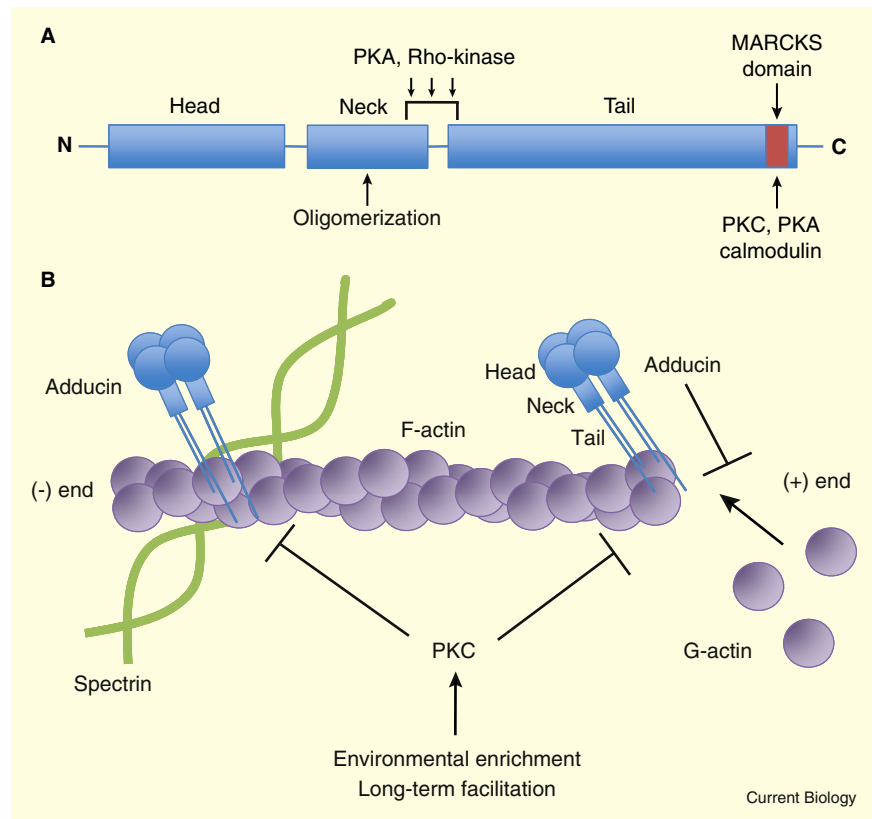


Figure 1. Organization and regulation of adducin function.

(A) Schematic of an adducin monomer, which contains a globular head region, a neck region required for oligomerization, and a tail region containing the MARCKS-related domain. Several key phosphorylation sites are indicated by arrows. (Modified from [13].) (B) Adducin caps the fast-growing end of filamentous F-actin and prevents the addition of monomeric G-actin. Environmental enrichment and long-term facilitation promote the phosphorylation of adducin by PKC, causing the dissociation of the actin-spectrin complex and inhibiting actin capping, thereby destabilizing the filament. (Modified from [13,20].)

proteins form tetramers of either α/β or α/γ heterodimers. The α - and γ -adducins are ubiquitously expressed, while β -adducin is enriched in erythrocytes and the brain, where it is present in dendritic spines and growth cones [13]. *Drosophila* has a single adducin homolog named *Hu-li tai shao* (Hts). The *hts* locus encodes four potential isoforms, one of which, Hts-M, is expressed in the larval brain [2]. Adducins contain a highly conserved carboxy-terminal region similar to the myristoylated alanine-rich C kinase substrate (MARCKS protein). The MARCKS-related domain is required but not sufficient for the actin-capping and spectrin-recruiting activities of adducins [13]. This region appears to be a key regulatory domain, with binding sites for protein kinase C (PKC), protein kinase A (PKA) and calcium-calmodulin [13], all of which play critical roles in synaptic plasticity (Figure 1A).

The recent *Neuron* papers used different model systems to illustrate the importance of β -adducin in synapse stability. Bednarek and Caroni [3] found that β -adducin knockout mice have normal active zone densities at the large mossy fiber terminals (LMTs) in the hippocampal CA3 region. However, the knockout has an increased rate of synapse and spine turnover, as well as enhanced gains and losses of filopodia and satellite terminals at LMTs, which suggests a loss of synaptic stability [3]. Such spine dynamics may regulate learning and memory, as the rapid formation of new dendritic spines, along with the maintenance of a specific subset, has been shown to play an important role in learning a new motor task [14]. Using the *Drosophila* model, Pielage *et al.* [2] show that the loss of presynaptic Hts-M results in a dramatic increase in the number of synaptic retractions, as well as a generalized overgrowth of

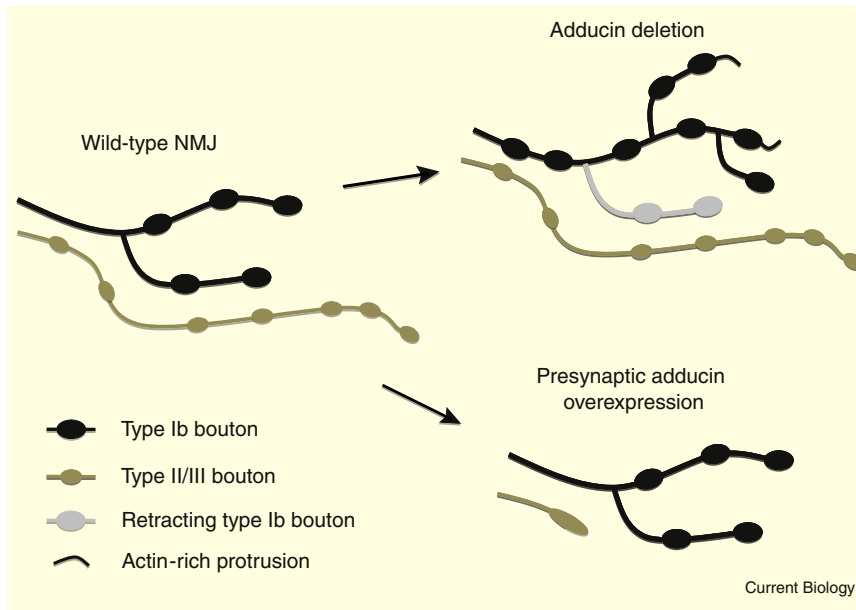


Figure 2. Adducin modulates synaptic growth at the *Drosophila* larval NMJ. Deletion of Hts-M/adducin results in an overgrowth of large-diameter type Ib boutons, as well as an increase in synaptic retractions and the appearance of actin-rich protrusions at the *Drosophila* larval NMJ. Presynaptic overexpression of Hts-M/adducin inhibits the formation of small-diameter type II and type III boutons.

large-diameter glutamatergic type Ib boutons at the larval neuromuscular junction (NMJ). Interestingly, the authors also saw unique small-caliber, actin-rich membrane protrusions from type Ib boutons in *hts* mutants that contained presynaptic markers in close proximity to glutamate receptors, indicating that these protrusions may be nascent synapses (Figure 2). The cytoskeletal proteins spectrin and ankyrin2 are required for synapse stability at the larval NMJ, but mutations in these genes do not result in the membrane protrusions seen in *hts* mutants, suggesting a unique role for Hts-M/adducin [2]. Furthermore, presynaptic overexpression of Hts-M in flies inhibits the formation of smaller, more dynamic type II and type III boutons. Unlike glutamatergic type Ib boutons, type II and type III boutons release peptide neurotransmitters, and their growth is more strongly influenced by changes in activity. Given that Hts-M/adducin has known actin-capping activity, it is likely that adducin acts to stabilize synapses in both mice and flies by preventing actin polymerization via restriction of growth at the fast-growing barbed ends of filaments.

The decreased stability of synapses in mouse and fly *adducin* mutants implies that synaptic plasticity and

learning may also be affected. Indeed, the β -*adducin* knockout mouse has defects in hippocampal LTP and LTD, as well as deficits in several learning assays [15,16]. Furthermore, the loss of β -*adducin* eliminates many of the benefits of environmental enrichment, during which animals are exposed to additional sensory, social and motor stimuli relative to standard housing conditions. The β -*adducin* knockout mice raised under enriched conditions show the expected increase in dendritic spine numbers in the CA1 region of the hippocampus, but lack a concomitant increase in the number of functional synapses [3]. Bednarek and Caroni [3] found that β -*adducin* knockout mice raised in standard housing conditions had no defects in contextual fear conditioning or novel object recognition. When raised under enriched conditions, however, the mutant mice had levels of learning below those of mice raised under standard conditions. These deficits may not be merely a result of altered LTP, as mice lacking Rab3A, a synaptic protein required for LTP in hippocampal mossy fibers, show improved learning under enrichment conditions [17].

To understand how adducin can affect synaptic stability it is important to determine how adducin activity itself

is regulated. PKC phosphorylation of the MARCKS-related domain inhibits the actin-capping and spectrin-recruiting activities of adducin in both mammals and invertebrates [13] (Figure 1B). In *Aplysia*, phosphorylation of adducin by PKC occurs during serotonin-induced long-term facilitation between sensory and motor neurons — a process that is associated with structural changes at synapses [18]. At the *Drosophila* larval NMJ, Hts-M is phosphorylated in the more dynamic type II and type III boutons, but is primarily dephosphorylated in larger type Ib boutons. This observation may explain the differences in dynamics seen between small- and large-caliber boutons at the NMJ in the study by Pielage *et al.* [2]. Phosphomimetic mutations in the MARCKS-related domain in *Drosophila* Hts-M result in increased synaptic accumulation of the protein, suggesting additional control at the level of adducin trafficking. In mice, adducin phosphorylation by PKC occurs upon environmental enrichment and is required for synapse disassembly [3]. Together, these results imply that phosphorylation of adducin by PKC leads to a loss of rigidity in the cytoskeleton, allowing synapses either to assemble or to disassemble. These structural changes are critical for modifications associated with learning and memory. Adducin activity can also be modified by PKA, Rho kinase, Fyn kinase, as well as calcium-calmodulin, all of which have been implicated in learning and memory [13,19].

These two recent studies add to the growing body of evidence that adducin is a key regulator of synapse stability. Through PKC phosphorylation, adducin can act as a switch to control critical steps of synapse disassembly and reassembly that occur during the rearrangement of neural connections. In the future, it will be interesting to determine to what extent adducin is involved in the initial establishment of neural circuits, as well as the relative contributions of adducin activity pre- and postsynaptically.

References

- Holtmaat, A., and Svoboda, K. (2009). Experience-dependent structural synaptic plasticity in the mammalian brain. *Nat. Rev. Neurosci.* 10, 647–658.
- Pielage, J., Bulat, V., Zuchero, J.B., Fetter, R.D., and Davis, G.W. (2011). Hts/Adducin controls synaptic elaboration and elimination. *Neuron* 69, 1114–1131.

3. Bednarek, E., and Caroni, P. (2011). beta-Adducin is required for stable assembly of new synapses and improved memory upon environmental enrichment. *Neuron* 69, 1132–1146.
4. Kuromi, H., and Kidokoro, Y. (1998). Two distinct pools of synaptic vesicles in single presynaptic boutons in a temperature-sensitive *Drosophila* mutant, shibire. *Neuron* 20, 917–925.
5. Shupliakov, O., Bloom, O., Gustafsson, J.S., Kjaerulf, O., Low, P., Tomilin, N., Pieribone, V.A., Greengard, P., and Brodin, L. (2002). Impaired recycling of synaptic vesicles after acute perturbation of the presynaptic actin cytoskeleton. *Proc. Natl. Acad. Sci. USA* 99, 14476–14481.
6. Gu, J., Lee, C.W., Fan, Y., Komlos, D., Tang, X., Sun, C., Yu, K., Hartzell, H.C., Chen, G., Bamberg, J.R., et al. (2010). ADF/cofilin-mediated actin dynamics regulate AMPA receptor trafficking during synaptic plasticity. *Nat. Neurosci.* 13, 1208–1215.
7. Zhou, Q., Xiao, M., and Nicoll, R.A. (2001). Contribution of cytoskeleton to the internalization of AMPA receptors. *Proc. Natl. Acad. Sci. USA* 98, 1261–1266.
8. Bennett, V., Gardner, K., and Steiner, J.P. (1988). Brain adducin: a protein kinase C substrate that may mediate site-directed assembly at the spectrin-actin junction. *J. Biol. Chem.* 263, 5860–5869.
9. Kuhlman, P.A., Hughes, C.A., Bennett, V., and Fowler, V.M. (1996). A new function for adducin. Calcium/calmodulin-regulated capping of the barbed ends of actin filaments. *J. Biol. Chem.* 271, 7986–7991.
10. Mejillano, M.R., Kojima, S., Applewhite, D.A., Gertler, F.B., Svitkina, T.M., and Borisy, G.G. (2004). Lamellipodial versus filopodial mode of the actin nanomachinery: pivotal role of the filament barbed end. *Cell* 118, 363–373.
11. Menna, E., Disanza, A., Cagnoli, C., Schenk, U., Gelsomino, G., Frittoli, E., Hertzog, M., Offenhauser, N., Sawallisch, C., Kreienkamp, H.J., et al. (2009). Eps8 regulates axonal filopodia in hippocampal neurons in response to brain-derived neurotrophic factor (BDNF). *PLoS Biol.* 7, e1000138.
12. Nestor, M.W., Cai, X., Stone, M.R., Bloch, R.J., and Thompson, S.M. (2011). The actin binding domain of beta-spectrin regulates the morphological and functional dynamics of dendritic spines. *PLoS One* 6, e16197.
13. Matsuoka, Y., Li, X., and Bennett, V. (2000). Adducin: structure, function and regulation. *Cell Mol. Life Sci.* 57, 884–895.
14. Xu, T., Yu, X., Perlik, A.J., Tobin, W.F., Zweig, J.A., Tennant, K., Jones, T., and Zuo, Y. (2009). Rapid formation and selective stabilization of synapses for enduring motor memories. *Nature* 462, 915–919.
15. Porro, F., Rosato-Siri, M., Leone, E., Costessi, L., Iaconcig, A., Tongiorgi, E., and Muro, A.F. (2010). beta-adducin (Add2) KO mice show synaptic plasticity, motor coordination and behavioral deficits accompanied by changes in the expression and phosphorylation levels of the alpha- and gamma-adducin subunits. *Genes Brain Behav.* 9, 84–96.
16. Rabenstein, R.L., Addy, N.A., Caldarone, B.J., Asaka, Y., Gruenbaum, L.M., Peters, L.L., Gilligan, D.M., Fitzsimonds, R.M., and Picciotto, M.R. (2005). Impaired synaptic plasticity and learning in mice lacking beta-adducin, an actin-regulating protein. *J. Neurosci.* 25, 2138–2145.
17. Castillo, P.E., Janz, R., Sudhof, T.C., Tzounopoulos, T., Malenka, R.C., and Nicoll, R.A. (1997). Rab3A is essential for mossy fibre long-term potentiation in the hippocampus. *Nature* 388, 590–593.
18. Gruenbaum, L.M., Gilligan, D.M., Picciotto, M.R., Marinesco, S., and Carew, T.J. (2003). Identification and characterization of Aplysia adducin, an Aplysia cytoskeletal protein homologous to mammalian adducins: increased phosphorylation at a protein kinase C consensus site during long-term synaptic facilitation. *J. Neurosci.* 23, 2675–2685.
19. Gotoh, H., Okumura, N., Yagi, T., Okumura, A., Shima, T., and Nagai, K. (2006). Fyn-induced phosphorylation of beta-adducin at tyrosine 489 and its role in their subcellular localization. *Biochem. Biophys. Res. Commun.* 346, 600–605.
20. Pariser, H., Herradon, G., Ezquerro, L., Perez-Pinera, P., and Deuel, T.F. (2005). Pleiotrophin regulates serine phosphorylation and the cellular distribution of beta-adducin through activation of protein kinase C. *Proc. Natl. Acad. Sci. USA* 102, 12407–12412.

The Picower Institute for Learning and Memory, Department of Biology and Department of Brain and Cognitive Sciences, Massachusetts Institute of Technology, Cambridge, MA 02139, USA.
E-mail: stevensr@mit.edu, troy@mit.edu

DOI: 10.1016/j.cub.2011.04.020