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Neuronal Homeostasis Through Translational Control

Richard A. Baines*

Neuroscience Group, Department of Biological Sciences, University of Warwick, Coventry, CV4 7AL, United Kingdom

Abstract

Translational repression is a key component of the mechanism that establishes segment polarity during early embryonic development in the fruitfly *Drosophila melanogaster*. Two proteins, Pumilio (Pum) and Nanos, block the translation of *hunchback* messenger RNA in only the posterior segments, thereby promoting an abdominal fate. More recent studies focusing on postembryonic neuronal function have shown that Pum is also integral to numerous mechanisms that allow neurons to adapt to the changing requirements placed on them in a dynamic nervous system. These mechanisms include those contributing to dendritic structure, synaptic growth, neuronal excitability, and formation of long-term memory. This article describes these new studies and highlights the role of translational repression in regulation of neuronal processes that compensate for change.

Index Entries: Excitability; glutamate; neural activity; neuromuscular junction; Nanos; paralytic; pumilio; translational repression.

Introduction

During a lifetime, neurons must face and adapt to the many changing demands placed on them. Although many of these changes go hand-in-hand with the developmental mechanisms that underpin circuit formation, growth, and synaptic plasticity, others can arise from unforeseen events, such as disease or injury. Despite these ever-changing demands, neurons show a remarkable capacity for compensation (reviewed in ref. *1*). Four recent studies *(2–5)*, each capitalizing on the molecular strengths offered by *Drosophila*, highlight the role of translational repression—mediated at least partly by Pumilio (Pum)—to orchestrate the mechanisms through which this compensation may be achieved.

Pumilio was first identified from a screen of *Drosophila* embryonic polarity mutants. Determination of anterior structures requires the activity of Hunchback (Hb) that is sufficient to

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*Author to whom correspondence and reprint requests should be addressed. E-mail: rbaines@bio.warwick.ac.uk

repress genes required for specification of the abdominal fate *(6–8)*. However, to allow proper development of the posterior, the activity of Hb must be suppressed in the abdomen. Repression is achieved through the inhibition of translation of *hb* messenger RNA (mRNA) mediated by a complex of proteins, including Pum *(9–13)*.

The first step of repression begins with the binding of Pum to specific sequence motifs, termed Nanos response elements (NREs), located in the 3'-untranslated region (UTR) of *hb* mRNA. The *hb* NRE contains two subelements, each including a defining UGU base sequence that is essential for binding of Pum *(9)*. Once bound, Pum is able to recruit two essential cofactors, Nanos (Nos) and Brain tumor (Brat), to form a quaternary complex with *hb* mRNA. This complex is sufficient to prevent translation. The mechanism of binding as well as translational repression has been reviewed in detail elsewhere *(14–16)*. The purpose of this article is to describe more recent studies that collectively show that Pum is also required for the correct development and functioning of the nervous system in flies.

Pumilio Shapes the Development of Dendritic Structure

The establishment of appropriate dendritic morphology is not only an essential prerequisite for circuit formation but also underlies changes in synaptic connectivity that arise from growth of an organism and changes that support learning. Although signaling between dendrites is an established concept *(17)*, the responsible mechanisms remain poorly understood. A characteristic of dendritic fields of mammalian neurons—for example, in both the cerebral cortex and retina—is that they occupy neighboring but nonoverlapping territories *(18–21)*. This resultant tiling of dendritic fields represents an economical coverage of two-dimensional receptive domains that is presumably maintained by persistent repulsive interactions. Clearly, proteins involved in this repulsion could be synthesized in the cell soma and transported to distal sites in the dendritic field. Alternatively, local RNA translation within dendrites might allow neurons to better maintain continual structural (and functional) plasticity only at required locations. Although the presence of mRNAs in dendrites is well-documented *(22,23)*, our understanding of the regulatory mechanisms controlling protein synthesis in these neuronal compartments is lacking.

Peripheral neurons located in the body wall of *Drosophila* larvae offer an experimentally accessible model for the analysis of dendritic development. Through expression of a green fluorescent protein-reporter, it is possible to visualize the dendritic fields of so-called dendritic arborization (da) neurons directly through the external cuticle. Similar to mammalian neurons, these neurons tile their dendritic fields. Removal of a single da neuron by targeted cell ablation results in a compensatory expansion of neighboring tiles to occupy the vacated territory. This observation clearly demonstrates that boundary disputes continually exist between adjacent dendritic fields *(24)*.

Ye and colleagues *(2)* used immunolocalization to demonstrate that both Pum and Nos are present in da neurons and, moreover, that Nos staining can be localized to punctate structures in the dendrites. The characteristics of these structures closely resemble those of RNA granules previously observed in mammalian cortical neurons *(25)*. Upregulation of either Pum or Nos (through expression of *pum* or *nos* transgenes, respectively) in a subset of these neurons (termed classes III and IV) is sufficient to reduce higher order branching of the dendritic field, whilst the morphology of the major branches remains unaffected.

Aberrations of dendritic patterning are also observed in loss-of-function alleles of both *pum* and *nos*, which, in a significant minority of neurons, results in an incomplete tiling between adjacent dendritic fields *(2)*. The findings that overexpression of *nos* is without significant effect in the absence of *pum* and that double loss-of-function mutants of both *nos* and *pum* exhibit no more severe phenotype

than either mutant alone indicate that both *pum* and *nos* are simultaneously required for normal dendritic patterning *(2)*. Therefore, although the mRNAs influenced have yet to be identified, the logical conclusion from this work is that translational repression, mediated by Pum and Nos, is an important part of the regulatory machinery that ensures appropriate dendritic development and remodeling in da neurons.

Pumilio Regulates Synaptic Development at the Neuromuscular Junction

During the course of development, complex organisms increase in size. Such growth is particularly challenging for the nervous system because changes in cell size demand compensatory changes in synaptic efficacy if excitation is to remain constant. Compensatory changes fall into one of three broad categories: (a) changes in presynaptic release that may or may not involve expansion of presynaptic terminals; (b) changes in postsynaptic receptor sensitivity; and (c) changes in voltage-gated conductances (*see* refs. *1* and *26*). Two recent studies showed that Pum is a common focus of all three mechanisms *(3,4)*.

The glutamatergic neuromuscular junction (nmj) of *Drosophila* has provided great insight into the molecular mechanisms underpinning both developmental and activity-dependent changes in synaptic efficacy that maintain synaptic depolarization within predetermined physiological limits (reviewed in ref. *26*). Of the approx 30 muscles comprising each hemi-segment of the *Drosophila* larva, Menon and coworkers *(3)* focused on muscle 12. This muscle is innervated by more than one motoneuron, the terminals of which can be distinguished based on size. Innervation by the RP5 motoneuron gives rise to larger boutons (termed Ib), whereas smaller boutons (termed Is) arise from additional motoneurons yet to be conclusively identified *(27)*.

In loss-of-function alleles of *pum*, the span of the nmj (the distance between the two furthest Ib boutons) is significantly reduced. The number of Ib boutons is also decreased, although the size of the remaining Ib boutons is larger *(3)*. This phenotype is indicative of a failure of the Ib boutons to separate during normal growth. Conversely, Is boutons are unaffected morphologically but are increased in number. That Pum seems essential for normal growth of the nmj parallels, its requirement for correct aborization of sensory neurons *(2)*, is indicative perhaps of a common pathway to control growth.

Presynaptic expression of a full-length *pum* transgene is sufficient to rescue the effects to only the Ib boutons, but not the increase in Is boutons, which seemingly result from a lack of postsynaptic *pum*. However, the most dramatic phenotype observed because of the loss of *pum* is a large increase in accumulation of the translation initiation factor eIF-4E in the postsynaptic muscle. This increase is restricted to the nmj, because levels of eIF-4E immunostaining remain normal throughout the rest of the muscle cell.

Upregulation of eIF-4E in *Drosophila* muscle has previously been reported to increase both bouton number and immunostaining for the DGluR-IIA glutamate receptor subtype in the postsynaptic membrane *(28)*. Against this backdrop, it is gratifying that increased staining for DGluR-IIA was also a consequence observed in the loss-of-function alleles of *pum (3)*. Overexpression of eIF-4E is also sufficient to reduce immunostaining for the cell adhesion molecule Fasciclin II at this nmj *(28)*, which may also contribute to the alteration of structure at this synapse. In the absence of *pum*, spontaneous release of transmitter (that persists in the absence of action potential firing) is significantly increased in frequency but not amplitude, indicating that the additional DGluR-IIA receptors formed are being targeted to *de novo* receptor fields to serve the expansion in Is boutons. Surprisingly, however, action-potential-evoked release of transmitter shows no increase in amplitude *(3)*.

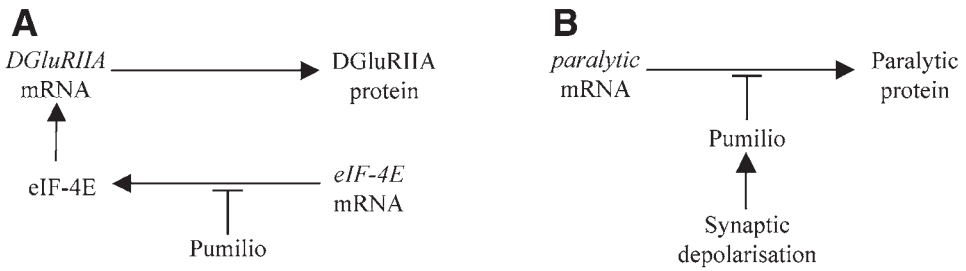

Fig. 1. Translational repression mediated by Pum regulates synaptic function and neuronal excitability. **(A)** In postsynaptic muscle cells, the presence of Pum translationally regulates the level of the translation factor eIF-4E. This factor is required for the translation of *DGluRIIA* messenger RNA to produce functional glutamate receptor subtypes that underlie synaptic transmission at the neuromuscular junction *(3)*. **(B)** In central neurons, Pum is both necessary and sufficient to regulate the magnitude of voltage-gated Na^+ conductance that is mediated by the protein product of the *paralytic* gene *(4)*. The level of *pum* mRNA (and presumably Pum) is regulated by synaptic depolarisation indicative that this translational cascade forms part of a regulatory mechanism that matches membrane excitability to synaptic depolarization.

Comparatively, upregulation of eIF-4E in postsynaptic muscle is sufficient to increase evoked junctional current without change to quantal size *(28)*. The finding that genetic overexpression of this receptor subtype in muscle is sufficient to phenocopy both consequences of upregulation of *eIF-4E* supports the finding that these changes are brought about by increased expression of DGluR-IIA *(29)*. Interestingly, loss-of-function mutants of *pum* do not fully recapitulate the effects to synaptic physiology following upregulation of eIF-4E, which underlines the fact that additional studies are required. Perhaps the most obvious reason for this discrepancy is that Pum may additionally regulate the translation of other mRNAs at the nmj, which act to prevent increase in quantal content.

Data provided by Menon et al. *(3)* support a model in which Pum translationally represses *eIF-4E*, which, in turn, regulates translation of DGluR-IIA receptors at the nmj (Fig. 1A). This control is likely to be activity-dependent, because postsynaptic aggregates of eIF-4E immunostaining are elevated in hyperexcitable genetic backgrounds *(28)*. The observation that a 51-nucleotide portion of the 3′-UTR of *eIF-4E* mRNA is capable of binding Pum supports this finding. This sequence contains an NRE motif, although, surprisingly, mutation of either or both UGU-containing subelements does not abolish binding. This finding raises an important issue regarding the binding specificity of differing mRNAs for Pum. Thus, in addition to being able to bind to *hb* (and *bicoid*) mRNAs through canonical NRE motifs *(9)*, Menon et al. *(3)* conclusively demonstrated that Pum can also bind to the *eIF-4E* 3′-UTR in their absence. Recent reports of crystal structures for the RNA-binding domain of *Drosophila* Pum revealed an extended region predicted to be capable of binding RNA *(30,31)*. It is tempting to speculate that specificity of binding to mRNAs may be dictated by the presence of cofactors, which include, but may not be limited to, Nos and Brat.

Pumilio Regulates Electrical Firing Properties in Motoneurons

The third category of compensatory change observed in nervous systems involves modification to voltage-gated conductances. For example, when deprived of synaptic excitation mammalian cortical neurons exhibit altered voltage-gated conductances, the most notable of which is a large upregulation of voltage-gated Na^+ conductance. These changes result in an increase in membrane excitability that

strives to maintain action potential firing within physiological levels *(32)*. More recently, the same phenomenon was described in motoneurons in *Drosophila*. Genetic manipulations that remove synaptic excitation during embryogenesis result in neurons with increased membrane excitability, which, again, largely results from increased voltage-gated Na^+ conductance *(33)*. On the other hand, in the same neurons, exposure to increased synaptic excitation is met by a reduced Na^+ conductance and decreased membrane excitability *(34)*. At least part of this mechanism is a rapid protein-kinase-A-mediated effect that is regulated by activity, which is sufficient to modulate voltage-gated Na^+ conductance. The mechanism is most likely through phosphorylation of the voltage-gated Na^+ channel *(34–37)*. However, whereas rapid changes in synaptic excitation may be best compensated for by equally rapid posttranslational modifications of key ionic conductances, longer term changes in neuronal activity may be better compensated for by changes in gene expression and/or translation.

An indication that translational repression may contribute to regulation of membrane excitability was the demonstration that neuronal excitability was increased at the *Drosophila* nmj in loss-of-function alleles of *pum (38)*. In the absence of *pum*, repetitive stimulation of motor axons results in the earlier onset of long-term facilitation, an effect that can be mimicked either by upregulation of voltage-gated Na^+ or suppression of K^+ conductances *(39,40)*. Strikingly, upregulation of Pum in motoneurons through expression of a full-length *pum* transgene is sufficient to completely abolish the onset of long-term facilitation *(41)*. A recent study by Mee et al. *(4)* provided a mechanistic understanding for these observations as well as conclusive evidence that Pum is able to regulate voltage-gated conductances.

Whole-cell voltage clamp recordings from identified *Drosophila* larval motoneurons demonstrates that the persistent component of the voltage-gated Na^+ conductance (termed $I_{Na(p)}$) is significantly greater in a loss-of-function allele of *pum*. Comparatively, overexpression of a full-length *pum* transgene is sufficient to reduce both $I_{Na(p)}$ and the transient component of this same current ($I_{Na(t)}$). These changes are associated with either an increase or decrease in membrane excitability, respectively *(4)*. The observation that the expression of *pum* is activity-dependent indicates that the compensatory effect forms part of a homeostatic mechanism. Therefore, in genetic backgrounds in which neuronal activity is hypoactive, the level of *pum* mRNA (determined by quantitative real-time polymerase chain reaction) is reduced approx threefold. In hyperactivity backgrounds, expression is elevated by the same approximate level *(4)*. The model supported by these data (Fig. 1B) is one in which increased exposure to synaptic excitation is compensated for by an increase in expression of *pum* and vice versa. Together, these changes are consistent with a Pum-dependent repression of *paralytic* mRNA that encodes the sole voltage-gated Na^+ conductance in these motoneurons. It remains to be shown whether this repression is translational or transcriptional.

The consequence of reducing Na^+ conductance is to lower membrane excitability, thereby preventing overexcitation resulting from the increased exposure to synaptic excitation. In an interesting twist, it is the 5′-UTR of *paralytic* mRNA that contains an NRE-like sequence, but binding studies have not been able to demonstrate a specific interaction between this motif and Pum. However, because these motifs are apparently not required for binding of Pum to *eIF-4E* mRNA *(3)*, lack of binding of the 5′-UTR may not be entirely unexpected. Binding of Pum to *paralytic* 3′-UTR that does not contain NRE motifs has yet to be tested.

Pumilio Is Required for Long-Term Memory

An ability to influence neuronal excitability, dendritic arborisation, and synaptic development suggests that Pum is a key player in the formation of memory. This involvement is demonstrated by the fourth study. Dubnau

and colleagues *(5)* employed a creative combination of forward- and reverse-genetics to isolate genes exhibiting altered regulation during learning. A micro-array comparison was performed to identify transcriptional changes between flies trained to avoid an olfactory cue by pairing with a noxious footshock either in spaced bouts (known to result in stronger memory formation) or in flies trained to avoid an olfactory cue by massed training. To identify genes that might not show transcriptional change but that are key to memory formation, a large-scale behavioral screen of single-gene mutants was also performed. Both approaches implicated *pum* as a requirement for normal long-term memory. Array analysis reported a significant increase in *pum* transcript following spaced training, whereas loss of *pum* resulted in poor memory scores on behavioral tests. Particularly notable, however, is that this study also identified additional genes predicted to have roles in local control of RNA translation, indicating that such mechanisms may be widespread in the nervous system. The additional genes identified included *staufen*, *oskar*, *CPEB*, *eIF-2G*, and *eIF-5C*.

How General Is Pumilio-Dependent Repression of Translation in Nervous Systems?

There are many reasons to suggest that Pum and Nos might play similar roles in the nervous systems of higher organisms. Perhaps the most compelling of these reasons is that numerous studies have shown that the same genes, the same cellular processes, and the same mechanisms are used to build a nervous system in the fly, the worm, and mammals. Pum was the first member of the Puf protein family, named after the founding members, **Pu**m and *Caenorhabditis elegans* **f**em-3 binding factor. Additional family members have been identified in plants, fungi, and vertebrates. The Puf domain consists of eight tandem repeats of approx 36 amino acids constituting the minimal RNA binding domain *(42–44)*. Two Pum homologs are present in both mouse and human, which share 75% identity with *Drosophila* Pum; the Puf domain is 91% identical *(45,46)*. Consistent with a role in regulation of neuronal function, human Pum proteins are expressed in central nervous system and, moreover, are capable of binding NRE-containing *hb* mRNA with an affinity exceeding that of *Drosophila* Pum *(9,47)*.

In addition to Pum, two *nos* genes have been identified in humans *(48)*, and three have been identified in mice *(49,50)*. Expression of *nos* is observed in murine brain and, particularly, in the hippocampus, which is a critical locus for the formation of long-term memory. However, knockout of *nos1* in mice did not lead to obvious gross anatomical or behavioral defects *(49)*. In this instance, lack of a phenotype might have been caused by functional redundancy between the multiple *nos* genes present in this animal. In the light of the present studies in *Drosophila*, it may be worthwhile to revisit this knockout and to conduct more detailed analysis on dendritic morphology, neuronal function, and learning.

Does Pumilio Regulate Translation of Trafficked mRNAs in Neurons?

Compartment-specific translation of mRNAs underlies at least two important roles in neuronal signaling. First, it allows for rapid changes to synaptic function, avoiding the need for relatively slow retrograde communication toward, and subsequent alteration of gene expression in, the distant cell body. Second, it has the potential to allow synapse-specific changes to occur (for reviews, *see* refs. *51* and *52*). The list of identified mRNAs found in dendrites (in a variety of neurons and species) continues to grow in number and includes those for the key signaling molecules calmodulin, CamKII, and glutamate receptors (both *N*-methyl-D-aspartate [NMDA] and non-NMDA). For local translation to occur, it is implicit that specific mRNAs be "tagged" for transport to dendritic regions, transported by RNA-binding proteins, and retained after

reaching their final destination. Targeting motifs are clearly contained within the 3′-UTR of α-CamKII mRNA, because replacement of this UTR results in a failure of mRNA to leave the cell body *(53)*. Well-characterized RNA binding proteins include *Drosophila* Staufen and Oskar and vertebrate cytoplasmic polyadenylation element-binding protein (for review, *see* ref. *54*). Throughout this journey, it is essential that transported and localized mRNAs be prevented from being translated until such time that their protein products are required. The four studies reported here provide convincing evidence to indicate that Pum may serve an important role in this regard.

References

1. Turrigiano G. G. and Nelson S. B. (2000) Hebb and homeostasis in neuronal plasticity. *Curr. Opin. Neurobiol.* **10,** 358–364.
2. Ye B., Petritsch C., Clark I. E., Gavis E. R., Jan L. Y., and Jan Y. H. (2004) *nanos* and *pumilio* are essential for dendrite morphogenesis in *Drosophila* peripheral neurons. *Curr. Biol.* **14,** 314–321.
3. Menon K. P., Sanyal S., Habara, Y., et al. (2004) The translational repressor Pumilio regulates presynaptic morphology and controls postsynaptic accumulation of translation factor eIF-4E. *Neuron.* **44,** 663–676.
4. Mee C. J., Pym E. C. G., Moffat K. G., and Baines R. A. (2004) Regulation of neuronal excitability through pumilio-dependent control of a sodium channel gene. *J. Neurosci.* **24,** 8695–8703.
5. Dubnau J., Chian A. S., Grady L., et al. (2003) The staufen/pumilio pathway is involved in *Drosophila* long-term memory. *Curr. Biol.* **13,** 286–296.
6. Tautz D. (1988). Regulation of *Drosophila* segmentation gene *hunchback* by two maternal morphogenetic centres. *Nature* **332,** 281–284.
7. Wharton R. P. and Struhl G. (1991) RNA regulatory elements mediate control of *Drosophila* body pattern by the posterior morphogen nanos. *Cell* **67,** 955–967.
8. Wharton R. P., Sonoda J., Lee T., Patterson M., and Murata Y. (1998) The Pumilio RNA-binding domain is also a translational repressor. *Mol. Cell* **1,** 863–872.
9. Zamore P. D., Williamson J. R., and Lehmann R. (1997) The Pumilio protein binds RNA through a conserved domain that defines a new class of RNA-binding proteins. *RNA* **3,** 1421–1433.
10. Wreden C., Verotti A. C., Schisa J. A., Lieberfarb M. E., and Strickland S. (1997) *Nanos* and *pumilio* establish embryonic polarity in *Drosophila* by promoting posterior deadenylation of *hunchback* mRNA. *Development* **124,** 3015–3023.
11. Sonoda J. and Wharton R. P. (1999) Recruitment of Nanos to hunchback mRNA by Pumilio. *Genes Dev.* **13,** 2704–2712.
12. Sonoda J. and Wharton R. P. (2001) *Drosophila* Brain Tumor is a translational repressor. *Genes Dev.* **15,** 762–773.
13. Chagnovich D. and Lehmann R. (2001) Poly(A)-independent regulation of maternal *hunchback* translation in the *Drosophila* embryo. *Proc. Natl. Acad. Sci. USA* **98,** 11,359–11,364.
14. Dean K. A., Aggarwal A. K., and Wharton R. P. (2002) Translational repressors in Drosophila. *Trends Neurosci.* **18,** 572-576.
15. Gavis E. R. (2001). Over the rainbow to translational control. *Nat. Struct. Biol.* **8,** 387–389.
16. Parisi M. and Lin H. (2000) Translational repression: A duet of nanos and pumilio. *Curr. Biol.* **10,** R81–R83.
17. Jan Y. H. and Jan L. Y. (2003) The control of dendrite development. *Neuron* **40,** 229–242.
18. Wässle H. and Boycott B. B. (1991) Functional architecture of the mammalian retina. *Physiol. Rev.* **71,** 447–478.
19. MacNeil M. A. and Masland R. H. (1998) Extreme diversity among amacrine cells: implications for function. *Neuron* **20,** 971–982.
20. Sestan N., Artavanis-Tsakonas S., and Rakic P. (1999) Contact-dependent inhibition of cortical neurite growth mediated by notch signalling. *Science* **286,** 741–746.
21. Logan M. A. and Vetter M. L. (2004) Do-it-yourself tiling: dendritic growth in the absence of homotypic contacts. *Neuron* **43,** 439–446.
22. Steward O. and Schuman E. M. (2001) Protein synthesis at synaptic sites on dendrites. *Annu. Rev. Neurosci.* **24,** 229–325.
23. Tang A. J. and Schuman E. M. (2000) Protein synthesis in the dendrite. *Phil. Trans. R. Soc. Lond. B.* **357,** 521–529.
24. Grueber W. B., Ye B., Moore A. W., Jan L. Y., and Jan Y. N. (2003) Dendrites of distinct classes of *Drosophila* sensory neurons show different capacities for homotypic repulsion. *Curr. Biol.* **13,** 618–626.

25. Knowles R. B., Sabry J. H., Martone M. E., et al. (1996) Translocation of RNA granules in living neurons. *J. Neurosci.* **16,** 7812–7820.
26. Davis G. W. and Bezprozvanny I. (2001) Maintaining the stability of neural function: a homeostatic hypothesis. *Annu. Rev. Physiol.* **63,** 847–869.
27. Davis G. W. and Goodman C. S. (1998) Synapse-specific control of synaptic efficacy at the terminals of a single neuron. *Nature* **392,** 82–86.
28. Sigrist S. J., Thiel P. R., Reiff D. F., Lachance P. E., Lasko P., and Schuster C. M. (2000) Postsynaptic translation affects the efficacy and morphology of neuromuscular junctions. *Nature* **405,** 1062–1065.
29. Sigrist S. J., Thiel P. R., Reiff D. F., and Schuster C. M. (2002) The postsynaptic glutamate receptor subunit DgluR-IIA mediates long-term plasticity in *Drosophila*. *J. Neurosci.* **22,** 7362–7372.
30. Edwards T. A., Pyle S. E., Wharton R. P., and Aggarwal A. K. (2001) Structure of Pumilio reveals similarity between RNA and peptide binding motifs. *Cell* **105,** 281–289.
31. Wang X., Zamore P. D., and Hall T. M. (2001) Crystal structure of a Pumilio homology domain. *Mol. Cell* **7,** 855–865.
32. Desai N. S., Rutherford L. C., and Turrigiano G. G. (1999) Plasticity in the intrinsic excitability of cortical pyramidal neurons. *Nat. Neurosci.* **2,** 515–520.
33. Baines R. A., Uhler J. P., Thompson A., Sweeney S. T., and Bate M. (2001) Altered electrical properties in *Drosophila* neurons developing without synaptic transmission. *J. Neurosci.* **21,** 1523–1531.
34. Baines R. A. (2003) Postsynaptic protein kinase A reduces neuronal excitability in response to increased synaptic excitation in the *Drosophila* CNS. *J. Neurosci.* **23,** 8664–8672.
35. Li M., West J. W., Lai Y., Scheuer T., and Catterall W. A. (1992) Functional modulation of brain sodium channels by cAMP-dependent phosphorylation. *Neuron* **8,** 1151–1159.
36. Smith R. D. and Goldin A. L. (1997) Phosphorylation at a single site in the rat brain sodium channel is necessary and sufficient for current reduction by protein kinase A. *J. Neurosci.* **17,** 6086–6093.
37. Catterall W. A. (2000) From ionic currents to molecular mechanisms: the structure and function of voltage-gated sodium channels. *Neuron* **26,** 13–25.
38. Stern M., Blake N., Zondlo N., and Walters K. (1995) Increased neuronal excitability conferred by a mutation in the *Drosophila* bemused gene. *J. Neurogenet.* **10,** 103–118.
39. Stern M. and Ganetzky B. (1989) Altered synaptic transmission in *Drosophila* hyperkinetic mutants. *J. Neurogenet.* **5,** 215–228.
40. Stern M., Kreber R., and Ganetzky B. (1990) Dosage effects of a *Drosophila* sodium channel gene on behavior and axonal excitability. *Genetics* **124,** 133–143.
41. Schweers B. A., Walters K. J., and Stern M. (2002) The *Drosophila melanogaster* translational repressor *pumilio* regulates neuronal excitability. *Genetics* **161,** 1177–1185.
42. Barker D. D., Wang C., Moore J., Dickinson L. K., and Lehmann R. (1992) Pumilio is essential for function but not for distribution of the *Drosophila* abdominal determinant Nanos. *Genes Dev.* **6,** 2312–2326.
43. Macdonald P. M. (1992) The *Drosophila* pumilio gene: an unusually long transcription unit and an unusual protein. *Development* **114,** 221–232.
44. Zhang B., Gallegos M., Puoti A., et al. (1997) A conserved RNA-binding protein that regulates sexual fates in the *C. elegans* hermaphrodite germ line. *Nature* **390,** 477–484.
45. Spassov D. S. and Jurecic R. (2002) Cloning and comparative sequence analysis of PUM1 and PUM2 genes, human members of the Pumilio family of RNA-binding proteins. *Gene* **299,** 195–204.
46. Spassov D. S. and Jurecic R. (2003) MousePum1 and Pum2 genes, members of the Pumilio family of proteins, show differential expression in fetal and adult hematopoietic stem cells and progenitors. *Blood Cells Mol. Dis.* **30,** 55–69.
47. White E. K., Moore-Jarrett T., and Ruley H. E. (2001) PUM2, a novel murine puf protein,and its consensus RNA-binding site. *RNA* **12,** 1855–1866.
48. Jaruzelska J., Kotecki M., Kusz K., Spik A., Firpo M., and Reijo Pera R. A. (2003) Conservation of a Pumilio-Nanos complex from *Drosophila* germ plasm to human germ cells. *Dev. Genes Evol.* **213,** 120–126.
49. Haraguchi S., Tsuda M., Kitajima S., et al. (2003) nanos1: a mouse nanos gene expressed in the central nervous system is dispensable for normal development. *Mech. Dev.* **120,** 721–731.
50. Tsuda M., Sasaoka Y., Kiso M., et al. (2003) Conserved role of nanos proteins in germ cell development. *Science* **301,** 1239–1241.
51. Lipshitz H. D. and Smibert C. A. (2000) Mechanisms of RNA localization and translational regulation. *Curr. Opin. Gen. Dev.* **10,** 476–488.
52. Steward O. and Schuman E. M. (2003) Compartmentalized synthesis and degradation of protein in neurons. *Neuron* **40,** 347–359.

53. Miller S., Yasuda M., Coats J. K., Jones Y., Martone M. E., and Mayford M. (2002) Disruption of dendritic translation of CaMKIIalpha impairs stabilization of synaptic plasticity and memory consolidation. *Neuron* **36,** 507–519.

54. Kiebler M. A. and DesGroseillers L. (2000) Molecular insights into mRNA transport and local translation in the mammalian nervous system. *Neuron* **25,** 19–28.