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Regulation of Neuronal Excitability through Pumilio-Dependent Control of a Sodium Channel Gene

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Dynamic changes in synaptic connectivity and strength, which occur during both embryonic development and learning, have the tendency to destabilize neural circuits. To overcome this, neurons have developed a diversity of homeostatic mechanisms to maintain firing within physiologically defined limits. In this study, we show that activity-dependent control of mRNA for a specific voltage-gated Na^+ channel [encoded by *paralytic* (*para*)] contributes to the regulation of membrane excitability in *Drosophila* motoneurons. Quantification of *para* mRNA, by real-time reverse-transcription PCR, shows that levels are significantly decreased in CNSs in which synaptic excitation is elevated, whereas, conversely, they are significantly increased when synaptic vesicle release is blocked. Quantification of mRNA encoding the translational repressor *pumilio* (*pum*) reveals a reciprocal regulation to that seen for *para*. Pumilio is sufficient to influence *para* mRNA. Thus, *para* mRNA is significantly elevated in a loss-of-function allele of *pum* ($pum^{bemused}$), whereas expression of a full-length *pum* transgene is sufficient to reduce *para* mRNA. In the absence of *pum*, increased synaptic excitation fails to reduce *para* mRNA, showing that Pum is also necessary for activity-dependent regulation of *para* mRNA. Analysis of voltage-gated Na^+ current (I_{Na}) mediated by *para* in two identified motoneurons (termed aCC and RP2) reveals that removal of *pum* is sufficient to increase one of two separable I_{Na} components (persistent I_{Na}), whereas overexpression of a *pum* transgene is sufficient to suppress both components (transient and persistent). We show, through use of anemone toxin (ATX II), that alteration in persistent I_{Na} is sufficient to regulate membrane excitability in these two motoneurons.

Key words: aCC; excitability; neural activity; paralytic; Pumilio; RP2

Introduction

Central neurons must face and adapt to changing patterns of synaptic drive. These adaptations are essential to prevent neurons from either falling silent as synaptic excitation falls or, conversely, becoming saturated during periods of intense neuronal activity. Changes in exposure to synaptic excitation are particularly extreme during early embryogenesis when neurons first form synaptic contacts, but also arise as a consequence of the synaptic remodeling that underpins memory and learning (for review, see Turrigiano, 1999; Burrone and Murthy, 2003; Turrigiano and Nelson, 2004]. Although such adaptive mechanisms, often termed homeostatic compensation, have been well documented in both mammals and invertebrates, the underlying mechanisms remain less well understood.

Mammalian cortical neurons maintained in culture compensate for changes in exposure to synaptic excitation by alteration of both intrinsic membrane excitability and responsiveness to excitatory neurotransmitters. Changes in membrane excitability are mediated by alterations in voltage-gated conductances, whereas change in responsiveness to neurotransmitter is mediated by altered AMPA receptor density and/or localization (O'Brien et al., 1998; Turrigiano et al., 1998; Desai et al., 1999). More recently, the same phenomenon has been observed in *Drosophila* motoneurons *in vivo*, which offers the significant opportunity to exploit molecular genetics to elucidate the underlying mechanism (Baines et al., 2001; Baines, 2003). Changes in synaptic excitation of two motoneurons (termed aCC and RP2) are compensated for by altered membrane excitability primarily mediated through changes in I_{Na} (Baines et al., 2001; Baines, 2003). Activity-dependent regulation of the cAMP–protein kinase A (PKA) pathway is both necessary and sufficient to mediate rapid changes in I_{Na} in aCC/RP2 (Baines, 2003), consistent with *in vitro* studies that show phosphorylation of rat Na^+ channels to be an effective determinant of channel conductance (Li et al., 1992; Smith and Goldin, 1997; Catterall, 2000). However, whereas rapid change in I_{Na} is predicted to compensate for equally rapid fluctuations in synaptic excitation, longer-term changes in neuronal activity might be better compensated for by changes in gene expression.

In addition to transcriptional control, regulation of translation plays a critical role in gene expression (Gavis, 2001). In *Drosophila*, the translational repressor Pumilio (Pum) is required for establishment of polarity during early embryogenesis (Tautz, 1988; Wharton and Struhl, 1991; Wharton et al., 1998). Binding of Pum to the nanos response element (NRE) motif located in the 3′ untranslated region (3′-UTR) of *hunchback* (*hb*) is the initial

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step in translation repression, setting up a gradient of transcript across the embryo. The ability of motoneurons to synaptically excite muscle is also reported to be influenced by levels of *pum* in the *Drosophila* CNS, which is indicative that this repressor might also contribute to regulation of neuronal excitability (Schweers et al., 2002).

A bioinformatic screen for NRE-like sequences in either 3′- or 5′-UTRs of *Drosophila* ion channel genes (our unpublished data) identified *paralytic* (*para*), the protein products of which carry the voltage-gated Na^+ current in embryonic and larval motoneurons (Baines and Bate, 1998). In this study, we show that *para* mRNA levels are activity dependent, increasing as synaptic excitation falls and vice versa. Levels of *pum* mRNA are also activity dependent but are reciprocal to that observed for *para*. Overexpression of a *pum* transgene is sufficient to reduce *para* mRNA and to reduce the magnitude of I_{Na} in aCC/RP2. The absence of *pum* [$pum^{bemused}$ (pum^{bem})] results in a complementary phenotype: increased *para* mRNA pan-neuronally and increased I_{Na} in aCC/RP2. In the absence of *pum*, genetic manipulations that result in increased synaptic excitation of aCC/RP2 fail to repress *para* mRNA levels, which is indicative that this translational repressor is both necessary and sufficient for activity-dependent regulation of I_{Na} in *Drosophila* motoneurons.

Materials and Methods

Fly stocks. Flies were fed on apple juice agar supplemented with yeast at 25°C. Wild type (WT) was Canton-S [except in Fig. 6*C*,*D*, in which RRC-GAL4 × UAS-GFPn was the WT control (Baines et al., 1999)]. 1407-GAL4 was used to express UAS transgenes in all of the CNS neurons. RN2-O GAL4 (homozygous viable second chromosome) was used to selectively express UAS transgenes in aCC and RP2 (Fujioka et al., 1999; Baines, 2003). Expression of RN2 GAL4 begins in early stage 16 embryos, a stage that precedes the onset of synaptogenesis (Baines and Bate, 1998). The *bemused* allele of *pumilio* (rebalanced over a TM3 GFP balancer) and full-length UAS-*pumilio* transgene used are detailed by Schweers et al. (2002). The isogenic wild type from which the *bemused* allele was produced served as control and is denoted bem^+. Synaptic transmission was blocked by either expression of UAS-tetanus toxin light chain (TeTx-A) or by use of a genetic null of n-synaptobrevin (n-syb), rebalanced over a GFP balancer (Sweeney et al., 1995). Synaptic transmission was increased by expression of UAS-*rutabaga* (a type I, Ca^{2+}–calmodulin-dependent, adenylate cyclase) (Zars et al., 2000) or by use of well characterized allele of *dunce* [a cAMP-specific phosphodiesterase (EC:3.1.4)] (Dudai et al., 1976; Davis et al., 1995; Baines, 2003). Expression of *para* was increased using a characterized genetic duplication: $Tp(1;2)r^{+75c}$ (Stern et al., 1990). PKA activity was promoted using UAS-*PKAact*[1] and inhibited using UAS-*PKAinh*[1] (formerly termed PKA[BDK22]); these transgenes are described by Davis et al. (1998). Para was removed using a small deficiency [Df(1) D34] (Baines and Bate 1998).

Embryo dissection. Larvae were dissected, and central neurons were accessed as described by Baines and Bate (1998). The larva was viewed using a water immersion lens (total magnification, 800×) combined with Nomarski optics (BX51 WI microscope; Olympus Optical, Tokyo, Japan).

Electrophysiology. All of the recordings were performed in young first instar larvae, 1–4 hr after hatching, at room temperature (22–24°C). Whole-cell recordings (current and voltage clamp) were achieved using thick-walled borosilicate glass electrodes (GC100TF-10; Harvard Apparatus, Edenbridge, UK), fire polished to resistances of between 15 and 20 MΩ. Cells were initially identified based on both size and dorsal position in the ventral nerve cord. Unequivocal identification was determined by labeling with sulfur rhodamine (0.3%; Molecular Probes, Eugene, OR), which was included in the patch saline. Recordings were made using an Axopatch-1D amplifier controlled by pClamp 8.1 (Axon Instruments, Foster City, CA). Only cells with an input resistance >1 GΩ were accepted for analysis. Traces were filtered at 2 kHz and sampled at 20 kHz. To better resolve Na^+ currents, an on-line leak subtraction protocol was used (P/4). Currents shown are the averages of five trials for each cell. To separate the persistent current ($I_{Na(p)}$), a voltage protocol was used that first stepped to +30 mV (50 msec) to inactivate the transient current ($I_{Na(t)}$). Determination of membrane excitability was performed using injection of depolarizing current (1–10 pA/500 msec) from a resting membrane potential (RMP) of −60 mV. RMPs were maintained at −60 mV by injection of a small amount of hyperpolarizing current (WT RMP without current injection = −44 ± 1.4 mV). Input resistance, which was determined by injection of 1 pA hyperpolarizing current, remained statistically unchanged in all of the genetic backgrounds tested (WT = 7.2 ± 0.9 GΩ).

Solutions. External saline for dissection and current clamp analysis of excitability consisted of the following (in mM): 135 NaCl, 5 KCl, 4 $MgCl_2 \cdot 6H_2O$, 2 $CaCl_2 \cdot 2H_2O$, 5 *N*-Tris[hydroxymethyl]methyl-2-aminoethanesulfonic acid, 36 sucrose, and 1 mecamylamine (used in the determination of excitability to block synaptic currents). For isolation of Na^+ currents, the following solution was used (in mM): 100 NaCl, 5 KCl, 50 TEA, 10 4-AP, 10 HEPES, 10 glucose, and 0.5 $CaCl_2 \cdot 2H_2O$. All of the solutions were pH 7.15. ATX II (Alomone Labs, Jerusalem, Israel) was applied, dissolved in the relevant saline.

Internal patch solution consisted of (in mM): 140 K^+ methylsulfonate (KCH_3SO_3), 2 $MgCl_2 \cdot 6H_2O$, 2 EGTA, 5 KCl, and 20 HEPES, pH 7.4. When recording Na^+ currents, $CsCl_2$ was substituted for KCH_3SO_3.

RNA isolation. RNA was extracted from 50 late stage 17 embryos (*para*) or from 50 isolated embryonic late stage 17 CNSs (*pumilio*) using the Qiagen RNeasy mini kit (Qiagen, Hilden, Germany) with minor modifications. Briefly, 50 μl of RLT solution containing 0.1 M β-mercaptoethanol was added to the embryos or isolated CNSs before homogenizing on ice using a sterile plastic Eppendorf (Hamburg, Germany) homogenizer. The homogenate was centrifuged (3 min at 13,000 rpm) to remove cell debris before addition of 50 μl of 70% ethanol (v/v). Homogenate was loaded onto an RNeasy column and washed, and RNA was eluted as per the manufacturer's protocol. RNA concentration was calculated by spectral analysis at 260 nm and quality assessed by agarose gel (1%) electrophoresis under denaturing conditions. Samples were subsequently stored at −80°C.

cDNA synthesis. cDNA synthesis was performed in a total volume of 20 μl. RNA (up to 5 μg) was mixed with random hexamer primers (0.2 μg; Roche, Mannheim, Germany) and made up to 11 μl with RNase-free water. After incubation (5 min at 65°C), the mix was chilled on ice (2 min). Reaction buffer [4 μl; (in mM): 250 Tris-HCl, 250 KCl, 20 $MgCl_2$, 50 DTT, pH 8.3] and deoxyribonucleoside triphosphates (dNTPs; 2 μl; 10 mM) made up to a final volume of 19 μl (RNase-free water) were added and incubated for 5 min at 25°C. Reverse transcriptase (200 U; RevertAid H Minus M-MuLV; Fermentas, Vilnius, Lithuania) was added before incubation steps of 10 min at 25°C, 60 min at 42°C, and 20 min at 65°C. From the total reaction volume of 20 μl, 1 μl of cDNA was used for each PCR.

PCR conditions and controls. Clone Manager Software (Scied Software, Durham, NC) was used to design PCR primers for *para*, *pumilio*, and *ribosomal protein 49* (*rp49*): *rp49* forward and reverse primers in 5′ to 3′ orientation, CCAAGGACTTCATCCGCCACC and GCGGGTGCGCTTGTTCGATCC; *para* forward and reverse primers, GATCTATATGGGCGTGCTCACCGAGAAGTG and TGCAGGCACACGTAATCGTCGTCGGATTG; and *pumilio* forward and reverse primers, TGAAGAGCCGGCGAGCTGAA and CTGGCCACCAGCTGACCAAT, respectively. Each reaction contained 5 μl of PCR Master Mix (3 mM $MgCl_2$, dNTPs, *Taq* polymerase; Biogene, Cambridge, UK), 500 nM forward and reverse primers, 1 μl of cDNA, and 1:1000 dilution of SYBR Gold (Biogene) made up to 10 μl with PCR-grade water. PCR was performed using a Roche LightCycler (Roche). After an initial denaturation step at 94°C for 60 sec, temperature cycling was initiated. Each cycle consisted of denaturation at 94°C for 0 sec, hybridization at 54°C for 5 sec for *para* and 57°C for *pumilio*, and elongation at 72°C for 10 sec. Determination of *rp49* was performed at either temperature. The fluorescent signal was acquired at the end of each hybridization step (F2/F1 channels, fluorimeter gains regulated on 1 for F1, 1 for F2, and 1 for F3). A total of 30 cycles was performed. The authenticity of the PCR products was verified by

melting-curve analysis and the presence of a single band of appropriate molecular size by agarose gel electrophoresis.

Real-time PCR quantification. mRNA levels are expressed as relative fold change normalized against *rp49* mRNA.

The comparative cycle threshold (Ct) method (User Bulletin 2, 1997; Applied Biosystems, Foster City, CA) was used to analyze the data by generating relative values of the amount of target cDNA. Relative quantification for any given gene, expressed as fold variation over control, was calculated from the determination of the difference between the Ct of the given gene (*para*, *pumilio*) and that of the calibrator gene (*rp49*). Ct values used were the means of triplicate replicates. Experiments were repeated at least five times.

Statistics. Data were compared using a nonpaired *t* test. Results were deemed significant at $p \leq 0.05$. All of the values shown are mean ± SE.

Results

Synaptic excitation regulates membrane excitability in *Drosophila* motoneurons

The synaptic drive to aCC/RP2 consists of large (50–300 pA) inward synaptic currents that are relatively long-lived (500–1000 msec) and mediated by acetylcholine (Baines et al., 1999) (Fig. 1*C*). Together with the relatively slow kinetics of these currents, these characteristics are indicative that these currents arise from the synchronous releases of acetylcholine from multiple convergent presynaptic inputs [for a more complete description of these currents, see Baines et al. (2001) and Baines (2003)]. Our previous studies demonstrate that endogenous membrane excitability (i.e., the ability to fire action potentials) of these two motoneurons is regulated by exposure to excitatory synaptic drive (Baines et al., 2001; Baines, 2003). Figure 1 shows additional data that reinforce these previous observations. In a range of genetic backgrounds that affect the magnitude of excitatory synaptic currents recorded in aCC/RP2 (Fig. 1*C*), the averaged current amplitude [these two neurons show no differences in synaptic inputs indicative of shared presynaptic interneurons (Baines, 2003)] shows a very strong correlation to the number of action potentials elicited by injection of a constant depolarizing current (10 pA/500 msec, a measure of membrane excitability) (Fig. 1*A*,*B*). The correlation is inverse in nature; increasing synaptic current amplitude results in reduced membrane excitability and vice versa (for more details, see Baines, 2003).

Synaptic excitation regulates abundance of *para* mRNA

The ability of a neuron to alter intrinsic membrane excitability in response to changing synaptic excitation is advantageous for the maintenance of global activity levels within neural circuits (Turrigiano and Nelson, 2000). Our previous work implicates a PKA-mediated regulation of I_{Na} as a mechanism able to compensate for rapid fluctuations in synaptic excitation (Baines, 2003). Undoubtedly, however, neurons must also adapt to changes in synaptic excitation that persist over the longer term, and we speculate that additional mechanisms might exist to compensate for such chronic changes. One conceivable mechanism is that of activity-dependent regulation of Na^+ channel gene expression. To test this hypothesis, we used real-time PCR [quantitative reverse-transcription PCR (QRT-PCR)] to quantify the abundance of *para* mRNA in the CNS of late stage 17 (19–21 hr after egg laying) *Drosophila* embryos, in which levels of synaptic excitation have been genetically manipulated. The voltage-gated Na^+ current in *Drosophila* central neurons is exclusively encoded by *para* during this early developmental stage (Hong and Ganetzky, 1994; Thackeray and Ganetzky, 1994; Baines and Bate, 1998).

To block synaptic neurotransmitter release, thereby removing synaptic excitation, we used two complementary approaches.

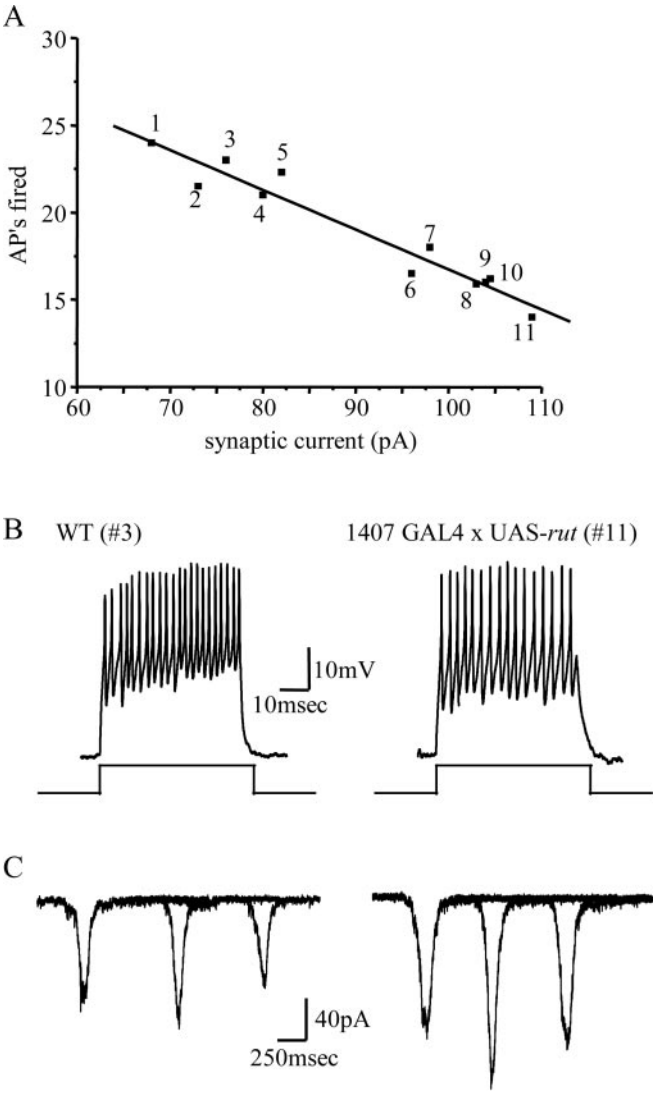

Figure 1. Exposure to synaptic excitation regulates membrane excitability in aCC/RP2. *A*, The number of action potentials (APs) fired, for a given depolarizing input (10 pA/500 msec), is inversely related to endogenous synaptic excitation in aCC/RP2 (line coefficient, 0.92). All of the determinations of AP firing were from a maintained RMP of −60 mV (see Materials and Methods). Input resistances did not differ significantly between genotypes shown. Synaptic current amplitude shown is the average current magnitude recorded from at least eight cells for each genotype ($n > 200$ currents). Neurons exposed to greater than normal synaptic excitation (WT, 76 ± 3.3 pA) exhibit reduced membrane excitability and vice versa. APs fired are means ($n \geq 8$ cells). Pan-neuronal expression of TeTx-A results in aCC/RP2 neurons firing 28 ± 2.4 APs while exhibiting no evoked synaptic currents (see also Baines et al., 2001). This "nonphysiological" condition is not shown because it markedly deviates from the line of best fit shown. This is most likely because this frequency of AP firing represents the physiological maximum for these cells at this developmental stage. *B*, Representative traces for injection of 10 pA/500 msec for the two genotypes stated. *C*, Endogenous synaptic currents, recorded in voltage clamp ($V_h = -60$ mV) for the same two genotypes. Three excitatory synaptic currents are shown overlaid in each example. Genotypes are as follows: (1) RN2-0 × UAS-*PKA*^{inh}, (2) B19-GAL4, (3) Canton-S, (4) RN2-0 × UAS-*dCREB*$^{act\ (1157\text{-}19)}$, (5) rut^1, (6) *dnc*^{2}, (7) RN2-0 × UAS-*PKA*^{act}, (8) RN2-0 × UAS-*dCREB*$^{act\ (1157\text{-}51)}$, (9) *dnc*^{2}, (10) RN2-0 × UAS-*dCREB*$^{inh\ (9)}$, and (11) 1407-GAL4 × UAS-*rut*.

First, we used pan-neuronal expression of TeTx-A, which is sufficient to block the evoked release of neurotransmitter (Sweeney et al., 1995; Baines et al., 1999). Second, we used a null allele of *n-synaptobrevin* that is essential for evoked synaptic vesicle release (Broadie et al., 1995). We observe that, in both genetic backgrounds, the abundance of *para* mRNA is significantly increased (2.5 ± 0.5- and 3.9 ± 0.4-fold, respectively; $p \leq 0.01$) relative to

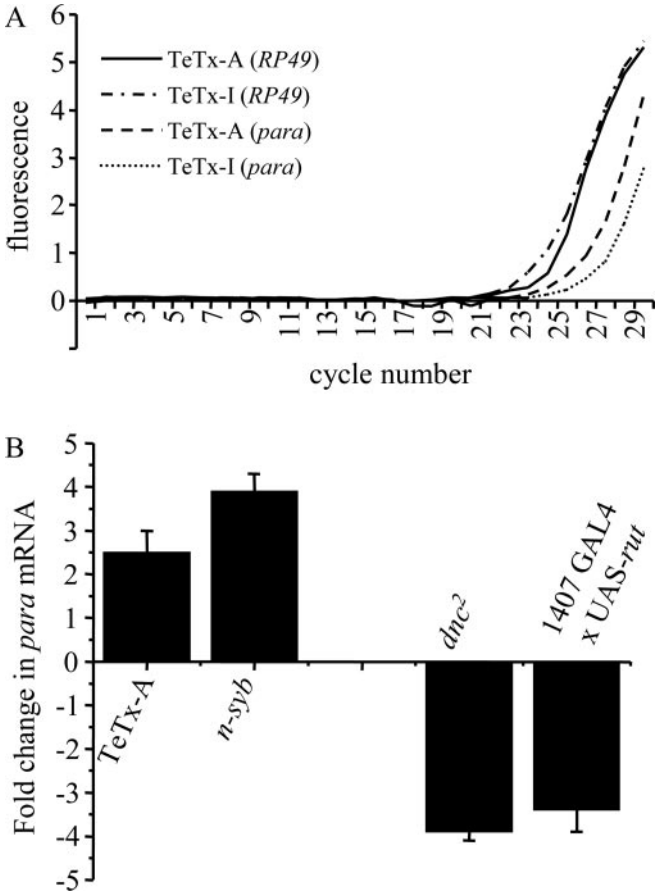

Figure 2. Abundance of *para* mRNA is activity dependent. *A*, Typical QRT-PCR output for determination of *para* mRNA isolated from whole embryos that expressed either active (TeTx-A) or inactive (TeTx-I) tetanus toxin in all of the neurons. For quantification, all of the values are normalized to *RP49* expression (for details of analysis, see Materials and Methods). *B*, Averaged changes in *para* mRNA, relative to controls (set to 0), show that abundance increases in backgrounds in which synaptic transmission is absent (TeTx-A and *n-syb*), and decreases when synaptic excitation is greater than normal (dnc^2 and 1407 GAL4 × UAS-*rut*). Values given are means ± SE ($n \geq 6$). All of the changes are significant from respective controls (inactive toxin expression, *n-syb*/+, WT, and 1407 GAL4; $p = 0.008$, 0.01, 0.002, and 0.01, respectively).

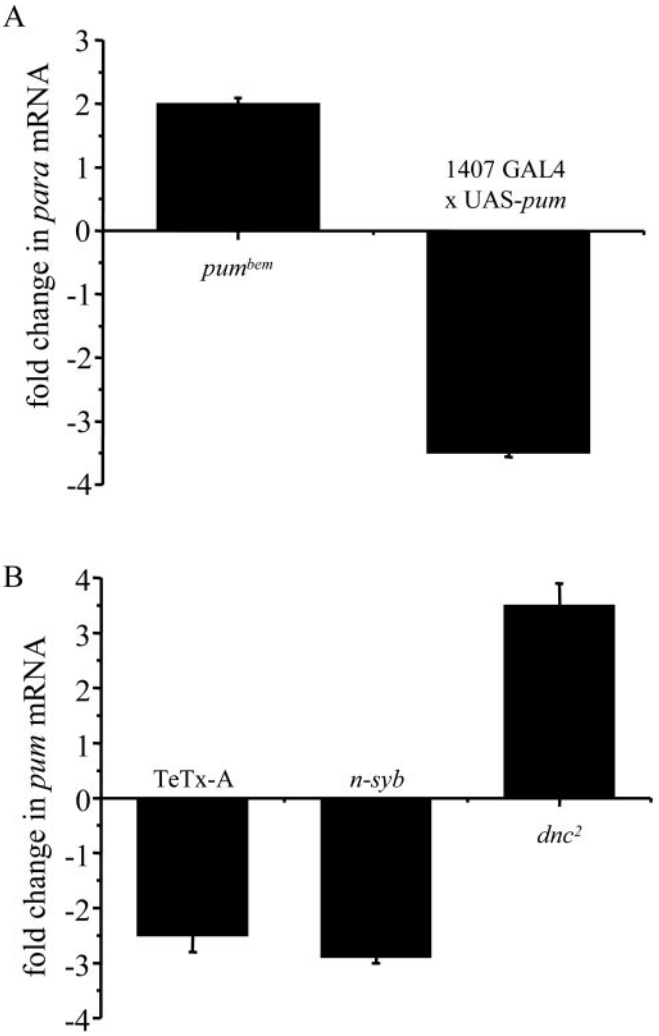

Figure 3. Expression of *pum* mRNA is activity dependent and regulates *para* mRNA abundance. *A*, The abundance of *para* mRNA in isolated CNS is significantly greater in the loss-of-function pum^{bem} allele. By comparison, overexpression of UAS-*pum* in all of the neurons of the CNS (1407 GAL4) is sufficient to significantly reduce *para* mRNA. Values given are means ($n \geq 6$). All of the changes are significant from respective controls (pum^{+} and 1407-GAL4; $p = 0.001$ and 0.0006, respectively). *B*, In genetic backgrounds in which evoked release of synaptic transmitter is blocked (TeTx-A and *n-syb*), *pum* mRNA is significantly reduced compared with controls (inactive toxin and *n-syb*/+; $p = 0.007$ and 0.01, respectively). In contrast, CNS isolated from dnc^2 embryos, which is known to enhance synaptic excitation of aCC/RP2 (Baines, 2003), results in an opposite and significant increase in *pum* mRNA compared with control (WT; $p = 0.007$). Controls are set to zero. Values given are means ± SE ($n \geq 6$).

controls (inactive tetanus toxin and *n-syb*/+, respectively) (Fig. 2). These observations correlate to our previous finding that I_{Na} is significantly increased in aCC/RP2 when TeTx-A is expressed in all of the central neurons (Baines et al., 2001). To potentiate synaptic excitation, we again used two independent but complementary approaches. Elevation of cAMP is seemingly sufficient to increase excitatory synaptic release in the *Drosophila* CNS (Baines, 2003). Thus, cAMP was elevated using an allele of *dunce* (dnc^2) that lacks a cAMP-specific phosphodiesterase (Dudai et al., 1976; Davis et al., 1995) and by pan-neuronal expression of *rutabaga* (*rut*; a type I, Ca^{2+}–calmodulin-dependent, adenylate cyclase) (Zars et al., 2000). Both genetic backgrounds exhibit increased synaptic excitation of aCC/RP2 (Fig. 1*A*), whereas *para* mRNA was significantly reduced in both (−3.9 ± 0.2- and −3.4 ± 0.5-fold; $p \leq 0.01$) relative to controls (WT and 1407 GAL4, respectively) (Fig. 2*B*). Measurement of I_{Na} in aCC/RP2 in the dnc^2 background shows a significant reduction compared with WT (Baines, 2003).

Manipulation of *pumilio* affects *para* mRNA

A characterization of a newly identified loss-of-function allele of *pumilio* (*bemused*) indicates, albeit indirectly, that this translational repressor is capable of altering excitability at the *Drosophila* neuromuscular junction (Schweers et al., 2002). Levels of Pum are greatly reduced in pum^{bem}, and, moreover, two *pum* transcripts are absent. In such mutants, stimulation of the axon innervating muscle 6 results in a hyperexcitability phenotype, whereas overexpression of full-length UAS-*pum* in the CNS results in an opposite phenotype, hypoexcitability (Schweers et al., 2002). To test whether activity-dependent changes in *para* mRNA abundance are downstream of *pum* in the CNS, we first quantified the levels of *para* mRNA in these same *pum* backgrounds. Figure 3*A* shows that, in the loss-of-function pum^{bem} background, *para* mRNA is significantly increased (2 ± 0.1-fold; $p \leq 0.01$) relative to control (bem^{+}). By comparison, overexpression of full-length UAS-*pum* in all of the neurons of the CNS resulted in a significant reduction of *para* mRNA (−3.5 ± 0.06-

fold; $p \leq 0.01$) relative to control (1407 GAL4). Thus, in genetic backgrounds in which *pum* is significantly reduced (pum^{bem}), *para* expression is increased, whereas overexpression of *pum* is sufficient to suppress *para* mRNA. Given that Pum is a known translational repressor, these results are consistent with the hypothesis that Pum might act to suppress translation of, and target for degradation, *para* mRNA.

Expression of *pum* is activity dependent

At the simplest level, if *pum* is a regulator of *para* in central neurons of *Drosophila*, then *pum* itself would be predicted to be regulated by synaptic activity. We tested this prediction by quantifying *pum* mRNA in the same synaptic activity backgrounds used to examine *para* mRNA abundance. Because *pum* is widely expressed in *Drosophila* embryos and not restricted to the CNS as in the case of *para*, this analysis used isolated CNSs. CNSs taken from late stage 17 embryos, which expressed either active TeTx-A in all of the neurons or a loss-of-function *n-syb* genotype, show a significant reduction in *pum* mRNA abundance (-2.5 ± 0.3- and -2.9 ± 0.1-fold, respectively; $p \leq 0.01$) (Fig. 3*B*) compared with controls (TeTx-inactive and *n-syb*/+). In contrast, CNS isolated from dnc^2 embryos, in which synaptic excitation of aCC/RP2 is increased (Baines, 2003), exhibited a significant increase in *pum* mRNA (3.5 ± 0.4-fold; $p \leq 0.01$) compared with WT control. Thus, these data strongly implicate that expression of *pum* is itself regulated in an activity-dependent manner. Moreover, the polarity of this regulation (activity increasing *pum* and vice versa) is reciprocal to that observed for activity-dependent changes in *para* mRNA and, as such, is consistent with a model that links increased *pum* to repression of *para* mRNA.

Pum is necessary for activity-dependent *para* regulation

To test directly whether Pum is required for activity-dependent changes in *para* mRNA abundance, we overexpressed UAS-*rut* in all of the neurons of the CNS (1407-GAL4) in the pum^{bem} background. Our prediction was that the reduction in *para* mRNA that normally results from overexpression of UAS-*rut* in a WT background (Fig. 2) would not occur if Pum is required. Figure 4 shows that this is indeed the case. In the absence of normal levels of *pum*, overexpression of *rut* in all of the neurons of the CNS is unable to reduce *para* mRNA levels. Thus, in addition to being sufficient to repress *para* mRNA when overexpressed, *pum* is also necessary for suppression of *para* mRNA that results from the overexpression of *rut*.

Pum regulates I_{Na} in aCC/RP2

Our previous electrophysiological studies show that altered synaptic excitation, cAMP, and/or PKA are sufficient to regulate I_{Na} in aCC/RP2 (Baines et al., 2001; Baines, 2003). To address whether manipulation of *pum* is equally able to regulate I_{Na} in these motoneurons, as our model predicts, we used whole-cell voltage clamp to measure voltage-gated I_{Na} while manipulating the level of *pum* expression. Voltage-gated I_{Na} in aCC/RP2 is composed of two principal components. In addition to a typical, rapidly inactivating transient current ($I_{Na(t)}$), there is an additional slower inactivating persistent current ($I_{Na(p)}$) (Fig. 5*A*,*B*). To show that both of these components are encoded by the *para* gene, we repeated these recordings in a *para* null (Df(1) D34). In this background, both the transient and persistent components of I_{Na} are absent (Fig. 5*C*), confirming previous reports that *para* encodes I_{Na} at early stages of development (O'Dowd et al., 1989). Recordings in this *para* null uncovers an outward current that exhibits similarities to the cation current (I_{cat}), which has been described in rat neocortical neurons (Alzheimer, 1994). This current has relatively slow activation kinetics and, as such, is unable to significantly affect $I_{Na(t)}$ but is able to counteract $I_{Na(p)}$ (see also Fig. 6*D*). Isolation of I_{cat} by addition of TTX (1 μM) to the Na^+ isolation saline used to record I_{Na} shows that it is unaffected by manipulation of *pum* (E. C. G. Pym and R. A. Baines, unpublished data).

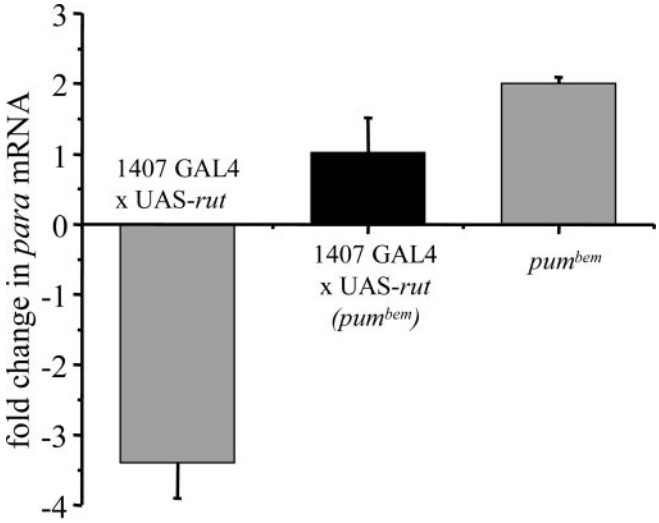

Figure 4. Pumilio is necessary for *rut*-dependent reduction of *para* mRNA. Overexpression of UAS-*rut* in all of the neurons of the CNS in the loss-of-function pum^{bem} allele fails to reduce the level of *para* mRNA. Changes in *para* mRNA observed in 1407-GAL4 × UAS-*rut* (wild-type background) and in pum^{bem} are shown for comparative purposes (gray boxes). Values given are means ± SE ($n \geq 6$). The change in *para* mRNA observed in 1407 × *rut* (pum^{bem}) is significant from that seen in 1407 × *rut* (WT) at $p = 0.01$.

We used established voltage protocols to separate out each I_{Na} current type (see Materials and Methods). In the loss-of-function pum^{bem} allele, the magnitude of $I_{Na(p)}$ but not $I_{Na(t)}$ is significantly increased relative to controls (bem^+) (Fig. 5*D*,*E*). Thus, in backgrounds in which *pum* is reduced, which our QRT-PCR analysis shows to result in increased *para* expression, at least one component of I_{Na} in aCC/RP2 is significantly increased. In comparison, overexpression of UAS-*pum* in just aCC/RP2 (using RN2-O GAL4) is sufficient to reduce both components of I_{Na} ($I_{Na(t)}$ and $I_{Na(p)}$) in these two motoneurons (Fig. 5*F*,*G*). Again, this effect is predictable based on our QRT-PCR analysis. Measurement of endogenous membrane excitability in aCC/RP2 [which is identical for both neurons (Baines, 2003)] shows that a reduction in *pum* expression (pum^{bem}) is sufficient to significantly increase membrane excitability (i.e., increased number of action potentials fired by injection of depolarizing current). In contrast, increased expression of *pum* (UAS-*pum*) is sufficient to reduce membrane excitability (Fig. 5*H*,*I*). Both effects are entirely consistent with the changes observed in I_{Na} (see below).

Increased *para* expression increases only the persistent Na^+ current

It is remarkable that, in the absence of *pum* (pum^{bem}), only the persistent component of I_{Na} is increased. That this should occur is indicative of an additional level of control of this current. To test this idea, we used the genetic duplication $Tp(1;2)r^{+75c}$ (Stern et al., 1990) to increase *para* expression in the CNS (an approximately twofold increase in *para* mRNA was observed by QRT-PCR in this genetic background; data not shown). Analysis of I_{Na} in aCC/RP2, in $Tp(1;2)r^{+75c}$, shows that only $I_{Na(p)}$, not $I_{Na(t)}$, is

significantly increased in amplitude (Fig. 6*A*,*B*), thereby phenocopying the effect of reduced *pum* expression. Injection of depolarizing current shows that this genetic duplication is, moreover, sufficient to increase membrane excitability in aCC/RP2 (Fig. 6*E*). To independently validate these results, we used the anemone toxin, ATX II, which is reported to increase only $I_{Na(p)}$, not $I_{Na(t)}$, in a wide variety of species (Mantegazza et al., 1998). In agreement with these previous studies, the presence of ATX II (300 nM) is sufficient to significantly increase $I_{Na(p)}$, but not $I_{Na(t)}$, in aCC/RP2 (Fig. 6*C*,*D*). Injection of depolarizing current shows that an ATX II-dependent increase in $I_{Na(p)}$ [we observed no change to other currents present in aCC/RP2 (E. C. G. Pym and R. A. Baines, unpublished data)] is sufficient to increase membrane excitability (Fig. 6*E*). Consistent with this conclusion is our additional observation that application of ATX II to our electrophysiological preparation potentiates endogenous synaptic depolarizations that are sufficient to fire action potentials (Baines, 2003) in aCC/RP2 (Fig. 6*F*). Thus, we conclude that increased $I_{Na(p)}$ in aCC/RP2, which we show results from either increases in *para* expression or a reduction in *pum* expression, mediates an increase in membrane excitability in these neurons. By comparison, reductions in *para* or increases in *pum* are sufficient to suppress membrane excitability through reductions in both $I_{Na(t)}$ and $I_{Na(p)}$.

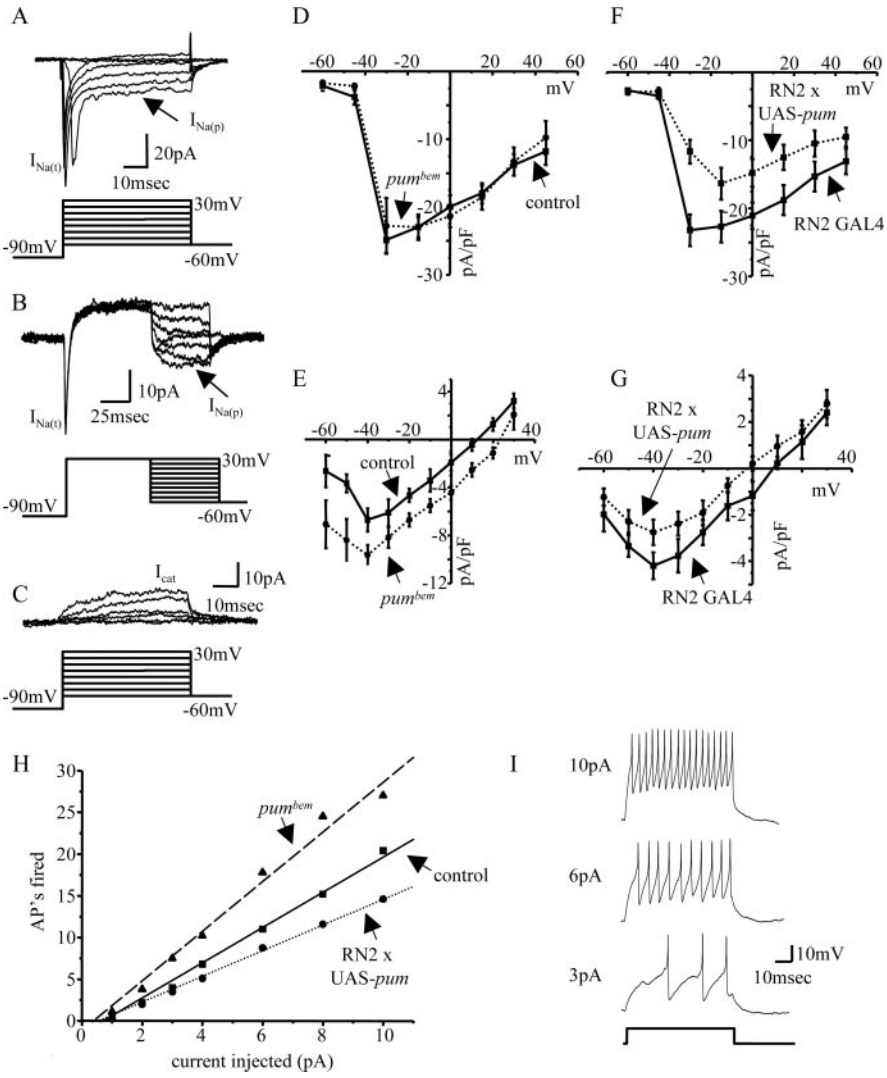

Figure 5. Voltage-gated I_{Na} is regulated by Pum in aCC/RP2. *A*, *B*, Voltage-gated I_{Na} in aCC/RP2 has at least two components. The first is a rapidly inactivating transient current (shown in *A*), whereas the second is a persistent current that inactivates slowly (shown in *B*). The outward current component in both traces is caused by a contamination by an I_{cat}-like cation current (Alzheimer, 1994) that we are unable to block at present. This current activates at approximately +20 mV and is not changed after manipulation of *pum* (determined by adding TTX to the Na^+-isolation saline that isolates just I_{cat}). *C*, Both components of I_{Na} are absent in recordings in a *para*-null [Df(1) D34] late stage 17 embryo. These recordings isolate I_{cat}. *D*, *E*, In the loss-of-function allele Pum^{bem}, the magnitude of $I_{Na(t)}$ does not change compared with control (Pum^+) (*D*), whereas the magnitude of peak $I_{Na(p)}$ is significantly increased (*E*) ($p \leq 0.05$). *F*, *G*, Overexpression of UAS-*pum* in aCC/RP2 (RN2-0 GAL4) is sufficient to reduce both components of I_{Na} (control is RN2-0 GAL4). *H*, Membrane excitability of aCC/RP2, determined by injection of constant current (1–10 pA/500 msec), shows a significant increase in Pum^{bem} and a significant decrease in overexpression of UAS-*pum*, respectively ($p \leq 0.05$). Control is WT. All of the values shown are means (±SE in *D*–*G*) for $n \geq 8$. AP, Action potential. *I*, Representative traces showing the firing of action potentials by successively greater depolarizing current injections (3, 6, and 10 pA/500 msec) in a WT aCC neuron.

Discussion

Embryonic neurons face rapidly changing synaptic excitation as neuronal circuits are first formed and then subjected to modulation by sensory feedback. To maintain stable circuit output while individual synapses are being either strengthened or weakened requires that the constituent neurons of these embryonic circuits exhibit adaptation to changing synaptic excitation. Known homeostatic mechanisms include activity-dependent gene expression (West et al., 2002), fixed ratio cotranslation of ion channel mRNAs (MacLean et al., 2003), and posttranslational modification of ion channel conductance (Li et al., 1992; Smith and Goldin, 1997; Catterall, 2000). Our results are of importance in that they increase this repertoire to include activity-dependent regulation of an ion channel mRNA by Pumilio, a known translational repressor.

Homeoststatic mechanisms maintain neuronal excitability

As nervous systems mature, neurons may change shape, lose or gain synaptic inputs, be exposed to changing patterns of synaptic drive, and experience constant turnover of ion channel proteins. Despite these changes, neurons are able to maintain relatively constant firing properties, indicative of intrinsic mechanisms that strive to maintain stability (Turrigiano and Nelson, 2004). A pertinent example is provided by neurons of the stomatogastric nervous system (STG) of *Crustacea*. STG neurons exhibit bursting behavior that is a consequence of phasic inhibitory inputs combined with intrinsic conductances. When isolated from these inputs in culture, these same neurons initially exhibit tonic firing. If isolation is continued for several days, however, these neurons regain an ability to fire in bursts (Turrigiano et al., 1994, 1995). Clearly, input and the lack of it have the effect of triggering a homeostatic mechanism that restores bursting behavior to these neurons. Although the particular aspect of synaptic input that

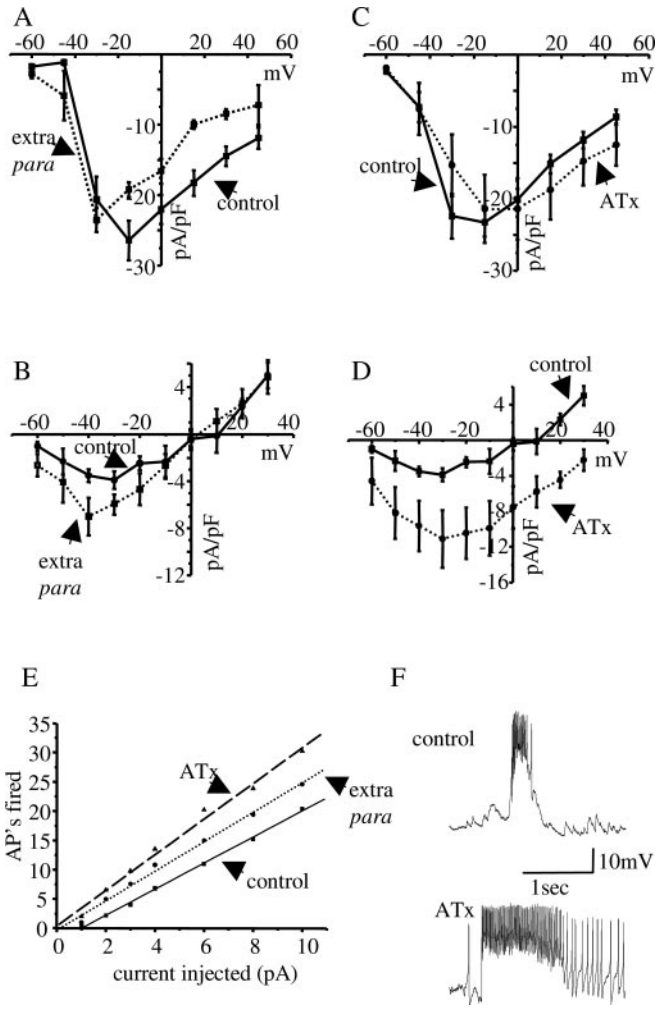

Figure 6. Increased $I_{Na(p)}$ is sufficient to increase membrane excitability in aCC/RP2. *A*, *B*, Increasing the copy number of *para* using a genetic duplication [Tp(1;2)r^{+75c}; extra *para*] results in no significant change in peak amplitude for $I_{Na(t)}$ (*A*) but a significant increase in peak $I_{Na(p)}$ (*B*) compared with a WT control ($p \leq 0.05$). *C*, *D*, Brief exposure (<5 min) to ATX II (300 nM) similarly fails to affect $I_{Na(t)}$ (*C*), but significantly increases peak $I_{Na(p)}$ ($p \leq 0.01$) (*D*). Control is RRC GAL4 × UAS-*GFPn*. All of the values shown are means ± SE for $n \geq 8$. The reversal of $I_{Na(p)}$ in the presence of ATX differs from control because the increase in current magnitude produced is sufficient to overcome a nonspecific I_{cat} that is also present in these neurons (Fig. 5*C*) (E. C. G. Pym and R. A. Baines, unpublished data). I_{cat} normally acts to oppose $I_{Na(p)}$ but, because of its slower activation kinetics, does not significantly influence $I_{Na(t)}$. *E*, Membrane excitability in aCC/RP2 is significantly increased ($p \leq 0.05$) in both the genetic duplication Tp(1;2)r^{+75c} (extra *para*) and in the presence of ATX II. Control is WT. All of the values shown are means ($n \geq 8$). AP, Action potential. *F*, Endogenous synaptic inputs to aCC/RP2 (for details, see Baines, 2003) are potentiated in the presence of ATX II (300 nM). Both traces are from the same WT aCC neuron (i.e., before and after ATX II exposure).

coordinates this homeostatic response remains obscure, a likely possibility is that of altered Ca^{2+} influx across the neuronal membrane as a direct consequence of depolarization. Although direct evidence for the involvement of Ca^{2+} is lacking, manipulation of membrane potential is sufficient to evoke homeostatic responses in both neurons and muscle (Leslie et al., 2001; Paradis et al., 2001; Burrone et al., 2002). In *Drosophila*, for example, genetically induced hyperpolarization of somatic muscle is sufficient to evoke increased presynaptic neurotransmitter release from innervating motoneurons to maintain depolarization within physiological limits (Paradis et al., 2001).

Mechanisms identified to mediate homeostatic control in mature neurons are likely to have features in common with the regulation of electrical properties in differentiating embryonic neurons. Our previous observations show that *Drosophila* embryonic motoneurons regulate intrinsic membrane excitability when faced with changing synaptic excitation (Baines et al., 2001; Baines, 2003). At least part of this mechanism involves activity-dependent regulation of the cAMP–PKA pathway (Baines, 2003). PKA activity in these motoneurons is sufficient to reduce membrane excitability through a reduction in I_{Na} (Baines, 2003). Our present results indicate that activity is also sufficient to regulate mRNA abundance for the same voltage-gated Na^+ channel. The genetic manipulations (*dnc*^{2} and overexpression of UAS-*rut*) that we use to increase synaptic excitation in the CNS probably do so through elevation of cAMP. Thus, a potential caveat to our use of these genetic manipulations is that the changes seen in *para* mRNA might not be a direct consequence of increased synaptic excitation but instead a consequence of increased cAMP. To counter this, we examined *para* mRNA in a third genetic background not predicted to influence cAMP directly, in which synaptic excitation of aCC/RP2 is increased. The mutation *slamdance*$^{iso7.8}$ (*sda*) encodes an aminopeptidase N, the loss of which is linked to induction of a seizure phenotype (Zhang et al., 2002). Synaptic excitation of aCC/RP2 is markedly increased in this genetic background, and analysis of *para* mRNA shows a significant reduction compared with WT (C. J. Mee and R. A. Baines, unpublished data). Together, our data are supportive of a model whereby exposure to synaptic excitation regulates I_{Na} in aCC/RP2 through activity-dependent changes in both activation of PKA (Baines, 2003) and abundance of *para* mRNA.

Posttranscriptional repression is a control mechanism of gene expression

The importance of RNA-binding proteins as regulators of cellular gene expression is underscored by the diverse roles that such proteins are implicated to control. In addition to the establishment of embryonic polarity (Tautz, 1988; Wharton and Struhl, 1991), posttranscriptional gene regulation is central to cell cycle control (Antic and Keene, 1997; Wang et al., 2000), neuronal differentiation (Blichenberg et al., 1999), cytokine expression (Atasoy et al., 1998), and DNA recombination (Hicks et al., 2000). In *Drosophila*, Pum is also implicated in the control of both long-term memory (Dubnau et al., 2003) and neuronal dendrite morphogenesis (Ye et al., 2004). Where studied, regulation is enabled by *cis*-regulatory sequences located in either the 3′- or 5′-UTRs of the transcript. The identities of these sequences are known to include the canonical NRE sequence recognized by Pum (Zamore et al., 1997) and a U-rich motif ($U_{4-6}A_{1-2}U$) designated the cytoplasmic polyadenylation element (Walker et al., 1996).

Our analysis of *para* mRNA shows an NRE-like sequence located in the 5′-UTR (our unpublished data) indicative that this mRNA might be subject to Pum-dependent translational repression. However, the presence of a *cis*-regulatory region homologous to the *hb* NRE motif is, by itself, insufficient evidence to implicate translational repression. To show this beyond doubt, a number of criteria must first be satisfied. These include a demonstration of specific binding of Pum to mRNA containing the *cis*-regulatory motif. Although gel-shift assays have been used to show an interaction between Pum and the 3′-UTR of *hb* (Zamore et al., 1997; Sonoda and Wharton, 1999), we have been unable, thus far, to show a strong interaction between Pum and the *para* NRE-like sequence. This lack of binding requires that we consider alternate mechanisms for the observed effect of Pum. One such mechanism could be that Pum is a transcriptional regulator of

para, although there is no evidence from previous work to indicate that Pum has any other activities in addition to that of a translational repressor. Our observation of altered *para* mRNA after manipulation of *pum* is consistent with both transcriptional and translational mechanisms. This is because Pum-dependent translational repression of *hb* may also increase the rate of degradation of *hb* mRNA by removal of the poly(A) tail (Wharton and Struhl, 1991; Wreden et al., 1997; Chagnovich and Lehmann, 2001). Of course, Pum could regulate *para* mRNA through interaction with an intermediate factor. Clearly, additional studies are required to establish the true nature of this regulatory mechanism.

Persistent Na^+ current influences neuronal excitability

Voltage-gated Na^+ channels are major determinants of neuronal excitability and as such have been identified as a convergent locus for intracellular regulation through PKA- and PKC-dependent mechanisms (Li et al., 1992; Smith and Goldin, 1997; Catterall, 2000). In a majority of neurons examined, phosphorylation mediates a reduction in maximal conductance in I_{Na} that, in *Drosophila*, has been shown to be sufficient to downregulate membrane excitability *in vivo* (Catterall, 2000; Cantrell and Catterall, 2001; Baines, 2003). Analysis of I_{Na} in aCC/RP2 shows two distinct components, a fast transient current that inactivates rapidly and a smaller persistent current that slowly inactivates. Although the transient current is suited to the initiation of single spikes, its function is compromised if membrane potentials remain depolarized. Motoneurons, including those in *Drosophila*, produce plateau potentials that amplify and sustain their motor output (Li and Bennett, 2003; Rohrbough et al., 2003; Li et al., 2004). Persistent sodium and calcium currents are principal contributing conductances that underlie these plateau potentials (Li and Bennett, 2003; Li et al., 2004). In the light of this, it is satisfying that we observe regulation of $I_{Na(p)}$ in a Pum-dependent manner. However, it is intriguing that, in the absence of *pum* (Pum^{bem}), or when *para* is upregulated [$Tp(1;2)r^{+75c}$], only $I_{Na(p)}$ is increased, whereas $I_{Na(t)}$ seemingly remains unchanged. This is even more puzzling when one considers that overexpression of *pum* is sufficient to downregulate both current components. That these two current components show differential regulation is suggestive that they may arise from different splice variants of *para*. *Para* is a highly complex gene with the capacity to produce multiple splice variants (Thackeray and Ganetzky, 1994, 1995). It is quite probable that these isoforms will, through differing kinetics and regulation, contribute to neuronal signaling in unique ways. Indeed, alternative splicing of exons a and i within the first intracellular loop is sufficient to alter $I_{Na(t)}$ ($I_{Na(p)}$ was not analyzed) (O'Dowd et al., 1995), and it is therefore not inconceivable that other isoforms will preferentially affect $I_{Na(p)}$.

In mammals, the kinetics of I_{Na} are also reported to be influenced by subunit composition. For example, the presence of the $Na_v1.6$ Na^+ channel subunit, in rat Purkinje neurons, confers a greater degree of persistent Na^+ current than in its absence (Raman et al., 1997). Thus, the increase in $I_{Na(p)}$ that we observe in aCC/RP2 may be accounted for by additional regulatory mechanisms that alter the predominant splice variants (in lieu of changing subunit composition) of the functionally expressed channels. Regardless of the precise mechanism, application of ATX II shows clearly that an increase in $I_{Na(p)}$ is sufficient to increase membrane excitability, whereas a reduction in total I_{Na} is sufficient to reduce excitability (Baines, 2003). In addition to Na^+ conductance, membrane excitability is also dependent on K^+ conductances and a complete understanding of how Pum regulates excitability in aCC/RP2 will require an analysis of how this protein regulates such conductances.

In summary, we present data to show that exposure to synaptic activity is able to regulate neuronal excitability in two identified *Drosophila* motoneurons through a Pum-dependent mechanism. That this mechanism is able to affect the abundance of *para* mRNA, together with the known function of this protein, implicates activity-dependent translational repression as a mechanism through which neurons maintain stability in firing when faced with changing synaptic excitation within the CNS.

References

Alzheimer C (1994) A novel voltage-dependent cation current in rat neocortical neurones. J Physiol (Lond) 479:199–204.

Antic D, Keene JD (1997) Embryonic lethal abnormal visual RNA-binding proteins involved in growth, differentiation, and post-transcriptional gene expression. Am J Hum Genet 61:273–278.

Atasoy U, Watson J, Patel D, Keene JD (1998) ELAV protein HuA (HuR) can redistribute between nucleus and cytoplasm and is up-regulated during serum stimulation and T cell activation. J Cell Sci 111:3145–3156.

Baines RA (2003) Postsynaptic protein kinase A reduces neuronal excitability in response to increased synaptic excitation in the *Drosophila* CNS. J Neurosci 23:8664–8672.

Baines RA, Bate M (1998) Electrophysiological development of central neurons in the *Drosophila* embryo. J Neurosci 18:4673–4683.

Baines RA, Robinson SG, Fujioka M, Jaynes JB, Bate M (1999) Postsynaptic vesicle release is essential for synaptogenesis in *Drosophila*. Curr Biol 9:1267–1270.

Baines RA, Uhler JP, Thompson A, Sweeney ST, Bate M (2001) Altered electrical properties in *Drosophila* neurons developing without synaptic transmission. J Neurosci 21:1523–1531.

Blichenberg A, Schwanke B, Rehbein M, Garner CC, Richter D, Kindler S (1999) Identification of a *cis*-acting dendritic targeting element in MAP2 mRNAs. J Neurosci 19:8818–8829.

Broadie K, Prokop A, Bellen HJ, O'Kane CJ, Schulze KL, Sweeney ST (1995) Syntaxin and synaptobrevin function downstream of vesicle docking in *Drosophila*. Neuron 15:663–673.

Burrone J, Murthy VK (2003) Synaptic gain control and homeostasis. Curr Opin Neurobiol 13:560–567.

Burrone J, O'Byrne M, Murthy VN (2002) Multiple forms of synaptic plasticity triggered by selective suppression of activity in individual neurons. Nature 420:414–418.

Cantrell AR, Catterall WA (2001) Neuromodulation of Na^+ channels: an unexpected form of cellular plasticity. Nat Rev Neurosci 2:397–407.

Catterall WA (2000) From ionic currents to molecular mechanisms: the structure and function of voltage-gated sodium channels. Neuron 26:13–25.

Chagnovich D, Lehmann R (2001) Poly(A)-independent regulation of maternal *hunchback* translation in the *Drosophila* embryo. Proc Natl Acad Sci USA 98:11359–11364.

Davis GW, DiAntonio A, Petersen SA, Goodman CS (1998) Postsynaptic PKA controls quantal size and reveals a retrograde signal that regulates presynaptic transmitter release in *Drosophila*. Neuron 20:305–315.

Davis RL, Cherry J, Dauwalder B, Han PL, Skoulakis E (1995) The cyclic AMP system and *Drosophila* learning. Mol Cell Biochem 149/150:271–278.

Desai NS, Rutherford L, Turrigiano GG (1999) Plasticity in the intrinsic excitability of cortical pyramidal neurons. Nat Neurosci 2:515–520.

Dubnau J, Chiang AS, Grady L, Barditch J, Gossweiler S, McNeil J, Smith P, Buldoc F, Scott R, Certa U, Broger C, Tully T (2003) The *staufen/pumilio* pathway is involved in *Drosophila* long-term memory. Curr Biol 13:286–296.

Dudai Y, Jan YN, Byers D, Quinn WG, Benzer S (1976) *dunce*, a mutant of *Drosophila* deficient in learning. Proc Natl Acad Sci USA 73:1684–1688.

Fujioka M, Emi-Sarker Y, Yusibova GL, Goto T, Jaynes JB (1999) Analysis of an *even-skipped* rescue transgene reveals both composite and discrete neuronal and early blastoderm enhancers, and multi-stripe positioning by gap gene repressor gradients. Development 126:2527–2538.

Gavis ER (2001) Over the rainbow to translational control. Nat Struct Biol 8:387–389.

Hicks GG, Singh N, Nashabi A, Mai S, Bozek G, Klewes L, Arapovic D, White

EK, Kroury MJ, Oltz EM, Van Kaer L, Ruley HE (2000) Fus deficiency in mice results in defective B-lymphocyte development and activation, high levels of chromosomal instability and perinatal death. Nat Genet 24:175–179.

Hong CS, Ganetzky B (1994) Spatial and temporal expression patterns of two sodium channel genes in *Drosophila*. J Neurosci 14:5160–5169.

Leslie KR, Nelson SB, Turrigiano GG (2001) Postsynaptic depolarization scales quantal amplitude in cortical pyramidal neurons. J Neurosci 21:RC170(1–6).

Li M, West JW, Lai Y, Scheuer T, Catterall WA (1992) Functional modulation of brain sodium channels by cAMP-dependent phosphorylation. Neuron 8:1151–1159.

Li Y, Bennett DJ (2003) Persistent sodium and calcium currents cause plateau potentials in motoneurons of chronic spinal rats. J Neurophysiol 90:857–869.

Li Y, Gorassini MA, Bennett DJ (2004) Role of persistent sodium and calcium currents in motoneuron firing and spasticity in chronic spinal rats. J Neurophysiol 91:767–783.

MacLean JN, Zhang Y, Johnson BR, Harris-Warrick RM (2003) Activity-independent homeostasis in rhythmically active neurons. Neuron 37:109–120.

Mantegazza M, Franceschetti S, Avanzini G (1998) Anemone toxin (ATX II)-induced increase in persistent sodium current: effects on the firing properties of rat neocortical pyramidal neurons. J Physiol (Lond) 507:105–116.

O'Brien RJ, Kamboj S, Ehlers MD, Rosen KR, Fischbach GD, Huganir RL (1998) Activity-dependent modulation of synaptic AMPA receptor accumulation. Neuron 21:1067–1078.

O'Dowd DK, Germeraad SE, Aldrich RW (1989) Alterations in the expression and gating of *Drosophila* sodium channels by mutations in the *para* gene. Neuron 2:1301–1311.

O'Dowd DK, Gee JR, Smith MA (1995) Sodium current density correlates with expression of specific alternatively spliced sodium channel mRNAs in single neurons. J Neurosci 15:4005–4012.

Paradis S, Sweeney ST, Davis GW (2001) Homeostatic control of presynaptic release is triggered by postsynaptic membrane depolarization. Neuron 30:737–749.

Raman IM, Sprunger LK, Meisler MH, Bean BP (1997) Altered subthreshold sodium currents and disrupted firing patterns in Purkinje neurons of Scn8a mutant mice. Neuron 19:881–891.

Rohrbough J, O'Dowd DK, Baines RA, Broadie K (2003) Cellular bases of behavioral plasticity: establishing and modifying synaptic circuits in the *Drosophila* genetic system. J Neurobiol 54:254–271.

Schweers BA, Walters KJ, Stern M (2002) The *Drosophila melanogaster* translational repressor *pumilio* regulates neuronal excitability. Genetics 161:1177–1185.

Smith RD, Goldin AL (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.

Sonoda J, Wharton RP (1999) Recruitment of Nanos to hunchback mRNA by Pumilio. Genes Dev 13:2704–2712.

Stern M, Kreber R, Ganetzky B (1990) Dosage effects of a *Drosophila* sodium channel gene on behavior and axonal excitability. Genetics 124:133–143.

Sweeney ST, Broadie K, Keane J, Niemann H, O'Kane J (1995) Targeted expression of tetanus toxin light chain in *Drosophila* specifically eliminates synaptic transmission and causes behavioral defects. Neuron 14:341–351.

Tautz D (1988) Regulation of *Drosophila* segmentation gene *hunchback* by two maternal morphogenetic centres. Nature 332:281–284.

Thackeray JR, Ganetzky B (1994) Developmentally regulated alternative splicing generates a complex array of *Drosophila para* sodium channel isoforms. J Neurosci 14:2569–2578.

Thackeray JR, Ganetzky B (1995) Conserved alternative splicing patterns and splicing signals in the *Drosophila* sodium channel gene *para*. Genetics 141:203–214.

Turrigiano GG (1999) Homeostatic plasticity in neural networks: the more things change, the more they stay the same. Trends Neurosci 22:221–227.

Turrigiano GG, Nelson SB (2000) Hebb and homeostasis in neuronal plasticity. Curr Opin Neurobiol 10:358–364.

Turrigiano GG, Nelson SB (2004) Homeostatic plasticity in the developing nervous system. Nat Rev Neurosci 5:97–107.

Turrigiano GG, Abbot LF, Marder E (1994) Activity-dependent changes in the intrinsic properties of cultured neurons. Science 264:974–977.

Turrigiano GG, LeMasson G, Marder E (1995) Selective regulation of current densities underlies spontaneous changes in activity in cultured neurons. J Neurosci 15:3640–3652.

Turrigiano GG, Leslie KR, Desai NS, Rutherford LC, Nelson SB (1998) Activity-dependent scaling of quantal amplitude in neocortical neurons. Nature 391:892–896.

Walker J, Dale M, Standart N (1996) Unmasking mRNA in clam oocytes: role of phosphorylation of a 3′ UTR masking element-binding protein at fertilization. Dev Biol 11:2510–2521.

Wang W, Caldwell MC, Lin S, Furneaux H, Gorospe M (2000) HuR regulates cyclin A and cyclin B1 mRNA stability during cell proliferation. EMBO J 19:2340–2350.

West AE, Griffith EC, Greenberg ME (2002) Regulation of transcription factors by neuronal activity. Nat Rev Neurosci 3:921–931.

Wharton RP, Struhl G (1991) RNA regulatory elements mediate control of *Drosophila* body pattern by the posterior morphogen nanos. Cell 67:955–967.

Wharton RP, Sonoda J, Lee T, Patterson M, Murata Y (1998) The Pumilio RNA-binding domain is also a translational repressor. Mol Cell 1:863–872.

Wreden C, Verotti AC, Schisa JA, Lieberfarb ME, Strickland S (1997) *Nanos* and *pumilio* establish embryonic polarity in *Drosophila* by promoting posterior deadenylation of *hunchback* mRNA. Development 124:3015–3023.

Ye B, Petritsch C, Clark IE, Gavis ER, Jan LY, Jan YN (2004) Nanos and Pumilio are essential for dendrite morphogenesis in *Drosophila* peripheral neurons. Curr Biol 14:314–321.

Zamore PD, Williamson JR, 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.

Zars T, Wolf R, Davis R, Heisenberg M (2000) Tissue-specific expression of a type I adenylyl cyclase rescues the *rutabaga* mutant memory defect: in search of the engram. Learn Mem 7:18–31.

Zhang H, Tan J, Reynolds E, Kuebler D, Faulhaber S, Tanouye M (2002) The *Drosophila slamdance* gene: a mutation in an aminopeptidase can cause seizure, paralysis and neuronal failure. Genetics 162:1283–1299.