The *Drosophila melanogaster* Translational Repressor Pumilio Regulates Neuronal Excitability

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**ABSTRACT**

Maintenance of proper neuronal excitability is vital to nervous system function and normal behavior. A subset of *Drosophila* mutants that exhibit altered behavior also exhibit defective motor neuron excitability, which can be monitored with electrophysiological methods. One such mutant is the P element insertion mutant *bemused (bem)*. The *bem* mutant exhibits female sterility, sluggishness, and increased motor neuron excitability. The *bem* P element is located in the large intron of the previously characterized translational repressor gene *pumilio (pum)*. Here, by several criteria, we show that *bem* is a new allele of *pum*. First, ovary-specific expression of *pum* partially rescues *bem* female sterility. Second, *pum* null mutations fail to complement *bem* female sterility, behavioral defects, and neuronal hyperexcitability. Third, heads from *bem* mutant flies exhibit greatly reduced levels of Pum protein and the absence of two *pum* transcripts. Fourth, two previously identified *pum* mutants exhibit neuronal hyperexcitability. Fifth, overexpression of *pum* in the nervous system reduces neuronal excitability, which is the opposite phenotype to *pum* loss of function. Collectively, these findings describe a new role of *pum* in the regulation of neuronal excitability and may afford the opportunity to study the role of translational regulation in the maintenance of proper neuronal excitability.

**NEURONAL** excitability is regulated by the balance of Na⁺ and K⁺ currents. Mutations that cause an increase in the ratio of Na⁺ currents to K⁺ currents cause neurons to become hyperexcitable, whereas mutations that decrease this ratio reduce neuronal excitability. For example, duplication of the Na⁺ channel gene *para* or loss-of-function mutations in K⁺ channel genes *Shaker (Sh)* and *Hyperkinetic (Hk)* each increase the ratio of Na⁺ channels to K⁺ channels and thereby result in hyperexcitable motor neurons (Jan et al. 1977; Kamb et al. 1988; Loughney et al. 1989; Stern and Ganetzky 1989; Stern et al. 1990). Application of quinidine, which inactivates the delayed rectifier K⁺ channel, causes a similar increase in excitability (Wu et al. 1989; Stern and Ganetzky 1992). Alternatively, loss-of-function mutations in *para* reduce the Na⁺ channel/K⁺ channel ratio and thus decrease motor neuron excitability (Suzuki et al. 1971; Ganetzky and Wu 1982; Loughney et al. 1989; Stern et al. 1990).

At one synapse, the Drosophila larval neuromuscular junction (nmj), the most readily observable effect of increases in neuronal excitability is an increased rate of onset of a phenomenon termed either long-term facilitation (LTF) or augmentation (Jan and Jan 1978; Wang et al. 1994). LTF at the nmj is a phenomenon in which repetitive nerve stimulation at a sufficient frequency and duration causes subsequent nerve stimulation to evoke a prolonged release of neurotransmitter. This prolonged neurotransmitter release results from prolonged Ca²⁺ sensitivity of the presynaptic nerve terminal and causes a corresponding significant increase in the amplitude and duration of the response of the muscle cell (Jan and Jan 1978). For example, flies overexpressing either the *para* Na⁺ channel or the guanylate cyclase activator *frequenin* (*frq*) or carrying loss-of-function mutations of the K⁺ channel gene *Hk* each increase the rate of LTF onset at the nmj and hence are hyperexcitable (Stern and Ganetzky 1989; Stern et al. 1990; Rivosecchi et al. 1994).

Another mutant that exhibits an increased rate of LTF onset at the nmj is *bemused (bem; Stern et al. 1995)*. The *bem* mutation is caused by insertion of a single *PlaW* element into region 85D1, 2 of the polytene chromosome (Bier et al. 1989; Stern et al. 1995). More precise analysis (described below) revealed that this P element is located within the *pum* transcription unit. The *pum* gene has been studied in great detail and elucidation of the molecular mechanism by which *pum* functions in Drosophila embryogenesis has been well characterized (Barker et al. 1992; Macdonald 1992). Pum protein binds directly to specific sequences in the 3’ untranslated region (UTR) of maternally supplied *hunchback* (*hb*) mRNA (known as nanos-response elements or NREs) and then recruits at least two other proteins, Nanos (Nos) and Brain Tumor (Brat), to the mRNA (Murata...
MATERIALS AND METHODS

Drosophila stocks: All fly stocks were maintained on standard cornmeal/agar Drosophila media at room temperature (20–22°C). The pum− and pumrenovt lines (Lin and Spradling 1997) were kindly provided by the lab of Dr. Haifan Lin (Duke University, Durham, NC). The nos-pum rescue construct (Barker et al. 1992) and pum− parental control lines were kindly provided by the lab of Dr. Ruth Lehmann (New York University, New York). The Bloomington Stock Center provided pum− and pum13. The pum− mutants are all caused by F element mutations within the pum transcription unit (Lin and Spradling 1997). The pum1088 P element is located in the intron between exons 3 and 4, whereas the P element in all of the other pum− alleles is inserted into the large intron between exons 8 and 9 (Parisi and Lin 1999). The pumrenovt is a pum1088 line produced by precise excision of the P element. The pum− allele is an ethyl methanesulfonate (EMS)-induced A-to-T mutation at nucleotide 3890, which causes a premature stop codon at amino acid 949 and encodes a Pum protein product without an RNA-binding domain (Teare and Nusslein-Volhard 1987; Forbes and Lehmann 1998). Similarly, pum− is an EMS-induced deletion of nucleotides 4224–4498, resulting in production of a Pum protein product that lacks the RNA-binding domain (Teare and Nusslein-Volhard 1987; Forbes and Lehmann 1998). Finally, pum13 is an EMS-induced point mutation within the pum RNA-binding domain that results in a single amino acid substitution, G1350D. The encoded protein is able to bind hβ mRNA but is unable to repress translation (Tearle and Nusslein-Volhard 1987; Wharton et al. 1998). No molecular information has been reported on pum+. The isogenic wild-type strain from which bem was produced is referred to as bem+. The isogenic wild-type strain from which pum−, pum+, and pum13 were produced is referred to as pum+. The bem− and pum− chromosomes served as controls in the genetic experiments presented.

Expression studies: Northern blots were performed using mRNA extracted from 40 flies or 800 heads of each genotype. Flies were decapitated by freezing in liquid nitrogen and subsequent vigorous shaking through a U.S. standard sieve no. 25, which has an opening size of 710 μm. Preparation of mRNA was accomplished with the QIAGEN (Valencia, CA) Oligotex mRNA kit, according to the manufacturer’s instructions. The probe used was a PCR product produced from nucleotides 3600–4400 of the pum cDNA (Barker et al. 1992), obtained from the Lehmann lab. Results were visualized using a phosphorimaging system and Fuji Mac-BAS software.

Electrophysiological analyses: Dissections and muscle recordings from third instar larvae were performed as described previously (Jan and Jan 1976; Stern et al. 1995). For measurement of LTF onset rates, larvae were bathed in saline containing 0.1 mm quinidine and 0.15 mm Ca2+. LTF onset rate was measured following nerve stimulation at the indicated frequencies. For measurement of failure rates, the nerves were stimulated for 10 sec at a frequency of 1 Hz. The number of stimuli per 10-sec stimulation train that failed to evoke any muscular response was recorded. Failure rate analysis was performed in the absence of quinidine and at a Ca2+ concentration of either 0.15 mm or 0.10 mm.

The pum and bem mutations were balanced over a TM6 balancer chromosome marked with the dominant Tubby (Tb) marker, which allowed larvae of the desired genotype to be recognized.

Behavioral analyses: Negative gravitaxis experiments were performed by placing single 6-day-old males of the appropriate genotype in an empty vial. The fly was banged to the bottom of the vial and we recorded the time required for the fly to right itself and climb 5 cm. Flight experiments were performed by emptying single flies from vials onto a flat and clean tabletop. When necessary, flies were encouraged to fly by prodding with a paintbrush. Any fly that was unable to achieve flight after 5 sec of prodding was deemed a nonflier.

Production of transgenic flies: The pum coding sequence was removed from the pNB40/R7-1 plasmid (Barker et al. 1992) with digestion with Nhel and XhoI. The pum sequence was then cloned into the XhoI site of pUAST (Brand and
found that \( pum^{7} \), \( pum^{9} \), and \( pum^{11} \) are each recessive for this fertility defect (data not shown), and all fail to complement \( bem \) female sterility (Table 1). These data strongly support the conclusion that \( bem \) female sterility is caused by improper \( pum \) function. Also, these results show that the Pum protein function in female fertility requires a functional RNA-binding domain.

**Rescue of \( bem \) female sterility:** If sterility in \( bem \) females is due to loss of \( pum \) function, then this phenotype should be rescued by expression of \( pum^{+} \) in transgenic flies. We found that fertility was significantly, but not completely, restored in \( bem \) females expressing a \( pum \) transgene under the transcriptional control of \( nos \) (Barker et al. 1992), which is expressed in the ovary (Figure 2). The difference in fertility seen between the rescued \( bem \) mutants and wild-type females might be an effect of insufficient Pum protein in the ovaries due to

**TABLE 1**

<table>
<thead>
<tr>
<th>Genotype</th>
<th>No. of offspring per female</th>
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<tbody>
<tr>
<td>( bem^{-} / bem^{-} ) (( n = 7 ))</td>
<td>( 60 \pm 10.6 )</td>
</tr>
<tr>
<td>( bem / bem^{+} ) (( n = 7 ))</td>
<td>( 74 \pm 9.3 )</td>
</tr>
<tr>
<td>( bem / bem ) (( n = 6 ))</td>
<td>( 1 \pm 0.4^{*} )</td>
</tr>
<tr>
<td>( pum^{790} / bem ) (( n = 9 ))</td>
<td>( 52 \pm 7.0 )</td>
</tr>
<tr>
<td>( pum^{790} / bem ) (( n = 10 ))</td>
<td>( 5 \pm 2.2^{*} )</td>
</tr>
<tr>
<td>( pum^{7} / bem ) (( n = 10 ))</td>
<td>( 9 \pm 3.4^{*} )</td>
</tr>
<tr>
<td>( pum^{7} / bem ) (( n = 10 ))</td>
<td>( 1 \pm 0.4^{*} )</td>
</tr>
<tr>
<td>( pum^{11} / bem ) (( n = 18 ))</td>
<td>( 6 \pm 1.0^{*} )</td>
</tr>
<tr>
<td>( pum^{11} / bem ) (( n = 9 ))</td>
<td>( 77 \pm 3.6 )</td>
</tr>
</tbody>
</table>

Means and standard errors (unpaired \( t \)-test) of offspring number from the indicated genotypes are presented. \( *P < 0.0001 \) vs. \( bem^{+} / bem^{-} \).
the presence of only one copy of the nos-pum rescue construct. Alternatively, this difference might reflect a role for pum in female fertility in a tissue that does not express nos. These results, in addition to the complementation results described above, demonstrate that the fertility defects seen in bem mutant females are indeed caused by improper pum function. These observations led us to investigate the possibility that the behavioral and electrophysiological defects seen in bem mutants are caused by disruption of Pum function as well.

Complementation of bem behavioral defects: We tested several pum alleles to determine if they failed to complement the behavioral phenotypes of bem. Flies heterozygous for the bem P element and pum2003, pum7, and pum9 were produced and we found that, in each case, the pum allele was unable to complement the negative gravitaxis and flight defects observed in bem (Figure 3; data not shown). These experiments demonstrate that the behavioral defects exhibited by bem mutants are caused by disruption of pum function. In contrast, pum1688 was able to complement bem behavioral defects (Figure 3A), similar to its ability to complement the fertility defects. pum1688 might exhibit different properties from other pumovt alleles because it is located in a different region of pum.

Neuronal excitability defects of pummutants: To investigate the possibility that previously characterized pum mutants exhibit a neuronal hyperexcitability similar

**Figure 2.** Ovarian expression of pum is able to rescue bem female sterility. The means and standard errors (unpaired t-test) of offspring number from single females of the indicated genotypes are presented. For bem/bem, n = 6 and for bem/bem; nos-pum, bem+/bem and bem/bem, n = 7. (*) P < 0.0001 vs. bem/bem.

**Figure 3.** Several pum alleles fail to complement bem behavioral defects. The means and standard errors (unpaired t-test) of climbing times from the indicated genotypes are presented. (A) Results obtained from tests with pummutants. (*) P = 0.0007, (**) P < 0.0001 vs. bem+/bem. The sample sizes were as follows: bem+/bem (n = 23), bem/bem (n = 12), pum2003/bem (n = 33), pum2003/bem (n = 33), pum2003/bem (n = 27), pum2003/bem (n = 27), pum2003/bem (n = 27), pum2003/bem (n = 15), pum2003/bem (n = 34). (B) Results obtained from tests with EMS-induced pum alleles. (*) P < 0.0001 vs. bem+/bem. The sample sizes were as follows: bem+/bem (n = 23), bem/bem (n = 12), pum7/bem (n = 29), pum7/bem (n = 27), pum7/bem (n = 27), pum7/bem (n = 41), pum9/bem (n = 37), pum9/bem (n = 17).
Figure 4.—pum alleles exhibit neuronal defects and fail to complement bem neuronal hyperexcitability. LTF onset rates were determined in the presence of 0.1 mM quinidine and at an external Ca\(^{2+}\) concentration of 0.15 mM. (A) Representative traces showing LTF onset at the nnj for the indicated genotypes. Black arrowheads indicate LTF onset. (B–D) Means and standard errors of LTF onset rates for the indicated genotypes at the indicated stimulation frequencies (hertz). (B) The neuronal excitability defects of pumovt mutants: For bem/bem, \(n = 22\); for bem/bem, \(n = 12\); for bem/bem, \(n = 8\); for bem/bem, \(n = 6\). (C) The inability of pum\(^{7}\) to complement bem neuronal hyperexcitability: For bem/pum\(^{7}\), \(n = 8\); for bem/pum\(^{7}\), \(n = 8\); for bem/pum\(^{7}\), \(n = 8\); for bem/pum\(^{7}\), \(n = 7\). (D) The inability of pum\(^{9}\) to complement bem neuronal hyperexcitability: For bem/pum\(^{9}\), \(n = 6\); for bem/pum\(^{9}\), \(n = 5\); for bem/pum\(^{9}\), \(n = 8\); for bem/pum\(^{9}\), \(n = 7\).

The LTF onset rates of pum\(^{2003}\) and pum\(^{4806}\) are virtually indistinguishable from the rate observed in bem mutants. These findings support the possibility that bem neuronal hyperexcitability is due to improper Pum function.

**Complementation of bem neuronal excitability defects:** Next, we investigated the ability of pum\(^{7}\) and pum\(^{9}\) to complement bem neuronal hyperexcitability. Third instar larvae of genotypes bem/pum\(^{7}\) and bem/pum\(^{9}\) were produced and the rate of LTF onset was determined. We found that these larvae exhibited a rate of LTF onset that was significantly faster than that of wild-type control larvae as well as that of larvae heterozygous for pum\(^{7}\), pum\(^{9}\), and bem (Figure 4, C and D). The observation that pum\(^{7}\) and pum\(^{9}\) are unable to complement the hyperexcitability defect seen in bem neurons shows that bem hyperexcitability is due to improper Pum function.

**Expression of pum mRNA in bem and pum\(^{48}\) mutants:** We found pum transcripts in both males and females (Figure 5A), which is predicted on the basis of the observation of bem mutant phenotypes in both males and females. Also, improper transcription of pum was found in bem mutants. In particular, the larger transcript in bem adults appeared to be smaller than the largest transcript in wild-type control adults. We also observed a striking difference between the transcripts present in bem and wild-type heads (Figure 5B). In particular, wild-type heads express four different pum mRNA species corresponding to the apparent molecular sizes of 9.0
and 6.7 kb as well as a doublet at 8.1 kb. We found that the 9.0-kb transcript and the upper band of the 8.1-kb doublet are absent from bem heads (Figure 5B). A final Northern blot was performed to investigate pum mRNA expression in the heads of previously generated and characterized pum<sup>wt</sup> mutants. pum<sup>203</sup>, which was shown to be unable to complement bem behavioral defects, and pum<sup>1688</sup>, which complements these defects, are each missing the same 9.0-kb head transcript that is absent from bem heads (Figure 5C). Although these findings show that the bem P element is indeed affecting pum expression in adult flies and especially in heads, they do not allow determination of which pum transcripts are needed for proper neuronal function. However, these results do show that the 9.0-kb transcript is not necessary in heads for proper behavior.

**Pum protein expression in bem and pum<sup>wt</sup> mutants:** Western blot analysis was performed to determine the effect of bem and pum<sup>wt</sup> mutations on Pum protein levels. We found that Pum protein is greatly reduced in abundance in heads from bem mutants as well as some pum<sup>wt</sup> mutants, when compared to bem<sup>+</sup> parental and pum<sup>wt</sup> revertant controls (Figure 6). The parental wild-type and revertant control heads abundantly express three different protein isoforms corresponding to the apparent molecular sizes of 156, 150, and 93 kD (resolution of these bands was greatly enhanced upon shorter exposure times, data not shown), whereas greatly reduced amounts of all three isoforms are present in the bem heads (Figure 6, also see Parisi and Lin 1999). Also, pum<sup>1688</sup> is missing the 156-kD isoform whereas pum<sup>1203</sup> and pum<sup>6897</sup> are missing both the 156- and 130-kD isoforms (Figure 6). Because pum<sup>1688</sup> is able to complement bem behavioral defects while pum<sup>1203</sup> is not, these results demonstrate that the 156-kD isoform is not necessary for Pum function in controlling behavior (Figure 3). The genetic and molecular data presented here show that all of the bem mutant phenotypes are due to improper pum function. Therefore, the bem mutation is allelic to pum and we recommend that bem henceforth be referred to as pum<sup>wt</sup>

**Overexpression of pum in the nervous system:** We utilized the UAS/GAL4 system to examine the effect of overexpressing Pum protein in the nervous system. Transgenic flies carrying UAS-pum were generated and crossed to flies carrying an elav-GAL4 transgene, which produces Gal4 protein specifically in postmitotic neurons. From electrophysiological analysis of third instar larvae, we found that overexpression of pum prevents LTF onset in motor neurons even after 90 sec of 10-Hz stimulation. In contrast, larvae that express either UAS-pum or elav-GAL4 alone exhibit normal LTF onset rates (data not shown).

During these studies we noted that larvae overexpress-
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Responded to nerve stimulation with a higher frequency of failure of excitatory junctional potentials (ejp's) than did controls in saline containing low Ca²⁺ (0.15 mM). In particular, we found that nerve stimuli were successful in eliciting an ejp only 20% of the time in the pum overexpression lines, whereas nerve stimuli elicited an ejp ~80% of the time at nmj’s from wild-type and control lines (Figure 7B). Thus, pum overexpression reduces transmitter release at low Ca²⁺. In contrast, we found that nmj’s from the hyperexcitable pum⁸⁸ loss-of-function mutants respond to nerve stimulation with an ejp 95% of the time. This result suggests that loss of pum in neurons increases transmitter release at low Ca²⁺ (Figure 7B). The difference between pum⁸⁸ and wild-type failure rates is even more evident at lower external Ca²⁺ (0.10 mM) at which stimulations to wild-type neurons successfully elicit an ejp following only 35% of the stimuli, whereas stimulations to pum⁸⁸ neurons elicit an ejp following 85% of the stimuli (Figure 7C). Flies overexpressing pum in their nervous system were also tested for behavioral defects and temperaturesensitive paralysis but no differences from wild type were evident (data not shown). The data collected from neurons overexpressing pum support the conclusion that the level of Pum protein expression in neurons regulates neuronal excitability. In particular, insufficient neuronal Pum causes hyperexcitability, whereas excess neuronal Pum reduces excitability.

**DISCUSSION**

**Discovery of a new pum allele:** We have shown by several criteria that bem is a new allele of pum. First, several pum alleles failed to complement the pum⁸⁸ fertility defects. Second, female fertility in pum⁸⁸ mutants was significantly restored by the expression of pum under the transcriptional control of the nos promoter. Third, several pum alleles failed to complement both the behavioral defects and defective neuronal excitability of pum⁸⁸ mutants. Fourth, both pum mRNA and protein are expressed improperly in the heads of pum⁸⁸ adults. Finally, we showed that previously identified pum mutants exhibit the same increased motor neuron excitability as seen in pum⁸⁸ and that overexpression of pum in larval neurons decreases motor neuron excitability. There-

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**Figure 7.—Overexpression of pum in the nervous system causes an increased failure rate in motor neurons. The number of stimuli (stimulus frequency 1 Hz for 10 sec) that evoke an ejp is indicated. The bath contained a Ca²⁺ concentration of either 0.15 mM (A and B) or 0.1 mM (C). (A) Representative traces of failures and successes from nerves of the indicated genotypes. Black arrowheads indicate failures. (B and C) Means and standard errors (unpaired t-test) of failure rates from nerves of the indicated genotypes are presented. Six larvae from each genotype were tested. (*) P < 0.0005 and <0.0001 vs. bem⁺/bem⁺ for B and C, respectively.
fore, we have uncovered a new role of *pum* in regulating neuronal excitability.

We have also begun to assign specific functions to individual Pum protein isoforms. In particular, we found that *pum<sup>1688</sup>* eliminates only the 156-kD isoform from heads and retains expression of both the 130- and 95-kD Pum isoforms, whereas expression of all three Pum isoforms in *pum<sup>1688</sup>* heads is greatly reduced. Since *pum<sup>1688</sup>* complements *pum<sup>1682</sup>* behavioral defects we can conclude that the 156-kD Pum isoform is not required for Pum function in controlling behavior.

**Mechanisms of *pum* action:** The Pum protein is a member of the PUF-domain-containing protein family, which is an evolutionarily conserved family of RNA-binding proteins found in organisms from yeast to humans (Coglievina *et al.* 1995; Miosga and Zimmermann 1996; Purnelle and Goffeau 1997; Zamore *et al.* 1997; Kraemer *et al.* 1999; Nakahata *et al.* 2001; Tadauchi *et al.* 2001). Whereas orthologs of *pum* have been isolated in several organisms, the molecular mechanism by which *pum* functions has been characterized in most detail in the process of posterior pattern formation during Drosophila embryogenesis. In this system, Pum binds to specific sequences (NREs) that are present in the 3’-UTR of *hh* mRNA (Wharton and Struhl 1991; Sonoda and Wharton 1999; Zamore *et al.* 1999). The *hh*-bound Pum then recruits Nos and Brat to the mRNA, forming a quaternary complex that results in repression of *hh* mRNA translation (Sonoda and Wharton 1999, 2001). The mechanism of repression results in part from deadenylation of the *hh* transcript (Wreden *et al.* 1997). This repression is required for the formation of posterior structures in the developing embryo (Barker *et al.* 1992). In *pum* mutants, *hh* mRNA is translated throughout the embryo, resulting in aberration of posterior development and the absence of posterior abdominal segments (Barker *et al.* 1992).

Pum has effects in Drosophila in addition to controlling the formation of posterior structures. In particular, certain *pum* alleles that exhibit defects in germ-line stem cell differentiation have been identified (Lin and Spradling 1997). In this process, *pum* and *nos* might act together to inhibit pole cell division by binding sequences similar to NREs present in *cyclin B* mRNA thereby repressing its translation (Sonoda and Wharton 2001). An unidentified pole cell-specific factor (possibly similar to *brat*) is also implicated by the observation that *pum* and *nos* do not affect *cyclin B* translation in somatic cells (Richter and Theurkauf 2001). In addition, *pum* has a role in optic nerve pathfinding and is also able to repress translation in photoreceptors in a *nos*-dependent manner (Schmucker *et al.* 1997; Wharton *et al.* 1998). However, neither the *pum* target mRNA in developing optic nerves nor the adult ocular target has been identified. These results taken together suggest that improper translational regulation of unknown target mRNA(s) during neuronal development or in the adult nervous system results in the increased motor neuron excitability seen in *pum<sup>1682</sup>* and *pum<sup>1688</sup>* mutants.

**Effects of translational regulation on neuronal excitability:** Much data concerning the role of translational regulation in neuronal function have been collected. Both translational machinery and mRNA are located in certain dendrites and axons as well as the cell body of neurons (Steward *et al.* 1996; Tiedge and Brosius 1996). In addition, it was shown in both Aplysia and rats that translation is required for learning, memory, and proper synaptic plasticity (Kang and Schuman 1996; Martin *et al.* 1997). Furthermore, recent studies have shown that translation at the Drosophila nmj affects both the anatomy and physiology of these synapses (Sigrist *et al.* 2000). Our studies suggest that Pum might regulate translation in the cell body, dendrites, or axons and that this translational regulation is important in maintaining proper neuronal excitability. For example, maintenance of proper neuronal excitability may be achieved by translational regulation of ion channel mRNAs directly or through regulation of an upstream ion channel regulator. Therefore, isolation of the *pum<sup>1688</sup>* mutation allows the opportunity to study with genetic methods the role played by translational regulation in maintaining proper neuronal excitability.

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