Conserved RNA-Binding Proteins Required for Dendrite Morphogenesis in

*Caenorhabditis elegans* Sensory Neurons


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RNA-Binding Proteins in Dendrite Morphogenesis

KEYWORDS

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ABSTRACT

The regulation of dendritic branching is critical for sensory reception, cell-cell communication within the nervous system, learning, memory, and behavior. Defects in dendrite morphology are associated with several neurological disorders, thus an understanding of the molecular mechanisms that govern dendrite morphogenesis is important. Recent investigations of dendrite morphogenesis have highlighted the importance of gene regulation at the posttranscriptional level. Since RNA-binding proteins mediate many posttranscriptional mechanisms, we decided to investigate the extent to which conserved RNA-binding proteins contribute to dendrite morphogenesis across phyla. Here we identify a core set of RNA-binding proteins that are important for dendrite morphogenesis in the PVD multidendritic sensory neuron in Caenorhabditis elegans. Homologs of each of these genes were previously identified as important in the Drosophila melanogaster dendritic arborization sensory neurons. Our results suggest that RNA processing, mRNA localization, mRNA stability, and translational control are all important mechanisms that contribute to dendrite morphogenesis, and we present a conserved set of RNA-binding proteins that regulate these processes in diverse animal species. Furthermore, homologs of these genes are expressed in the human brain suggesting that these RNA-binding proteins are candidate regulators of dendrite development in humans.
INTRODUCTION

Dendrites are neuronal structures that receive sensory and synaptic information and are often elaborately branched to cast a wide receptive field or receive synaptic input from many other cells. Dendritic morphogenesis is a critical step in nervous system development, learning, and memory such that dendritic defects are associated with myriad neurological disorders such as autism, Alzheimer’s disease, and schizophrenia (Jan and Jan 2010; Kulkarni and Firestein 2012). Therefore it is important to understand the molecular genetic mechanisms that underlie dendrite morphogenesis. While several insights into the molecular controls of dendrite morphogenesis have come from studies that have focused on transcriptional control (Parrish et al. 2006; Ou et al. 2008; Jan and Jan 2010; Iyer et al. 2013), there is increasing evidence that posttranscriptional mechanisms such as mRNA localization and localized translational control are important as well (reviewed by Holt and Schuman 2013). For example, the translational repressor Nanos (Nos) regulates dendrite morphogenesis and branching complexity of *Drosophila* class IV da neurons. Importantly, dendrite morphogenesis depends on proper localization of *nos* mRNA to dendrites as well as translational regulation of *nos* mRNA, both of which are mediated by *cis*-elements in the *nos* 3’ untranslated region (UTR; Brechbiel and Gavis 2008). Therefore, elucidating posttranscriptional mechanisms that regulate dendrite morphogenesis is an important research goal.

RNA-binding proteins (RBPs) are important posttranscriptional regulators of gene expression that are involved in mRNA splicing, transport, localization, stability, and translational control. Animal genomes encode a diverse suite of several hundred RBPs
(Gamberi et al. 2006; Lee and Schedl 2006; Hogan et al. 2008; Kerner et al. 2011). Although there are some examples of mutations in RBP-encoding genes that are associated with neurological disorders (Zhou et al. 2014), it is not clear how many RBPs regulate dendrite morphogenesis. Thus far, only one study has reported a systematic screen of a majority of RBP-encoding genes for function in dendrite morphogenesis. Olesnicky et al. (2014) reported that 63 RBP-encoding genes in the Drosophila genome are important for dendrite development in the larval dendritic arborization (da) neurons. However, it is not clear the extent to which RBPs involved in dendrite development are conserved across animal species. To gain insight into this question, we identified C. elegans homologs of the RBPs identified in the Olesnicky et al. (2014) study and tested each for a role in dendrite development in the worm using the multidendritic PVD neuron as a model.

The C. elegans PVD neuron is an excellent model for the molecular genetic investigation of dendrite morphogenesis. The bilateral PVDs, which are located between the epidermis and the body wall muscles, have extensively branched dendritic trees that function as mechanoreceptors, nociceptors, proprioceptors, and cold temperature receptors (Way and Chalfie 1989; Tsali and Hobert 2003; Halevi et al. 2002; Oren-Suissa et al. 2010; Smith et al. 2010; Chatzigeorgiou and Schafer 2011; Albeg et al. 2011). PVD dendritic trees are stereotypic with primary (1°) branches that project anteriorly and posteriorly from the cell body and menorah- or candelabra-shaped structures extending from the primary branches, which include an orthogonal series of secondary (2°), tertiary (3°), and quaternary (4°) branches (Oren-Suissa et al. 2010;
Antonacci et al.  G3

Smith et al. 2010; Figure 1A). Thus, PVD function and morphology are similar to *Drosophila* da neurons and mammalian polymodal nociceptors (Albeg et al. 2011).

While little is known about PVD dendrite morphogenesis, we do know that a tripartite ligand-receptor complex, which includes epidermal ligands MNR-1 and SAX-7 and the PVD receptor DMA-1, promotes PVD dendrite branching (Liu and Shen 2012; Salzberg et al. 2013; Dong et al. 2013). In addition, nascent secondary branches are stabilized by HPO-30, a PVD-expressed claudin-like transmembrane protein, that likely anchors dendrites to the epidermis (Smith et al. 2013). We also know that the UNC-6/Netrin guidance molecule, the UNC-40/DCC receptor, and the UNC-5/Netrin receptor mediate dendrite self-avoidance (Smith et al. 2012). However, it is still unclear how PVD shape changes are mediated downstream of DMA-1 or Netrin signaling. Mechanical changes to dendrite morphology are, at a most basic level, the result of cytoskeleton dynamics. Interestingly, an RNA interference (RNAi) screen for PVD dendrite defects identified several genes that encode cytoskeleton-associated proteins, such as UNC-116/Kinesin-1 heavy chain, DLI-1/Dynein light intermediate chain, and BICD-1/Bicaudal-D (Aguirre-Chen et al. 2011). An open question is whether the DMA-1, HPO-30, Netrin, or other signaling pathways affect posttranscriptional gene regulation of genes involved in cytoskeleton dynamics, or other genes, to mediate PVD dendrite morphogenesis.

Since RBPs are important posttranscriptional regulators, an investigation of the role of RBPs in dendrite morphogenesis may provide insights to this question. Thus far, no RBPs have been implicated in dendrite development in PVDs or other neurons in *C. elegans* although a PVD-specific expression profile suggests that 47 RBP-encoding genes are enriched in expression in the PVD over other cells (Smith et al. 2010).
Here we identify the *C. elegans* homologs of *Drosophila* RBP genes described as important for da neuron dendrite morphogenesis (Olesnicky *et al.* 2014) and test each of them for a role in dendrite morphogenesis in PVDs. Using a combination of genetic mutations and RNA interference assays, we show that reduction or elimination of function of 12 of the candidate RBP-encoding genes reveals a reduction in the number of dendritic termini in PVD neurons. Some of these genes regulate the number of secondary and tertiary dendrite branches as well. We show that each of these genes is expressed within the PVD neuron and we use time-course analyses to show that while most of these RBPs affect terminal dendrite branch formation, two of the RBPs are required for dendrite maintenance, and one RBP is required for the timing of PVD dendrite morphogenesis. We examine the subcellular localization of each RBP within PVD neurons and discuss potential molecular roles of each RBP in this context and in the context of previous research. Because each of these RBPs functions in dendrite development in fly and worm and has at least one strong mammalian homolog, it suggests that these RBPs may be important in the development of dendrites in mammals as well. This claim is bolstered by human expression data showing that all of the human homologs of RBPs identified in this screen are expressed in the human brain.
MATERIALS AND METHODS

_C. elegans_ strains

Strains were derived from the Bristol strain N2, grown at 20°, and constructed using standard procedures (Brenner 1974). Mutants screened for dendrite defects are listed in Table S1. Mutant strains obtained from the Mitani Lab through the _C. elegans_ National Bioresource Project of Japan, the _C. elegans_ Reverse Genetics Core Facility at the University of British Columbia, the _C. elegans_ Reverse Genetics Core Facility at the Oklahoma Medical Research Foundation, and the Million Mutation Project (Thompson _et al._ 2013) were outcrossed at least four times. PVD dendrites were marked by _wdIs52[P_F49H12.4::GFP]_ or _wdIs51[P_F49H12.4::GFP]_ (Watson _et al._ 2008) for screening, _rwIs1[P_mec-7::RFP]_ (Smith _et al._ 2010) for gene expression studies, and _wyls587[ser-2prom3::myr-mCherry]_ (Liu and Shen 2012; Dong _et al._ 2013) for subcellular localization and rescue studies. The _sup-26_ expression pattern was determined using _smIs259[P_sup-26::sup-26::GFP]_ (Mapes _et al._ 2010). All other transgenes used in this study were constructed as described below. RNAi was conducted with strain: _wdIs52; sid-1(pk3321); uIs69[Pmyo-2::mCherry + Punc-119::sid-1]_ (Calixto _et al._ 2010).

**Imaging and quantification of PVD dendrite morphology**

Worms were picked at the life stages indicated, mounted on slides with 2% agarose pads, and immobilized with 600µM levamisole. Initial screening, time-course studies, and rescue experiments were conducted using a 40x or 63x objective on a Zeiss Axioskop or Leica DM5000B epifluorescence microscope. Dendrites, gene expression
patterns, and subcellular localization were imaged with a Leica SP5 spectral confocal microscope at 63x with 0.5µm per step and Leica LAS software. Secondary, tertiary, and terminal (quaternary and senary) dendrites were counted from the PVD cell body to the posterior end separately on the dorsal side, the ventral side, or both. While numerous researchers contributed to the primary screen, positive hits were independently verified by confocal microscopy by a single researcher who did not participate in the primary screen. Statistical tests were performed and graphs created with Prism 6.0f software (GraphPad Software, Inc.).

Construction of transgenes and DNA microinjection

All primers used in construction of transgenes described here are given in Table S2. For cgh-1, dcr-1, and mtr-4, presumptive promoter regions were PCR-amplified and subcloned into the SphI/KpnI sites of the Fire Lab vector pPD117.01, which carries a multiple cloning site upstream of GFP with a let-858 3’ UTR. For all other genes, presumptive promoter regions, which include at least 1000bp upstream of the start codon, or the entire upstream intragenic region, or previously published sequences, were PCR-amplified and subcloned into the pDONR221 vector using Gateway BP Clonase II (Invitrogen). Promoters were then subcloned into pDJK237, a promoterless plasmid with a Gateway cassette upstream of GFP with a 3’ UTR from let-858 derived from pPD117.01, using Gateway LR Clonase II Plus (Invitrogen).

cDNAs were gifts of Y. Kohara or Ding Xue and James Mapes (sources given in Table S2), or were amplified from a cDNA library derived from him-5(e1490) created by Trizol/chloroform and first-strand synthesis by Superscript Reverse Transcriptase III
Antonacci et al. (Invitrogen) and oligo dT primers. In all cases where multiple isoforms exist, the longest isoform was selected. cDNAs were PCR-amplified without stop codons and were cloned in pDONR221 using Gateway BP Clonase II (Invitrogen). The ser-2 promoter 3 fragment (Tsalik and Hobert 2003) was PCR-amplified and cloned into pDONR P4-P1r and the GFP coding sequence with the let-858 3' UTR from pPD117.01 was PCR-amplified and cloned into pDONR P2r-P3 using Gateway BP Clonase II (Invitrogen). The cDNAs, ser-2 promoter 3, and GFP were all cloned into pDEST R4-R3 using Gateway LR Clonase II Plus (Invitrogen).

DNA microinjection was performed using standard practices (Mello and Fire 1995). For gene expression pattern studies, unc-76(e911); rwls1 hermaphrodites were injected with 20ng/µl of GFP plasmid and 60ng/µl of unc-76(+) plasmid. For subcellular localization and rescue studies, wyIs587; unc-76(e911) worms or wyIs587; unc-76(e911); RBP mutation-bearing worms were injected with 10-20ng/µl of GFP plasmid and 60ng/µl of unc-76(+) plasmid.

RNAi
RNAi feeding was performed essentially as described (Kamath and Ahringer 2003). wdls52; sid-1(pk3321); uls69[Pmyo-2::mCherry + Punc-119::sid-1] L4 hermaphrodites were placed on Petri plates with nematode growth medium seeded with dsRNA-expressing E. coli. The P0 worms were transferred to a new plate after 24 hours and the progeny from that second plate scored at the young adult stage. dsRNA-expressing E. coli were obtained from the Ahringer lab library (Kamath et al. 2003; Fraser et al. 2000) or were constructed by PCR-amplification of a region of the target gene and Gateway Clonase-
mediated insertion of that PCR amplicon into a double-T7 pPD129.36 plasmid modified with a Gateway cassette (Invitrogen). In cases where target genes produce multiple isoforms, RNAi strategies targeted mRNA sequences common to all isoforms. See Table S2 for specific information on the RNAi clones used in this study.

**Blast Searches**

Blast searches were performed using the command line NCBI-BLAST package (version 2.2.25; Camacho et al. 2009). *C. elegans* protein sequences were aligned to custom BLAST databases using the BLASTp algorithm with default parameters. BLAST databases were assembled from annotated *Drosophila melanogaster* proteins (genome release 6.01, downloaded from flybase.org on 8/4/2014) and the human protein Refseq database (downloaded from NCBI on 8/4/2014) for alignments between *C. elegans* and *Drosophila* and human sequences respectively. Alignment Expect (E) values for all comparisons are reported.

**Orthology Mapping and Expression of Human Orthologs**

To identify human orthologs, the *C. elegans* genes were mapped on to the Database of Orthologous Groups (OrthoDB; Waterhouse et al. 2013) to determine the OrthoDB identifier for each gene. All human genes assigned to the same OrthoDB identifier were taken as orthologs of the *C. elegans* gene and used for additional analysis. Data from the Tissue-specific Gene Expression and Regulation (TiGER) database (Liu et al. 2008) was used to assay whether the identified human orthologs are expressed in human brain tissue. Raw data files were downloaded from the TiGER website.
Antonacci et al. (http://bioinfo.wilmer.jhu.edu/tiger/, accessed on 6/27/2014) and analyzed using a custom Perl script.

RESULTS

A screen for conserved RNA-binding proteins that function in *C. elegans* PVD sensory neuron dendrite morphogenesis

While RNA-binding proteins (RBPs) have been shown to be important for the regulation of dendrite development (Ye et al. 2004; Vessey et al. 2008; Bestman and Cline 2008; Brechbiel and Gavis 2008; Olesnicky et al. 2012; Olesnicky et al. 2014), it is not clear the extent to which RBPs are conserved in this process across species. Thus far, only one study has aimed to identify all predicted RBP-encoding genes in a genome that contribute to dendrite morphogenesis. Olesnicky et al. (2014) screened the vast majority of the predicted RBP-encoding genes in the *Drosophila* genome for a role in dendrite morphogenesis of the larval class IV da sensory neurons and found that 63 of these genes were important. To determine if these conserved RBPs function in dendrite morphogenesis in other species, we tested the homologs of these 63 RBPs for function in dendrite development using the *C. elegans* multidendritic PVD sensory neuron.

Using BLASTp, we identified 54 *C. elegans* homologs of the 63 RBPs reported to function in dendrite morphogenesis in *Drosophila* (Olesnicky et al. 2014). The relative smaller number of candidate RBPs in *C. elegans* is due to several cases in which two *Drosophila* RBPs are homologous to a single *C. elegans* RBP and one case in which a *Drosophila* RBP, specifically Oskar, does not have a clear homolog in *C. elegans*. We
next identified mutations and RNA interference (RNAi) treatments to eliminate or knock down the function of each candidate RBP gene in *C. elegans*. To assay each RBP gene for a role in PVD dendrite morphogenesis, young adult mutant or RNAi-treated animals carrying a GFP marker for PVD neurons were imaged and the number of dendritic termini counted on the dorsal and ventral sides from the cell body to the posterior end of the animal (see Materials and Methods). Thirty of the 54 RBP genes were tested using genetic mutations while we used RNAi to test the remaining 24. Twenty-eight of the mutations we used are presumptive null alleles characterized by small deletions or nonsense substitutions whereas the other two mutations were previously reported to cause a reduction of function. RNAi was used to test genes for which mutations were unavailable or to bypass pleiotropy and possibly reveal dendrite phenotypes in cases where genetic mutations resulted in embryonic or larval lethality. To increase the efficacy of RNAi in neurons we used a neuron-sensitized knockdown strategy, which expresses the dsRNA transporter gene *sid-1* in the neurons of an otherwise *sid-1* mutant animal (Calixto et al. 2010; see Materials and Methods). *mec-3(RNAi)* was used as a positive control for RNAi treatments and produced a reduced branching phenotype in PVDs similar to previously published phenotypes (Aguirre-Chen et al. 2011). The complete list of RBPs tested, BLASTp E values, alleles used, and RNAi treatments is given in Table S1.

We found that the loss or reduction of function of 12 RBP genes, individually, resulted in a statistically significant reduction in PVD dendritic termini compared to control animals (Table 1 and Figure 1 and Figure 2A). Whereas control animals at the young adult stage have an average of 23 dendritic termini in the region scored,
mutations in \textit{cgh-1, cpb-3, dcr-1, larp-5, mbl-1, mtr-4, rsp-3, rsp-6, set-2} and \textit{sup-26} all result in at least a 20% reduction in dendrite termini (Figure 1B and Figure 2A). While RNAi screening was less effective at identifying RBP genes important for dendrite morphogenesis (see Discussion), we did find that \textit{ddx-17} and \textit{Y55F3AM.3} RNAi-treated animals showed a 14% and 11% respective reduction in the number of dendritic termini compared to untreated control animals (Figure 1C and Figure 2A). After completing the RNAi screen we tested several point mutations in \textit{Y55F3AM.3} generated by the Million Mutation Project (Thompson \textit{et al.} 2013) to identify a reduction-of-function allele. One allele, \textit{gk454899}, affects a conserved residue across \textit{Drosophila}, zebrafish, and mammals and resulted in a 19% reduction of dendritic termini compared to control animals (Table S1 and Figure 1B and Figure 2A).

The \textit{Y55F3AM.3} RNAi and mutant studies provide independent verification of the role of this RBP gene in PVD dendrite morphogenesis. To obtain independent verification of the role of other RBP genes in dendrite development, we obtained additional alleles where available. One additional allele of \textit{mbl-1} and \textit{set-2} and three additional alleles of \textit{sup-26} confirmed a reduction of dendritic termini (data not shown). We found that \textit{set-2(ok952)} animals had a weak reduction in the number of dendrites compared to the \textit{n4589} allele (data not shown), which is consistent with a previous report that the \textit{ok952} deletion is a hypomorphic allele (Xiao \textit{et al.} 2011).

We next tested to see if the loss or reduction of function of these 12 RBP genes also resulted in defects in lower order dendritic branching in PVDs by counting the number of secondary and tertiary branches. Loss or reduction of function of five of the 12 genes, \textit{dcr-1, larp-5, mbl-1, mtr-4, and sup-26}, resulted in a reduction in the number
of secondary branches. Interestingly, loss of function of two of the 12 genes, cgh-1 and cpb-3, resulted in an increase in the number of secondary branches (Figure 2B). Loss of cgh-1, cpb-3, and set-2 resulted in an increase of tertiary branches while loss of larp-5, mbl-1, mtr-4, and sup-26 resulted in a reduction of tertiary branches (Figure 2C). Thus, we find that loss of cgh-1 or cpb-3 results in an increase of secondary and tertiary dendrite branches, but also causes a roughly 30% reduction of terminal branches (Figure 2). This similarity in the cgh-1 and cpb-3 mutant phenotypes is interesting given their predicted molecular functions (see Discussion). Loss of larp-5, mbl-1, mtr-4, or sup-26 results in a decrease of all orders of dendrite branches (Figure 2). Notably, we did not find any mutations or RNAi treatments that resulted in supernumerary dendritic termini at the young adult stage. The pleiotropic defects in PVD neurons resulting from loss of RBP gene function are summarized in Table 2.

Finally, we examined all 12 RBP mutants or RNAi treatments for qualitative patterning phenotypes in the PVD. We note that mbl-1 null mutants display a particularly striking and reproducible terminal dendrite branching defect. While terminal branches near the cell body are similar to controls in terms of length and distribution, mbl-1 mutants have progressively fewer and shorter terminal branches towards the posterior end of the PVD neuron (Figure 1). This suggests that mbl-1 is not explicitly required for dendrite branching in general but is specifically important for the patterning of branching posterior to the cell body.

**RBPs are required for formation, timing, or maintenance of dendrite branches**
A reduction in the number of terminal dendritic branches could be due to a failure to form the branches, a delay in branch formation relative to other hallmarks of animal development, or a failure to maintain branches. To distinguish between these possibilities we conducted a time course analysis of dendrite development by counting dendritic termini of the PVD neuron at the mid-L4 stage, the young adult stage, and 18 hours past the young adult stage (adult).

We found a statistically significant reduction of dendritic termini in *cpb-3, dcr-1, larp-5, mbl-1, mtr-4, set-2, rsp-6,* and *Y55F3AM.3* mutants relative to the control at all stages (Figure 3A). This suggests that these mutants fail to form the appropriate number of terminal branches and that there is neither a delay nor a maintenance defect.

The time course analyses suggest that *sup-26* and *rsp-3* are required for dendrite maintenance during the L4 stage. We found that young adult *sup-26* mutant animals have a statistically significant, roughly 10% reduction in dendritic termini compared to mid-L4 stage mutant animals (Figure 3B). The number of dendrite termini in *sup-26* mutants then increases significantly during the adult stage but does not reach the same level as controls (Figure 3B). Similar to *sup-26* mutants, *rsp-3* mutants exhibit a statistically significant loss of roughly 9% of dendritic termini in PVD neurons from the mid-L4 to the young adult stage (Figure 3B). Adult *rsp-3* mutant animals exhibit a mild but statistically significant increase in the number of dendrites from the young adult stage but never recover beyond the number of dendritic termini observed in mid-L4 animals (Figure 3B). Together, this suggests that *sup-26* and *rsp-3* mutants lose more terminal dendrite branches than they form during the late-L4 stage. However, branch loss ceases during the adult and some growth ensues, albeit weakly in *rsp-3* mutants.
We also find that sup-26 and rsp-3 mutants have significantly fewer dendrites than controls at the mid-L4 stage (Figure 3B) shortly after fourth order dendrite branch outgrowth is initiated in the early L4 stage (Smith et al. 2010). This suggests that sup-26 and rsp-3 mutants are defective in maintenance of terminal branches as soon as they begin to develop or that these mutants fail to form the appropriate number of dendritic termini initially.

Interestingly, we find that cgh-1 mutants are delayed in dendrite development with a 51% reduction at the mid-L4 stage but only a 35% reduction at the young adult stage. As adults and late adults (48 hours past the young adult stage), there is no longer a reduction in dendritic termini in cgh-1 mutants relative to controls. In fact, cgh-1 mutants have statistically significantly more terminal branches than controls at these later stages (Figure 3C). Collectively, the time course analyses demonstrate at least three different etiologies for dendrite defects; hence, the RBPs identified in this study likely employ different molecular mechanisms for dendrite morphogenesis.

**RBP genes required for dendrite development are expressed in the PVD neuron**

To determine the expression patterns for each of the RBP genes identified in our screen, we expressed the coding sequence of GFP under the control of presumptive promoters for each RBP gene (see Materials and Methods). Each of the 12 RBP gene regulatory regions expressed GFP in the PVD neuron (Figure 4). Most of the genes (cgh-1, cpb-3, dcr-1, mtr-4, rsp-3, rsp-6, set-2, and sup-26) are expressed broadly throughout development (data not shown) excluding the germ line, which often silences repetitive DNA such as the extrachromosomal arrays generated by DNA microinjection
in worms (Mello et al. 1991; Kelly and Fire 1998). In contrast, \textit{ddx-17, larp-5, mbl-1}, and \textit{Y55F3AM.3} are expressed mostly or exclusively in neurons including the PVD (data not shown). These data demonstrate that RBP genes required for PVD dendrite development are expressed in the PVD and our findings are consistent with a cell-autonomous function of the RBPs within the PVD neuron. However, RBP gene expression is not specifically restricted to the PVD neuron, suggesting that these RBP genes may play additional roles in other neurons and, in some cases, non-neural tissues.

\textbf{Subcellular localization of RBPs suggests how they influence dendrite development}

Because RBPs play diverse molecular roles related to posttranscriptional gene regulation, the subcellular localization of any given RBP may offer some insight as to which role(s) it does or does not play. To determine where within the PVD each RBP is localized, we expressed each RBP as a fusion to GFP in PVD neurons using the \textit{ser-2prom3} regulatory element (Tsalik and Hobert 2003; see Materials and Methods).

\textit{DDX-17, MBL-1, MTR-4, RSP-3, RSP-6, SET-2, and Y55F3AM.3} translation fusions to GFP show clear nuclear expression (Figure 5). This is consistent with these RBPs playing roles in RNA processing within the nucleus (see Discussion). Conversely, we find that \textit{CGH-1, CPB-3, LARP-5, and SUP-26} translational fusions to GFP are localized to the cytoplasm (Figure 6). More specifically, \textit{CGH-1::GFP} is enriched in the perinuclear region and small puncta are present throughout the cytoplasm including particles in dendrites (Figure 6). Similarly, \textit{CPB-3::GFP} is also found in puncta in the
cytoplasm of the cell body and dendrites. However, CPB-3 puncta are substantially larger and lack perinuclear enrichment (Figure 6). Unlike CGH-1 and CPB-3, SUP-26::GFP is restricted to the cytoplasm of the cell body and is not found in dendrites. SUP-26 has a striking perinuclear enrichment with intermediate-sized puncta, relative to CGH-1 and CPB-3, located further from the nucleus but still within the cell body (Figure 6). Finally, LARP-5::GFP is diffuse throughout the cytoplasm, including the dendrites, and the nucleus and does not localize to discrete puncta (Figure 6).

We were not able to generate a visible DCR-1::GFP fusion protein expressed in the PVD neuron. However, anti-DCR-1 immunohistochemistry reveals that DCR-1 is in puncta in the cytoplasm and nucleus of C. elegans germ cells (Beshore et al. 2011). This is consistent with known roles of DCR-1/Dicer in the microRNA and RNAi pathways as well as restricting the accumulation of dsRNA from bidirectional transcription (Bernstein et al. 2001; Grishok et al. 2001; White et al. 2014).

To test if these RBPs function cell-autonomously in the PVD neuron and to determine if RBP::GFP fusion protein subcellular localization is biologically relevant, we expressed each RBP::GFP transgene specifically in the PVD neuron and tested for its ability to rescue PVD dendrite defects in RBP mutant animals (see Materials and methods). We found that PVD-specific expression of CGH-1, MBL-1, and Y55F3AM.3 fusion proteins to GFP confer a statistically significant complete rescue of the PVD dendritic termini defects in the respective mutants (Figure 7). This strongly suggests that CGH-1, MBL-1, and Y55F3AM.3 each function cell-autonomously in the PVD to control dendrite development. Furthermore, the subcellular localizations inferred from
these RBP::GFP fusions proteins is likely accurate because these proteins are functional.

We also found that \textit{cpb-3}, \textit{mtr-4}, \textit{rsp-3}, \textit{rsp-6}, \textit{set-2}, and \textit{sup-26} dendrite defects are partially rescued by PVD-specific expression of the corresponding RBP::GFP fusion proteins. The reduction of PVD dendritic termini associated with each mutant is significantly ameliorated by expression of the corresponding RBP::GFP fusion protein, but does not return to control levels (Figure 7). This suggests that these RBPs function within the PVD neuron to control dendrite development. However, the partial rescue does not allow us to exclude the possibility that RBP expression in other cells is also important for PVD dendrite development. Alternatively, partial rescue may be the result of GFP slightly reducing the activity of the RBPs or non-native expression levels of the RBP::GFP fusion proteins.

We were unable to recover transgenic lines expressing LARP-5::GFP suggesting that this transgene is toxic. However, we were able to generate transgenic animals expressing GFP::LARP-5. Interestingly, we found that \textit{larp-5} mutants expressing a GFP::LARP-5 fusion protein in PVD neurons were not rescued; rather, these animals had significantly lower numbers of dendritic termini than \textit{larp-5} mutants alone (Figure 7). It is possible that a GFP tag on LARP-5 disrupts its function. Alternatively, the results may indicate that LARP-5 levels must be precisely controlled for proper dendritic development. This would not be unusual given that the expression levels of multiple RBPs are critical for \textit{Drosophila} da sensory neuron development. For example, overexpression of \textit{nanos}, \textit{pumilio}, and \textit{brat} all result in more severe dendrite defects than the mutants alone (Ye \textit{et al.} 2004; Olesnicky \textit{et al.} 2012). We were unable to test
DDX-17::GFP for cell-autonomous function and relevance of its subcellular localization since this RBP was identified from RNAi, and *ddx-17* mutants die early in development.

**DISCUSSION**

**Conservation of the role of RBPs in dendrite morphogenesis**

Sixty-three RBP genes were reported to function in *Drosophila* da neuron morphogenesis suggesting that many aspects of posttranscriptional regulation are important to this process (Olesnicky *et al.* 2014). Here we test 54 homologs of the *Drosophila* suite of dendrite RBPs and show that 12 of them, or roughly 22%, are important for PVD dendrite morphogenesis. This relatively low rate of functional conservation may not be unexpected. Only 21% of *C. elegans* genes with an ortholog in another eukaryote give obvious RNAi phenotypes (Kamath *et al.* 2003). In addition, the plasticity of genetic networks between *C. elegans* and *C. briggsae*, which share similar body plans and ecological niches despite roughly 20 million years of evolution, is significant; over 25% of orthologs in these worm species have different functions (Verster *et al.* 2014).

There are few reasons why such a small percentage of the RBP genes required for *Drosophila* da neuron morphogenesis have homologs with conserved function in *C. elegans* PVD neurons. Although da neurons and PVD neurons are both complex multidendritic sensory neurons, the PVD has a simpler and more stereotyped shape, which may not require as many posttranscriptional regulatory factors. Another significant difference between da neurons and PVDs is that da neurons undergo
extensive pruning and shape changes during development while the PVD neuron does not undergo drastic morphological changes (Williams and Truman 2005; Williams and Truman 2005; Albeg et al. 2011). Perhaps the reduced need for shape changes in PVDs also reduces the need for posttranscriptional gene regulation mechanisms mediated by RBPs.

While this study specifically tested 54 RBP genes using predominantly predicted null alleles and a neuron-sensitized RNAi strategy, the 12 genes reported are likely an underestimate. While 10 out of 30 genetic mutations tested positive for dendrite defects, our primary RNAi screen identified only two (ddx-17 and Y55F3AM.3) of 24 positive hits. Based on the rate of positive hits using genetic mutations, we would have expected a higher percentage of the genes tested by RNAi to also be positive. Moreover, after completing the RNAi screen we identified some mutations from the Million Mutation Project (Thompson et al. 2013) that affect genes for which no deletion allele was available. Using these alleles we confirmed Y55F3AM.3 and added larp-5, which was not a hit in the initial RNAi screen. Thus we conclude that false negatives by RNAi are likely and difficult to avoid. Some of the genes tested by genetic null mutation may also be reported as false negatives in our screen due to maternal rescue in cases where homozygous sterile mutants are scored from heterozygous mothers (see below for discussion of drsh-1/Drosha). Finally, although most alleles tested are predicted nulls, at least two have been reported to be hypomorphic alleles. Homologs of stau-1 are involved in dendrite development in various species, yet we did not find statistically significant defects in PVD neurons using the hypomorphic allele tm2266 (Tang et al. 2001; Goetze et al. 2006; Barbee et al. 2006; LeGendre et al. 2013). The sym-2(mn617)
allele is also likely to be a hypomorph (Yochem et al. 2004) and did not produce PVD defects in our screen. While the sym-2 homolog in *Drosophila*, glo, is required for dendrite development (Brechbiel and Gavis 2008; Olesnicky et al. 2014), this has not been demonstrated for the human homolog, heterogeneous nuclear ribonucleoprotein F.

Although the dendritic roles of several RBP genes identified in the Olesnicky et al. (2014) RNAi screen were confirmed by genetic mutation or second RNAi line, it is possible that some false positives were reported due to a previously unknown problem with some RNAi fly stocks used. A recent study showed that many randomly selected Vienna *Drosophila* RNAi Center “KK” series RNAi stocks produce nonspecific phenotypes when crossed to certain Gal4 drivers due to an additional and previously uncharacterized RNAi hairpin vector insertion site (Green et al., 2014). We therefore acknowledge this caveat to our assessment of the number of conserved RBPs that function in dendrite morphogenesis in both *Drosophila* da neurons and *C. elegans* PVDs. Considering the results of the *Drosophila* da neuron dendrite screen and this study, the percent of RBPs that play a conserved role in dendrite morphogenesis is likely to exceed the 22% reported here.

**mRNA localization and translational regulation contribute to dendrite morphogenesis**

It is well documented that mRNAs are transported within RNA-protein complexes (RNPs) into dendrites and there is increasing evidence that translational control of these mRNAs contributes to dendrite morphology (reviewed by Bramham and Wells 2007;
Pimentel and Boccaccio 2014; Tom Dieck et al. 2014; Di Liegro et al. 2014). Therefore we were not surprised that this study identified RBP genes, such as cgh-1, cpb-3, lar-5, and sup-26, which are implicated in mRNA transport, localization, and/or translation.

*cgh-1* encodes an RNA helicase similar to mammalian DDX6/RCK/p54. Both are associated with various RNP granules and are required for mRNA translational repression, possibly by stalling translation by polysomes associated with RNPs (reviewed by Rajyaguru and Parker 2009; Pimentel and Boccaccio 2014). We found that functional CGH-1::GFP protein is enriched in the cell body and in puncta within PVD dendrites (Figure 6 and Figure 7) suggesting that CGH-1 may act as a translational repressor of target mRNAs during their transport into dendritic compartments.

*cpb-3* encodes a homolog of mammalian cytoplasmic polyadenylation element binding protein (CPEB), which acts as a translational repressor of target mRNAs but also promotes translation when phosphorylated (reviewed by Villalba et al. 2011). Although invertebrate CPEBs, such as CPB-3, lack the site of phosphorylation (Hasegawa et al. 2006), it is possible that their dual-function as a repressor and activator may be regulated by an alternative mechanism. CPEB has known roles within the nervous system where it is required for learning and memory by regulating synaptic plasticity (Si et al. 2003; Miniaci et al. 2008). In addition, CPEB regulates the transport of mRNAs in dendrites and disruption of CPEB function in frogs led to stunted development of dendritic arbors (Huang et al. 2003; Bestman and Cline 2008). Since CPEB-containing granules are found within dendrites in frogs and a functional CPB-3::GFP protein is found within dendrites in *C. elegans* (Bestman and Cline 2009; Figure
6 and Figure 7), it suggests that CPEB-dependent translation is important for providing localized sources of proteins within the dendritic compartment, which in turn regulates local dendrite development.

*sup-26* encodes an RNA recognition motif-containing protein similar to *Drosophila* Shep and mammalian RBMS1/2/3. *C. elegans* SUP-26 has been shown to directly bind to the 3’ UTR of a target mRNA and repress its translation, possibly through a physical interaction with the poly-A binding protein (PAB-1; Mapes *et al.* 2010). *Drosophila shep* was recently shown to be important for neuronal remodeling, (Chen *et al.* 2014) suggesting a broader role in nervous system development. A functional SUP-26::GFP fusion protein is largely perinuclear and absent from dendrites (Figure 6 and Figure 7) and thus may play a role in repressing the translation of target mRNAs upon nuclear export and before they associate with RNPs that take over the role of mRNA localization and translational control.

LARP-5 is homologous to mammalian LARP4 and LARP4B, which encode proteins that bind to the poly-A binding protein, associate with polysomes, and promote mRNA stability and translation (Schäffler *et al.* 2010; Yang *et al.* 2011). siRNA-mediated knockdown of LARP4 leads to decreased mRNA levels while overexpression of LARP4 increases mRNA levels, strongly suggesting that LARP4 can prolong mRNA half-life (Yang *et al.* 2011). In *C. elegans*, LARP-5::GFP is localized to the cytoplasm including the dendrites (Figure 6), which is consistent with association with poly-A binding protein and polysomes and suggests that LARP-5 may promote the stability of target mRNAs that are required for dendrite development. However, the subcellular localization of LARP-5 must be considered in context of the caveat that GFP::LARP-5 does not rescue
dendrite defects of *larp-5* mutants and actually leads to a significant further reduction of dendritic termini. Thus the subcellular localization we observe may not reflect endogenous LARP-5 protein localization (Figure 7).

Taken together, we favor a model whereby SUP-26 translationally represses mRNAs upon nuclear export and prior to loading with additional RBPs, possibly including CGH-1, CPB-3, and/or LARP-5, which regulate mRNA transport, stability, and translation. In the absence of SUP-26, target mRNAs may be prematurely translated in the cell body where they cannot influence dendrite development. In support of this idea, *sup-26* mutants have fewer branches at all orders (Figure 2). In the absence of CGH-1 and CPB-3, target mRNAs may be translated prematurely within dendrites, which could plausibly explain the excess secondary and tertiary branches observed in *cgh-1* and *cpb-3* mutant PVDs (Figure 2). Terminal branches eventually do form in *cgh-1* mutants (Figure 3C) suggesting that over time target mRNAs or their protein products do eventually reach the correct location or exhibit the correct activity. Finally, we suggest that a loss of LARP-5 activity results in decreased mRNA stability and thus a reduction of branching at all orders (Figure 2). These findings highlight the importance of mRNA localization, stability, translational repression, and localized protein synthesis within the developing dendritic arbor.

The role of mRNA splicing in dendrite morphogenesis

One striking finding of this screen is that six (MBL-1, RSP-3, RSP-6, SET-2, Y55F3AM.3, and DDX-17) of the 12 RBPs that we identified as important for PVD
dendrite morphogenesis are known or thought to be involved in mRNA splicing, and more specifically alternative splicing.

*mbl-1* encodes a homolog of *Drosophila* Muscleblind (Mbl) and mammalian Muscleblind-like proteins (Mbnl), which are Zinc finger containing proteins that regulate alternative splicing (Begemann *et al.* 1997; Kanadia *et al.* 2003; Ho *et al.* 2004; Pascual *et al.* 2006; Wang *et al.* 2008; Sasagawa *et al.* 2009; Spilker *et al.* 2012; Wang *et al.* 2012). Although *C. elegans* MBL-1 has not been shown definitively to participate in alternative splicing, strong homology to fly Mbl and mammalian Mbnl proteins strongly suggests that this molecular function is conserved (Pascual *et al.* 2006; Spilker *et al.* 2012).

*rsp-3* and *rsp-6* provide another strong link to alternative splicing. These genes encode members of the serine/arginine-rich (SR) family of splicing factors that regulate constitutive and alternative splicing (reviewed by Risso *et al.* 2012). *rsp-3* encodes a protein similar to *Drosophila* SF2 and human SRSF1/9, and *rsp-6* encodes a homolog of *Drosophila* X16 and mammalian SRSF3/7 splicing factors (Krainer *et al.* 1991; Longman *et al.* 2000; Vorbrüggen *et al.* 2000; Kawano *et al.* 2000; de la Mata and Kornblihtt 2006).

While little is known about *Y55F3AM.3* and *ddx-17*, the paucity of information does suggest that these genes may be involved in alternative splicing. *Y55F3AM.3* is an RRM-containing protein with a human homolog (RBM39) that co-localizes with spliceosomal proteins and affects alternative splicing for some target genes (Imai *et al.* 1993; McCracken *et al.* 2005; Ellis *et al.* 2008; Dowhan *et al.* 2005; Huang *et al.* 2012). *ddx-17* encodes a DEAD-box RNA helicase protein similar to human DDX17/p72 (and
DDX5/p68), which is a regulator of alternative splicing and co-purifies with the U1snRNP and SR protein SRrp86 (Hönig et al. 2002; Lee 2002; Li et al. 2003). Furthermore, one study suggests that DDX5/p68 facilitates the binding of Mbnl proteins to splicing targets (Laurent et al. 2012), adding another link to alternative splicing.

Finally, set-2 encodes an RRM-containing Histone 3 lysine 4 (H3K4) methyltransferase similar to yeast and fly Set1 and human SETD1A and SETD1B (Xu and Strome 2001; Briggs et al. 2001; Miller et al. 2001; Lee and Skalnik 2005; Xiao et al. 2011; Table 1). Set1 is recruited by RNA polymerase II to mediate H3K4 trimethylation (me3) on nearby nucleosomes. Thus, H3K4me3 is a landmark of actively transcribed genes or genes that experience transcription initiation (Miller et al. 2001; Krogan et al. 2003; Tenney and Shilatifard 2005; Guenther et al. 2007). In addition, H3K4me3 enhances splicing efficiency and affects alternative splicing, possibly through its association with the U2 snRNP (Sims et al. 2007; Luco et al. 2010). Furthermore, H3K4me3 is enriched at alternative transcriptional start sites suggesting it may regulate alternative promoter use. This is interesting because the use of alternative transcriptional start sites contributes more to transcriptome diversity than alternative splicing does in the mouse cerebellum (Pal et al. 2011). Since loss of set-2 activity greatly reduces H3K4me3 in worms (Greer et al. 2010), it is attractive to suggest that set-2 may play a role in regulating alternative splicing and/or alternative transcription start sites.

There are several possibilities for how alternative splicing and use of alternative promoters may affect dendrite development. The simplest possibility is that one or more specific alternatively spliced products are important for dendrite development and that
defects in alternative splicing reveal defects in dendrite morphology (Olesnicky et al. 2014). Support for this comes from the well-documented prevalence of transcriptome diversity in the nervous system, which is thought to be important for contributing to cellular complexity (Yeo et al. 2004; Lipscombe 2005; Li et al. 2007; Norris and Calarco 2012). For example, alternative splicing of the mammalian Bcl11A transcriptional factor-encoding gene produces a long form that negatively regulates dendrite outgrowth and a short form that antagonizes the long form and thus promotes dendrite outgrowth (Kuo et al. 2009).

An alternative possibility is that some splicing occurs in the dendrites rather than in the nucleus. Several mammalian splicing components have been shown to exit the nucleus and retain function in the dendroplasm, perhaps to provide localized mature mRNAs for localized translation of specific transcripts (Glanzer et al. 2005; Bell et al. 2008). However, all of the putative splicing regulators in this study show a tight nuclear localization as observed with RBP::GFP fusion proteins (Figure 5). Since each of these fusion proteins rescues the mutant dendrite defects (except DDX-17, which was not amenable to testing), it suggests that the strong nuclear localization reflects normal activity (Figure 7). Thus if any of these factors are involved in activities within dendrites, they must be present in concentrations that are below the level of detection by confocal microscopy. Furthermore, cytoplasmic splicing has thus far not been described in C. elegans.

Proteins associated with splicing may also be important for mRNA localization and/or translational regulation. For example, mammalian Mbnl proteins, which are homologs of MBL-1, are important for targeting hundreds of mRNAs to membranes
where they are translated (Adereth et al. 2005; Osborne et al. 2009; Du et al. 2010; Masuda et al. 2012; Wang et al. 2012). Again, we do not favor this model for how the splice factors identified in this study affect dendrite development because their localization is restricted to the nucleus (Figure 5). However, the localization of mRNAs can be influenced by RBPs that never leave the nucleus. For example, localization of Ash1 mRNA in yeast requires the function of Loc1p, an RBP that is exclusively nuclear (Long et al. 2001). Loc1p is required for other RBPs to assemble onto ASH1 mRNA, which then remove Loc1p, and guide its localization (Niedner et al. 2013). Similarly, ZBP2 is a predominantly nuclear RBP that is required for beta-actin mRNA localization in fibroblasts and neurons (Gu et al. 2002). ZBP2 facilitates the binding of ZBP1 to beta-actin mRNAs, which then escorts the mRNA and represses translation (Pan et al. 2007). Thus, there is precedent that nuclear RBPs, including at least one implicated in mRNA splicing, can affect mRNA localization without leaving the nucleus.

Knockdown of several RBP genes encoding splicing factors in Drosophila led to excess dendrite branches in da neurons (Olesnicky et al. 2014). Among these were mbl and x16, both of which are implicated in alternative splicing (Vorbrüggen et al. 2000; Wang et al. 2012). However, mutations in the worm homologs of these genes, mbl-1 and rsp-6, result in a decrease in the number of dendritic termini in PVDs. While splicing is important in both species, it is possible that the target genes of alternative splicing are not conserved and that these targets regulate different aspects of dendrite morphogenesis.

RNA processing and dendrite morphogenesis
Antonacci et al.  G3

*mtr-4* encodes a homolog of yeast Mtr4p, an RNA helicase that modulates the polyadenylation activity of the Trf4/Air2/Mtr4 polyadenylation (TRAMP) complex. TRAMP adds short poly-A tails to mRNAs and small noncoding RNA (such as snoRNAs and tRNAs) to mark them for degradation or processing by the nuclear exosome (Liang *et al.* 1996; Jia *et al.* 2011; Schmidt and Butler 2013). While it is difficult to speculate on how a general process such as RNA processing and degradation may lead to a specific phenotype such as dendrite morphology, at least one study has shown that exosome activity is important for nervous system development. Loss of zebrafish *EXOSC3* activity, which is required for exosome function, resulted in spinal motor neuron developmental defects and degeneration (Wan *et al.* 2012).

**Is the microRNA/RNAi pathway important for dendrite development?**

We found that *dcr-1/Dicer* mutants have a reduction in the number of terminal dendrite branches. Although this might suggest that the process of dendrite morphogenesis is partly regulated by microRNAs, this is inconsistent with our finding that a null mutation in *drsh-1/Drosha* does not cause dendrite defects. One possibility is that *drsh-1* mutants, which are recovered from heterozygous mothers, are rescued from defects by maternal *drsh-1(+)*. Maternal rescue of adult stage developmental events in *drsh-1* mutants has been suggested previously (Denli *et al.* 2004). There is some evidence that microRNAs are important for dendrite morphogenesis (Bicker *et al.* 2014). For example, rat miR-134 is localized to dendrites in the hippocampus and negatively regulates the size of dendritic spines. Brain-derived neurotrophic factor positively regulates the size of dendritic spines by alleviating miR-134 repression (Schratt *et al.* 2006). The microRNA
*bantam* regulates the scaling of da neuron arbors in *Drosophila*. However, *bantam* functions within the epidermis, not the da neuron (Parrish et al. 2009). It is unclear from our work if *dcr-1* functions in the neuron or non-cell-autonomously. Alternatively, dendrite development may be partly regulated by endogenous RNAi mechanisms, which are *dcr-1*-dependent but *drsh-1*-independent, or *dcr-1* may have additional roles that do not involve small noncoding RNAs.

**RBPs are candidates for dendrite development in humans**

By comparing results from genetic screens for dendrite morphogenesis in two similar but distinct neuronal types in distantly related species, we have identified 12 RBPs that we suggest are likely to be involved in dendrite development in many other species as well. Furthermore these 12 proteins may constitute a minimal set of evolutionarily conserved RBPs required for dendrite development across animal phyla. This is bolstered by the fact that human orthologs of these RBP genes are expressed in the human brain based on the Tissue-specific Gene Expression and Regulation (TiGER) database (Table 3; Liu et al. 2008). It will be interesting to see if future functional studies in vertebrates demonstrate a role for these RBPs in dendrite development or if human studies identify any of these RBP genes as mutated in neurological disorders in humans.
ACKNOWLEDGEMENTS

We thank Delaine Winkelblech and Brent Wallace for technical support, Tracy Kress for insightful discussions, the *Caenorhabditis* Genetics Center, the Mitani Lab as part of the National Bio-Resource Project of Japan, James Mapes, Ding Xue, Cody Smith, David Miller, Caitlin Taylor, and Kang Shen for strains, and Yuji Kohara, James Mapes, and Ding Xue for cDNA clones. This work was supported by the National Science Foundation (IOS proposal number 1257703 to DJK and ECO), a CC Mrachek Fellowship to DJK, a CC Figge-Bourquin Summer Research Grant to LK, CC Venture Grants to MAS and GK, and the CC and UCCS Dean’s offices.
Table 1. Genes identified as positive hits in a genetic screen for PVD dendrite defects

<table>
<thead>
<tr>
<th>C. elegans RBP</th>
<th>Drosophila RBP</th>
<th>E value</th>
<th>Predicted Protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>CGH-1</td>
<td>Gem3</td>
<td>6.00E-61</td>
<td>ATP-dependent DEAD-box RNA helicase</td>
</tr>
<tr>
<td>CPB-3</td>
<td>Orb</td>
<td>7.00E-64</td>
<td>Cytoplasmic polyadenylation element binding protein</td>
</tr>
<tr>
<td>DCR-1</td>
<td>Dcr-1</td>
<td>3.00E-120</td>
<td>RNase III family member; ortholog of Dicer</td>
</tr>
<tr>
<td>DDX-17</td>
<td>CG10777</td>
<td>5.00E-160</td>
<td>ATP-dependent DEAD-box RNA helicase</td>
</tr>
<tr>
<td>LARP-5</td>
<td>CG11505</td>
<td>8.00E-33</td>
<td>La-related protein with a LARP5 domain</td>
</tr>
<tr>
<td>MBL-1</td>
<td>Mbl</td>
<td>5.00E-45</td>
<td>CCCH Zinc-finger RNA-binding domain regulator of splicing</td>
</tr>
<tr>
<td>MTR-4</td>
<td>L(2)35Df</td>
<td>0</td>
<td>RNA helicase; homolog of yeast Mtr4p which is part of the TRAMP complex that is involved in various RNA processing events</td>
</tr>
<tr>
<td>RSP-3</td>
<td>SF2</td>
<td>4.00E-59</td>
<td>SR splicing factor required for constitutive splicing and for influencing alternative splicing</td>
</tr>
<tr>
<td>RSP-6</td>
<td>X16</td>
<td>3.00E-22</td>
<td>SR splicing factor required for constitutive splicing and for influencing alternative splicing; implicated in transcriptional termination</td>
</tr>
<tr>
<td>SET-2</td>
<td>Set1</td>
<td>4.00E-68</td>
<td>RRM domain-containing histone H3K4 methyltransferase</td>
</tr>
<tr>
<td>SUP-26</td>
<td>Shep</td>
<td>6.00E-52</td>
<td>RRM domain-containing protein;</td>
</tr>
</tbody>
</table>
translational repressor

Y55F3AM.3  CG11266  1.00E-97  RRM domain-containing protein with splicing factor RBM39 linker; co-localizes with spliceosomal proteins

The *C. elegans* RBPs identified as important for dendrite morphogenesis in PVD neurons are given with an E value from a BLASTp search on *D. melanogaster* unique protein isoform database. Predicted protein function is given based on Wormbase (WS244). Abbreviations: Asp-Glu-Ala-Asp (DEAD) box; motif with three Cysteine and one Histidine residue (CCCH); RNA recognition motif (RRM); Trf4/Air2/Mtr4p Polyadenylation complex (TRAMP); Serine/Arginine rich (SR); Histone 3 Lysine 4 (H3K4).
Table 2. Summary of pleiotropic defects in *C. elegans* PVD architecture resulting from loss of RBP function

<table>
<thead>
<tr>
<th>RBP gene</th>
<th>Reduction of Terminal branches</th>
<th>$3^\circ$ branch defect</th>
<th>$2^\circ$ branch defect</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>cgh-1</em></td>
<td>28%</td>
<td>24% increase</td>
<td>21% increase</td>
</tr>
<tr>
<td><em>cpb-3</em></td>
<td>33%</td>
<td>19% increase</td>
<td>12% increase</td>
</tr>
<tr>
<td><em>dcr-1</em></td>
<td>40%</td>
<td>-</td>
<td>14% decrease</td>
</tr>
<tr>
<td><em>ddx-17(RNAi)</em></td>
<td>11%</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><em>larp-5</em></td>
<td>20%</td>
<td>19% decrease</td>
<td>28% decrease</td>
</tr>
<tr>
<td><em>mbi-1</em></td>
<td>41%</td>
<td>20% decrease</td>
<td>23% decrease</td>
</tr>
<tr>
<td><em>mtr-4</em></td>
<td>20%</td>
<td>17% decrease</td>
<td>29% decrease</td>
</tr>
<tr>
<td><em>rsp-3</em></td>
<td>32%</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><em>rsp-6</em></td>
<td>36%</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><em>set-2</em></td>
<td>27%</td>
<td>10% increase</td>
<td>-</td>
</tr>
<tr>
<td><em>sup-26</em></td>
<td>26%</td>
<td>15% decrease</td>
<td>34% decrease</td>
</tr>
<tr>
<td><em>Y55F3AM.3</em></td>
<td>19%</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>
Table 3. Human orthologs of *C. elegans* RBPs implicated in PVD dendrite development

<table>
<thead>
<tr>
<th><em>C. elegans</em> RBP</th>
<th>OrthoDB ID</th>
<th>Human Ortholog</th>
<th>E Value</th>
<th>Brain Expression</th>
</tr>
</thead>
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<tr>
<td>CGH-1</td>
<td>EOG7D85W7</td>
<td>DDX6</td>
<td>1.00E-179</td>
<td>Expressed</td>
</tr>
<tr>
<td>CPB-3</td>
<td>EOG751NG9</td>
<td>CPEB1</td>
<td>5.00E-71</td>
<td>Expressed</td>
</tr>
<tr>
<td>DCR-1</td>
<td>EOG78PV82</td>
<td>DICER1</td>
<td>2.00E-167</td>
<td>Expressed</td>
</tr>
<tr>
<td>DDX-17</td>
<td>EOG7HTHGB</td>
<td>DDX17</td>
<td>2.00E-163</td>
<td>Expressed</td>
</tr>
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<td>EOG7HTHGB</td>
<td>DDX5</td>
<td>5.00E-163</td>
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<tr>
<td>LARP-5</td>
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<td>LARP4B</td>
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<tr>
<td>MBL-1</td>
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<td>MBNL2</td>
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<td>Not assayed</td>
</tr>
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<td>5.00E-31</td>
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<td>MBNL1</td>
<td>5.00E-30</td>
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</tr>
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<td>MTR-4</td>
<td>EOG7XSTCX</td>
<td>SKIV2L2</td>
<td>0.0</td>
<td>Expressed</td>
</tr>
<tr>
<td>RSP-3</td>
<td>EOG76X620</td>
<td>SRSF1</td>
<td>5.00E-56</td>
<td>Expressed</td>
</tr>
<tr>
<td></td>
<td>EOG76X620</td>
<td>SRSF9</td>
<td>3.00E-53</td>
<td>Not assayed</td>
</tr>
<tr>
<td>RSP-6</td>
<td>EOG73NG5V</td>
<td>SRSF3</td>
<td>9.00E-23</td>
<td>Expressed</td>
</tr>
<tr>
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<td>EOG73NG5V</td>
<td>SRSF7</td>
<td>3.00E-22</td>
<td>Expressed</td>
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<td>SET-2</td>
<td>EOG72JWHB</td>
<td>SETD1A</td>
<td>6.00E-65</td>
<td>Expressed</td>
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<td>SETD1B</td>
<td>7.00E-60</td>
<td>Expressed</td>
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<td>SUP-26</td>
<td>EOG71G9V3</td>
<td>RBMS1</td>
<td>4.00E-53</td>
<td>Expressed</td>
</tr>
<tr>
<td></td>
<td>EOG71G9V3</td>
<td>RBMS2</td>
<td>8.00E-52</td>
<td>Expressed</td>
</tr>
<tr>
<td></td>
<td>EOG71G9V3</td>
<td>RBMS3</td>
<td>9.00E-52</td>
<td>Expressed</td>
</tr>
<tr>
<td>Y55F3AM.3</td>
<td>EOG71K63D</td>
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<td></td>
<td>EOG71K63D</td>
<td>RBM23</td>
<td>3.00E-53</td>
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</tbody>
</table>
Human orthologs were identified using the Database of Orthologous Groups and are displayed with the corresponding OrthoDB IDs. *C. elegans* proteins were aligned to the human protein RefSeq database using BLASTp and E values are given. Gene expression in the brain was determined using the TiGER database (Liu et al., 2008).
FIGURE LEGENDS

A

1°

CB

3°

2°

4°

B

ctl
cgh-1
cpb-3
dcr-1
larp-5
mbl-1
mtr-4
rsp-3
rsp-6
set-2
sup-26
Y55F3AM.3

C

RNAi ctl
mec-3(RNAi)
ddx-17(RNAi)
**Figure 1.** Loss or reduction of RBP genes results in a decrease in dendritic termini in PVD neurons. (A) PVD dendritic tree morphology includes primary (1°) branches extending from the cell body (CB) and a series of perpendicular secondary (2°), tertiary (3°), and quaternary (4°) branches. (B) Animals carrying a GFP marker for PVD neurons and a mutation in the RBP gene indicated have reduced dendritic termini compared to the control (ctl). (C) Animals carrying a GFP marker for PVD neurons and treated with RNAi for the genes indicated have reduced dendritic termini compared to the control. *mec-3(RNAi)* is a positive control for RNAi and reduced dendrite phenotypes. Posterior is to the right in all images. Bar = 25µm.
**Figure 2.** Quantification of PVD dendrite phenotypes in RBP mutants and RNAi knockdowns. Points within each scatter column represent counts of (A) dendritic termini, (B) secondary branches, or (C) tertiary branches from the PVD cell body to the tail on the dorsal or ventral side of the worm. Lines within each column represent the means and the 95% confidence interval of the mean. Results in red and blue are significantly
lower or higher respectively than controls (ctl) in grey based on a one-way ANOVA test with a Fisher’s Least Significant Difference multiple comparisons test with a 95% confidence interval.
**Figure 3.** A time course analysis of dendrite development reveals defects in dendrite formation, dendrite maintenance, and timing. Control (*ctl*) or mutant animals were scored for the number of PVD dendritic termini, as before, at the mid-L4, young adult, adult (18 hours after the young adult), and sometimes late adult (48 hours after the young adult) stages. All data points for mutants are significantly different from controls based on a one-way ANOVA test with a Fisher’s Least Significant Difference multiple comparisons test with a 95% confidence interval. Data points are the mean values where \( n = 80 \) for each genotype. Error bars show the standard error of the mean. (A) Mutants indicated have a dendrite formation defect. (B) Mutants indicated have a dendrite maintenance defect in the L4 stage. Mutant values at each time point are significantly different from each other time point based on a one-way ANOVA test with a Fisher’s Least Significant Difference multiple comparisons test with a 95% confidence interval. (C) *cgh-1* mutants have a delay in dendritic termini formation.
Figure 4. RBP genes that are important for PVD dendrite morphogenesis are expressed in the PVD neuron. Presumptive promoter regions for each RBP gene indicated were fused to GFP to determine if they are expressed in the PVD neuron, which were marked with $P_{mec-7}$::RFP. Arrowheads mark the PVD cell body. $sup-26$ expression was determined from a $P_{sup-26}$::sup-26 cDNA::GFP construct. Bar = 10µm.
<table>
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<tr>
<th>DDX-17</th>
<th>MBL-1</th>
<th>MTR-4</th>
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</table>
Figure 5. Nuclear localization of several RBPs in PVD neurons. cDNAs for RBP genes indicated were fused to GFP under the control of a PVD-specific promoter and reveal a nuclear localization. PVDs were marked by \textit{ser-2prom3::myr-mCherry}. Bar = 5\mu m.
Figure 6. Cytoplasmic localization of CGH-1, CPB-3, LARP-5, and SUP-26 in PVD neurons. cDNAs for RBP genes indicated were fused to GFP under the control of a PVD-specific promoter and reveal cytoplasmic localization. PVDs were marked by ser-2prom3::myr-mCherry. Arrowheads indicate GFP-positive particles within dendrites. Bar = 5µm.
Figure 7. PVD-specific expression of RBP::GFP fusion proteins rescues dendrite defects in most of the RBP mutants. Control (ctl) animals (grey), mutant animals (red), and mutant animals bearing an extrachromosomal array (Ex) that expresses the corresponding RBP::GFP fusion protein in the PVD neuron (blue) were scored for the number of dendritic termini using the ser-2prom3::myr-mCherry PVD marker. Points within each scatter column represent counts of dendritic termini from the PVD cell body to the tail on the dorsal or ventral side of the worm. Lines represent the means and the 95% confidence interval of the mean. All of the mutants are significantly lower than the control. * Mutants expressing RBP::GFP are significantly different from mutants and significantly different from controls indicating a partial rescue. ** Mutants with RBP::GFP
are significantly different from mutants and not significantly different from controls indicating a complete rescue. *** GFP::LARP-5 causes a significant reduction in dendritic termini compared to the lar5 mutant alone. All statistics are based on a one-way ANOVA test with a Fisher’s Least Significant Difference multiple comparisons test with a 95% confidence interval.
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