The Proprotein Convertase KPC-1/Furin Controls Branching and Self-avoidance of Sensory Dendrites in Caenorhabditis elegans

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Abstract

Animals sample their environment through sensory neurons with often elaborately branched endings named dendritic arbors. In a genetic screen for genes involved in the development of the highly arborized somatosensory PVD neuron in C. elegans, we have identified mutations in kpc-1, which encodes the homolog of the proprotein convertase furin. We show that kpc-1/furin is necessary to promote the formation of higher order dendritic branches in PVD and to ensure self-avoidance of sister branches, but is likely not required during maintenance of dendritic arbors. A reporter for kpc-1/furin is expressed in neurons (including PVD) and kpc-1/furin can function cell-autonomously in PVD neurons to control patterning of dendritic arbors. Moreover, we show that kpc-1/furin also regulates the development of other neurons in all major neuronal classes in C. elegans, including aspects of branching and extension of neurites as well as cell positioning. Our data suggest that these developmental functions require proteolytic activity of KPC-1/furin. Recently, the skin-derived MNR-1/menorin and the neural cell adhesion molecule SAX-7/L1CAM have been shown to act as a tripartite complex with the leucine rich transmembrane receptor DMA-1 on PVD mechanosensory to orchestrate the patterning of dendritic branches. Genetic analyses show that kpc-1/furin functions in a pathway with MNR-1/menorin, SAX-7/L1CAM and DMA-1 to control dendritic branch formation and extension of PVD neurons. We propose that KPC-1/furin acts in concert with the ‘menorin’ pathway to control branching and growth of somatosensory dendrites in PVD.

Introduction

Multicellular organisms sense their environment through specialized nerve cells termed sensory neurons. The diversity in shape and structure that dendrites of sensory neurons (hereafter named ‘dendritic arbors’) assume reflects the variety of stimuli they receive [1]. Work over the past two decades has established that dendritic arbor development of sensory neurons relies on conserved molecular mechanisms [2,3]. However, dendritic arbors do not only exist in the periphery, but also in the central nervous system where they function to receive synaptic input from other neurons. The molecules and mechanisms that orchestrate dendritic arbor development, e.g. cell adhesion molecules and molecular motor proteins, are often similar between the peripheral and central nervous system [see ref. 3 for these and more examples]. Importantly, failed dendrite development has been linked to neurological diseases of the central nervous system, ranging from Autism Spectrum Disorders (ASD) to Schizophrenia [4,5].

The understanding of sensory dendrite morphogenesis has been greatly advanced through studies in the fly Drosophila melanogaster (reviewed in [3]). These studies revealed the importance of transcriptional cascades, cytoskeletal proteins, the secretory pathway, microtubular transport, and the basement membrane for development of the so-called dendritic arborization (da) neurons in flies [3,6–8]. In the nematode Caenorhabditis elegans, the PVD mechanosensory neurons have been used to study development of dendritic arbors owing to their stereotypic branching patterns (Figure 1A,B) [9,10]. A PVD cell on each side of the animal sends a primary dendrite both anteriorly and posteriorly that branches off perpendicular higher order branches in a stereotypical fashion (Figure 1A, B). The resulting structures resemble candelabra and have hence been named menorahs [11]. Like fly da neurons, transcriptional cascades and microtubule based motor proteins have been shown to play a role during PVD dendritic arbor formation suggesting that basic principles of dendritic arbor formation are evolutionarily conserved [12–14]. Recently, a tripartite complex consisting of the extracellular...
Author Summary

Sensory neurons receive input from other neurons or sample their environment through elaborate structures termed dendritic trees. The correct patterning of dendritic trees is crucial for the proper function of the nervous system, and ample evidence points to the involvement of dendritic defects in a wide range of neuropsychiatric diseases. However, we still do not understand fully how this process is regulated at the molecular level. We discovered an important role for the protein-processing enzyme KPC-1/furin in the development of touch-sensitive dendritic trees in the roundworm C. elegans. Animals lacking this enzyme show multiple defects in the size, shape and number of these dendritic branches as well as other neurons. We further show that the gene encoding KPC-1 is expressed widely in the nervous system and that it is required within the branching neuron to exert its function on dendritic growth. Finally, we reveal a genetic connection between KPC-1 function and genes of the menin pathway, which was recently discovered to also play an essential role in dendrite development. Thus, our findings add new insight into the molecular understanding of dendrite formation.

molecule MNR-1/menin, the neural cell adhesion molecule SAX-7/L1CAM both of which act from the hypodermis (skin) and the leucine rich repeat DMA-1/LRR transmembrane receptor on PVD dendrites have been shown to be critical for PVD development [15,16].

Here we report the identification of the furin homolog kpc-1 in C. elegans as a factor that acts in concert with the ‘meninor’ pathway to shape sensory dendrite development. Furin is a serine protease of the proprotein convertase family, that following autocatalytic activation cleaves proteins at characteristic dibasic motifs (reviewed in [17–19]). We show that in C. elegans a reporter for kpc-1/furin is expressed broadly in the nervous system and is necessary for multiple neuronal positioning, neurite extension and branching events, including PVD dendritic growth and self-avoidance. Moreover, kpc-1 can act cell autonomously to shape PVD dendrites in a manner that is dependent on the ‘meninor’ pathway.

Results

In a screen for genes involved in PVD development [16] we also identified mutant alleles of the proprotein convertase kpc-1, which encodes the C. elegans furin homolog based on sequence similarity and domain organization [20,21]. Mutant animals show no obvious morphological or defects in body size. However, they do display severely defective dendritic arbors in both PVD and the analogous anterior FLP neurons (Figure 1B–E). The identified recessive alleles include two nonsense (dz177, dz182) and a missense allele (dz185) (Figure 1F, Figure S1). We also obtained a deletion (gh8) and two additional missense alleles (gh333538, gh779937) (Figure 1F,G, Figure S1). The deletion and nonsense alleles truncate or delete the catalytic domain of KPC-1/furin, respectively, and their phenotypes seem indistinguishable in severity, suggesting that all three alleles represent strong if not complete loss of function alleles. The missense alleles dz185 and gh333538 are hypomorphic alleles because their phenotype was (1) less severe when compared to the presumptive gh8 null allele and (2) more severe when placed in trans to a deficiency (hdf17) that spans the genomic region of kpc-1/furin (Figure 1H). The missense mutations affect residues in a perfectly conserved alpha helix that positions the histidine of the catalytic triad in furin [22] (Figure 1G), suggesting that proteolytic activity of KPC-1/furin is necessary for PVD development.

To define when kpc-1/furin function is required during development we first conducted timed RNAi experiments as described [16]. We found that knock down of kpc-1/furin starting at early larval stages when PVD sensory dendrites begin to develop [13] resulted in robust defects in PVD in adult animals (Figure 1I). In contrast, knock down starting at the L3 stage resulted in weaker phenotypes in adults (Figure 1I). RNAi mediated knockdown initiated only 12–24 h later at the L4 larval stage failed to result in any PVD defects. Importantly, RNAi mediated knockdown of GFP in PVD neurons suggested that changes in RNAi efficacy cannot account for lack of defects upon induction of kpc-1 knock down at later larval stages (Figure 1I). Consistent with these observations we find defects in PVD dendrite arborization in kpc-1 mutants already during the L3 larval stage rather than later as a result of a maintenance defect (Figure S2). Collectively, these findings suggest that kpc-1/furin functions during the earlier stages of PVD development and may not play a major role in dendrite maintenance.

We next sought to determine whether kpc-1/furin functions in PVD neurons or in surrounding tissues such as the hypodermis (skin) or muscle. To this end we first conducted transgenic rescue experiments. We found rescue of the PVD defects in kpc-1 mutants when a kpc-1 cDNA was expressed under control of a PVD-specific heterologous promoter but not when expressed in muscle or the hypodermis (Figure 2A,B). Expression of this PVD-specific kpc-1 transgene in a wild-type background did not have any detectable effect on PVD architecture (Figure S3). To further investigate where kpc-1 may function we constructed transgenic animals that carried a kpc-1 reporter where a 5.8 Kb fragment of the endogenous upstream regulatory region of kpc-1 drives expression of green fluorescent protein. This transcriptional kpc-1::GFP reporter was widely expressed from early developmental stages in the nervous system including in PVD (Figure 2C–E, Figure S4). We conclude that kpc-1/furin acts cell-autonomously to shape the dendritic arbors of PVD neurons.

The observed extensive neuronal expression of the kpc-1 reporter prompted us to examine other neuron types besides PVD for defects in migration, neurite extension and branching in kpc-1 mutant animals. We detected significant defects in multiple neuron types in kpc-1 mutants. For example, the sensory AQR neurons failed to branch appropriately in the nerve ring whereas VC motoneurons showed defects in the extension and formation of characteristic branches near the vulva (Figure 3). Yet, kpc-1 did not appear to be required for all branching processes because branching in AY interneurons was not affected by overexpression of the secreted kal-1 cell adhesion molecule [23] did not require kpc-1 and, AVL neurons showed ectopic branches but no defects in normal branch formation (Figure 3). Whereas DA/DB motoneurons did not display obvious defects, D-type motoneurons showed gaps in the dorsal cord, possibly due to defective neurite extension, and increased numbers of inappropriately positioned commissures on the left side of the animals (Figure 3). Similarly, AY interneurons displayed a characteristic axonal ‘short stop’ phenotype, likely as a result of defects in extension [23]. In addition, we observed neuronal cell positioning defects of ALM touch receptor neurons and HSN motoneurons (Figure 3). Thus, kpc-1’s function in nervous system development extends well beyond its role in dendrite morphogenesis and affects many but not all neurons in the major neuronal classes (sensory, motor and interneurons).
Figure 1. **KPC-1 is required for development of dendritic arbors.**

A. Schematic of the PVD somatosensory neuron with dendrites (including primary/1, secondary/2, tertiary/3 and quaternary/4 branches) and the axon indicated in green and black, respectively. B–C Lateral view of an adult wild type (B) and kpc-1(dz182) mutant animal (C). PVD sensory neurons are visualized by a fluorescent reporter (wdl52). Anterior is to the left and scale bars indicate 50 μm in all panels. An arrowhead denotes the PVD axon. Open arrow heads indicate ectopic 3′ branches.

D–E Images of an adult wild type animal (D) and a kpc-1(dz182) mutant animal (E). FLP sensory neurons are visualized by a fluorescent reporter (muIs32). F Schematic of KPC-1 with domains (SP: signal peptide, PD: P domain, TM: transmembrane domain) and exon boundaries (dashed lines) indicated. KPC-1 exists in a long and a short splice variant. All experiments were conducted with the short KPC-1A variant. Mutant alleles and predicted molecular changes are shown. NTP (N terminal propeptide) indicates cleavage sites that are necessary for autoproteolytic activation of the proprotein.

G Partial sequence alignment of the peptidase domain in KPC-1 (NP_001021101), human furin (NP_002560.1), mouse furin (NP_001074923.1), and yeast Kex2 (NP_014161) protein. Asterisks indicate the aspartic acid and histidine of the catalytic triad. Alignments were obtained using Multalin (http://multalin.toulouse.inra.fr) and rendered with Boxshade (http://www.ch.embnet.org). For a Full alignment see Figure S1.

H Quantification of the number of secondary branches in the genotypes indicated. hDf17 is a chromosome I deficiency allele that deletes the entire kpc-1 locus. Data are represented as mean ±/− SEM. Statistical comparisons were performed using one-sided ANOVA with the Tukey correction. Statistical significance is indicated (*, P≤0.05; **, P≤0.01; ****, P≤0.0001; ns: not significant (P>0.05)). N = 22 animals (302 dendritic branches) of wild type control; N = 22 animals (548 dendritic branches) of hDf17.
Importantly, kpc-1 appears to be involved in both control of neurite branch formation and extension (see also below).

To define the function of kpc-1 in dendrite development in more detail we subjected mutants to a morphometric analysis of PVD dendrites. These studies showed an increase in secondary and ectopic tertiary branches with a concomitant decrease in the number of tertiary and quaternary branches in kpc-1 mutants (Figure 4A-F). The secondary branches in kpc-1 mutants often failed to reach the vicinity of the sublateral nerve cords (Figure 4F) where they normally bifurcate to form tertiary branches [13]. Instead, they frequently sprouted ectopic tertiary branches in places that normally do not support tertiary branches (Figure 4F). Interestingly, the average length of branches was significantly reduced in kpc-1/+ and kpc-1/furin mutants (Figure 5A-E), as was the aggregate length of each branch order (2, 3 and 4 branches) separately or combined (Figure 5F). To distinguish whether this phenotype is a reflection of a decrease or increase in growth or retraction, we conducted time-lapse analyses of kpc-1 mutants and measured growth and retraction of secondary branches. We found significantly slowed growth in kpc-1 mutants compared to wild type animals whereas the speed of retraction remained unchanged (Figure 6, Movies S1 and S2). None of the observed PVD defects were shared in mutants of the three other proprotein convertases that genetically act in dendritic branches—kpc-1(gk333538), kpc-3(dz170), and kpc-4(bli-4) (Figure 7). The shorter primary branch length in kpc-1 mutants, hence lower average length of branches, is likely the result of a decrease in branch growth and not an increase in branch retraction. The decrease in branch growth contrasts with the increase in branch retraction in the ‘menorin’ pathway (Figure 7C), indicating that kpc-1 acts genetically in the same pathway and that kpc-1 and the ‘menorin’ pathway are functionally similar to the kpc-1/dma-1 double mutant (Figure 4). A possible exception was the reduced number of secondary branches in the kpc-1; dma-1 double compared to both single mutants and wild type animals (Figure 4A). This synthetic phenotype suggests that the formation of secondary dendrites is controlled by a genetic parallel or convergent pathway in kpc-1 and dma-1.

Discussion

In a screen for genes required for patterning of the PVD somatosensory neuron, we isolated mutant alleles in kpc-1, which encodes the C. elegans furin homolog [20,21]. The phenotypes in PVD patterning that we observed in kpc-1/+ and kpc-1/furin mutants displayed striking similarities to mutants in the ‘menorin’ pathway,
which comprises the three cell adhesion molecules MNR-1/menorin, SAX-7/L1CAM and DMA-1/LRR-TM [15,16]. Our double mutant analyses revealed genetic interactions that were distinct for branch formation and extension. For branch formation, we suggest that kpc-1/Furin acts in a genetic pathway with mnr-1/menorin, sax-7/L1CAM and dma-1/LRR. First, double mutants between kpc-1/furin and either mnr-1/menorin or sax-7/L1CAM were not more severe than the single mutants. Second, dma-1/LRR appeared generally more severe than the kpc-1/mnr-1 or kpc-1/sax-7 double mutants and epistatic to kpc-1/furin. Third, gain of function experiments demonstrated that mnr-1/menorin function requires kpc-1/furin. Overall, these genetic

Figure 2. KPC-1 functions in PVD to shape dendritic arbors. A Epifluorescence micrographs of kpc-1(dz182) mutant animals and transgenically rescued kpc-1(dz182) animals. Scale bars indicate 50 μm. B Quantification of transgenic rescue. Transgenic (T) and nontransgenic (NT) siblings were quantified in kpc-1 mutant animals in which a kpc-1 wild type DNA was driven from a hypodermal (dpy-7prom) [31], muscle (myo-3prom) [32] or PVD specific promoter (ser-2prom) [9]. Defective animals were defined as in Figure 1I. C Schematic of the kpc-1 locus on linkage group (LG) I. The extent of the rescuing fosmid WRM0635bG07 is indicated in green as is the transcriptional kpc-1::GFP reporter that contains 5.8 kb of regulatory sequence upstream of the ATG (kpc-1prom5.8::GFP). A transcriptional reporter containing 2.8 kb of regulatory sequence (kpc-1prom2.8::GFP) was not expressed in PVD suggesting that regulatory elements for PVD expression reside between −2.8 kb and −5.8 kb (Figure S4). D Composite epifluorescent micrograph of an adult transgenic animal carrying the kpc-1prom5.8::GFP transgene. Expression is seen in the pharynx (ph) and the nervous system, e.g. the retrovesicular ganglion (rvg), ventral cord motor neurons (mn) and in PVD mechanosensory neurons. In addition, expression is seen in vulval epithelial tissues as well as the spermatheca (not shown here). A scale bar indicates 30 μm. E Higher magnification epifluorescent micrograph of an adult transgenic animal carrying the kpc-1prom5.8::GFP transgene. Expression is seen in PVD and its dendritic tree (arrowheads). A scale bar indicates 10 μm.

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interactions are strikingly similar to those between \textit{mnr-1/menorin}, \textit{sax-7/L1CAM} and \textit{dma-1/LRR} [15,16] and suggest that \textit{kpc-1/furin}, \textit{mnr-1/menorin} and \textit{sax-7/L1CAM} act in a linear pathway but that a parallel pathway may exist that also acts through \textit{dma-1/LRR} during branch formation. For branch extension, the scenario is slightly different. First, \textit{kpc-1/furin} mutant branch extension phenotypes were generally more similar to \textit{dma-1/LRR} than to \textit{mnr-1/menorin} or \textit{sax-7/L1CAM} mutant phenotypes. Second, mutations in \textit{mnr-1/menorin} or \textit{sax-7/L1CAM} but not \textit{dma-1/LRR} partially suppressed \textit{kpc-1/furin} mutant branch extension phenotypes in higher order branches. This could suggest higher activity of a parallel pathway during extension that is normally inhibited by \textit{mnr-1/sax-7}-function, but also requires \textit{dma-1/LRR}. Taken together, the findings presented here suggest that \textit{kpc-1} collaborates with the menorin pathway to sculpt the PVD dendritic arbor through distinct genetic mechanisms for branch formation and extension.

Our experiments showed that \textit{kpc-1/furin} functions cell autonomously to coordinate formation, extension and self-avoidance of PVD somatosensory dendritic branches. However, the functions of \textit{kpc-1/furin} are not limited to shaping PVD and FLP neurons during development. First, a recent report described a function for KPC-1 in the remodeling of sensory dendrites of IL2s (a set of sensory neurons in the head of \textit{C. elegans}) as a result of changes in environmental conditions [21]. This report provided also evidence for a role of \textit{kpc-1} in the patterning of PVD and FLP

\begin{figure}
\centering
\includegraphics[width=\textwidth]{figure3.png}
\caption{\textit{kpc-1} displays defects in neurite branching, extension and cell positioning of certain sensory neurons, interneurons and motoneurons. Summary of neuronal defects observed in several classes of neurons. Schematics of normal neuronal morphology is shown and contrasted with abnormal neuronal morphology indicated in red or with red arrowheads. N = 30 in all cases except AII and ALM (N = 25). Statistical significance was calculated using Fisher’s exact test and is indicated (*, P $\leq$ 0.05; **, P $\leq$ 0.01; ***, P $\leq$ 0.001; ****, P $\leq$ 0.0001, ns: not significant (P $> 0.05$)). doi:10.1371/journal.pgen.1004657.g003}
\end{figure}
neurons. Second, our detailed survey of the neuroanatomy of several classes of neurons in kpc-1/furin mutants revealed that kpc-1/furin plays a more general role in nervous system development than previously acknowledged. For example, AIY interneurons display neurite extension defects and the D-type motoneurons exhibit gaps in the dorsal nerve cord that appear to be the result of defects in neurite extension. On the other hand, kpc-1/furin is required for the formation of certain characteristic neuronal branches like in AQR sensory neurons or VC4/5 motoneurons, while preventing ectopic branching in other neurons such as the AVL neuron. In addition, kpc-1 functions during cell migration of the touch neuron ALM and HSN motoneurons. How could kpc-1/furin regulate such seemingly diverse developmental processes as formation and extension of neurite branches or neuronal positioning? One hint may come from our time-lapse analyses, which established that PVD branches grow slower in kpc-1/furin mutants compared to wild type animals. It remains unclear how KPC-1/Furin controls branch formation, extension or the speed of growth in such diverse cellular contexts on a mechanistic level. One possibility is that KPC-1 regulates extracellular adhesion of

Figure 4. kpc-1 acts in a genetic pathway with mnr-1/menorin, sax-7/L1CAM, and the leucine rich repeat (LRR) transmembrane receptor dma-1. A–D Quantification of branch numbers in kpc-1(gk8), mnr-1(dz175), sax-7(nj48), and dma-1(tm5159) single and double mutant animals. Data are represented as mean ± SEM of the number of branches found in the 100 μm segment immediately anterior to PVD cell body. Statistical comparisons were performed using one-sided ANOVA with the Tukey correction and statistical significance is indicated (*, P<0.05; **, P<0.01; ***, P<0.001; ****, P<0.0001; ns: not significant (P>0.05)). N = 21 animals (1371 dendritic branches) of wild type control; N = 21 animals (1158 dendritic branches) of kpc-1(gk8); N = 21 animals (1154 dendritic branches) of mnr-1(dz175); N = 22 animals (1251 dendritic branches) of sax-7(nj48); N = 22 animals (520 dendritic branches) of dma-1(tm5159); N = 19 animals (1119 dendritic branches) of kpc-1(gk8); mnr-1(dz175); N = 22 animals (319 dendritic branches) of kpc-1(gk8); sax-7(nj48); N = 22 animals (1213 dendritic branches) of dma-1(tm5159). Data for wild type control and kpc-1 are identical to Figure 1 and shown for comparison only. E–L Maximum intensity projections of representative L3 larval animals of the genetic backgrounds as indicated, paired with schematics of the respective tracings (E’–L’). Traces were color coded as follows: dark blue: 1 μ, cyan: 2 μ, red: 3 μ, yellow: 4 μ, rose: ectopic 3 μ, dark blue: mispositioned 3 μ (combined with ectopic 3 μ for quantification purposes), green: 5 μ.

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Figure 5. *kpc-1* controls dendrite branch extension. A Quantification of primary branch length, expressed as a ratio of A/B to normalize for animal size (as indicated in schematic) is shown for the genotypes indicated. Percentage of animals falling within a bin (of A/B ratio) is given (illustrated with the corresponding shade of grey). Statistical significance was calculated using the F test to compare variances (*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; ****, $P < 0.0001$; ns: not significant ($P > 0.05$)).

B–E Quantification of average branch length in *kpc-1*(gk8), *mnr-1*(dz175), *sax-7*(n48), and *kpc-1*/Furin in Neuronal Development.
the neuron/growth cone to the substrate either directly or indirectly to mediate these functions, much like it has been suggested for metalloproteases (reviewed in [24]).

An important question is hence to determine the target(s) that are proteolytically processed by KPC-1/furin. In vertebrates, furin or furin-like proteases are known to cleave members of the TGFbeta family of morphogens as well as neuregulins in the secretory pathway [17,18]. Yet, mutations in genes required for neuropeptide processing and secretion, including the EGL-21/carboxypeptidase E, the PAMN-1/peptidyl-α-hydroxylysin-amidating lyase [25] or unc-31/CAPS which is required for dense core vesicle secretion [26] did not result in comparable defects in PVD (Figure S6). Similarly, neither mutations in genes of the TGFbeta pathway (including the TGFbeta ligands tig-2, dbl-1, unc-129 or the sole type II TGFbeta receptor daf-4) nor in the C. elegans homolog of the repulsive guidance molecule (RGM), or furin-like proteases are known to cleave members of the TGFbeta family of morphogens as well as neuregulins in the secretory pathway [17,18]. Yet, mutations in genes required for neuropeptide processing and secretion, including the EGL-21/carboxypeptidase E, the PAMN-1/peptidyl-α-hydroxylysin-amidating lyase [25] or unc-31/CAPS which is required for dense core vesicle secretion [26] did not result in comparable defects in PVD (Figure S6). Similarly, neither mutations in genes of the TGFbeta pathway (including the TGFbeta ligands tig-2, dbl-1, unc-129 or the sole type II TGFbeta receptor daf-4) nor in the C. elegans homolog of the repulsive guidance molecule (RGM), known to be cleaved by furin [27] displayed similar defects in PVD (data not shown). Several of the genes that have been shown to be required for PVD development contain predicted cleavage sites for furin-like proprotein convertases (Table S3). Thus, a candidate gene approach testing those genes and alternative genetic or proteomic approaches will be required to identify the in vivo target(s) of KPC-1 that are important for PVD dendritic arborization.

**Materials and Methods**

**Strains and genetics**

Worms were grown on OP50 *Escherichia coli*-seeded nematode growth medium plates at 20°C. Strains used in this work include: N2 (wild type reference), kpc-1(dz177), kpc-1(dz182), kpc-1(dz185), kpc-1(gk333538), kpc-1(gk779937), kpc-1(gk8), sax-7(nj48), sax-7(dz156), mnr-1(dz175) and dma-1(tm5159). PVD neurons were visualized by the integrated transgene *adIs52 (Is[F49H12.4::GFP]) and FLP neurons with *muIs32 (Is[mec-7prom::GFP])*. Transgenic strains for cell-specific rescue were established by injecting the respective plasmids at 5 ng/µl together with rol-6 (sa1006) at 50 ng/µl as a dominant injection marker into kpc-1(dz182). The transcriptional reporter *kpc-1prom5.8::GFP* was injected at 5 ng/µl together with *txr-3prom::mCherry* at 5 ng/µl into N2 wild type animals. For details on strains and transgenesis see also Supplementary Text S1.

**Identification of kpc-1**

In a clonal F1 Ethyl methanesulfonate (EMS) screen [16] we identified three alleles of *kpc-1*. Two alleles with similar phenotypes, *dz177* and *dz182* were mapped and cloned using a one-step whole genome sequencing approach [28]. Within the mapped region both *dz177* and *dz182* carried nonsense mutations in *kpc-1* on chromosome I at positions 11,676,957 (C to T) and 11,679,245 (G to A) (WS220), respectively. One additional allele, *dz185* failed to complement *dz182* for the PVD phenotype and contained a missense mutation in *kpc-1* at position 11,678,076 (A to T); three additional alleles were obtained from the C. elegans strain collection: the gk8 deletion allele and the missense alleles gk333538 and gk779937, which change 11,678,078 (G to A) and 11,678,071 (G to A), respectively. Transgenic animals carrying a wild type copy of the *kpc-1* locus (fosmid WRM0635bG07) fully rescued the PVD defect in *dz182* mutants (Figure 2C). For details on mapping and identifying the molecular lesions of different alleles see Supplementary Text S1, and Tables S1 & S2.

**Molecular cloning**

The *kpc-1* cDNA was amplified with gene specific primers from a N2 mixed stage cDNA sample and cloned *KpnI/EcoRI* downstream of the *txr-3prom* regulatory element [29]. For the cell specific heterologous rescue the *kpc-1* cDNA was placed under
control of the \textit{dpy-7prom}, \textit{myo-3prom}, or \textit{ser-2prom}\textsuperscript{3} promoters, respectively. The transcriptional reporter was constructed by cloning 5.8 kb upstream of the predicted \textit{kpc-1} translational start site into \textit{pPD95.75} (gift of A. Fire).

**Imaging and quantification of branching**

Synchronized starved L1 larvae were allowed to grow for 30 hrs (corresponding to mid- to late L4) at which time they were mounted and fluorescent images of immobilized animals (1–5 mM levamisol, Sigma) were captured using a Zeiss Axioimager Z1 Apotome. \textit{Z} stacks were collected and maximum projections were used for tracing of dendrites as described [16]. For time lapse imaging, animals at the L3 stage (by gonadal development) were immobilized as described [30]. PVD neurons were imaged for six to eight hours in 5 min intervals starting at the beginning of secondary branch development. \textit{Z}-projections (0.5 \textmu m/step) spanning the focal depth of the neuron were collected using a 63× objective. At least four movies per genotype were obtained using an inverted Nikon TE2000-S microscope equipped with a Perkin-Elmer UltraVIEW spinning disk unit. Volocity software (version 6.2.1) was used to collect the raw files. Processing was carried out using the Image-J 1.46r software.

![Figure 7. \textit{kpc-1} serves a function in dendritic self-avoidance. A] Epifluorescent micrographs of a lateral view of wild type and \textit{kpc-1(dz185)} hypomorphic animals. Filled white arrowheads indicate axons and empty white arrowheads gaps between tertiary dendrites, which are known to be maintained by self-avoidance [33]. Scale bar is 20 \textmu m. B Quantification of self-avoidance index which was determined as previously described [34] and is defined as the ratio of the number of gaps between adjacent tertiary branches divided by the number of candelabra in a 100 \textmu m segment anterior to the cell body. Statistical comparisons were performed using the Student \textit{t}-test and statistical significance is indicated (****, \textit{P} \textless 0.0001). C Maximum intensity projection of a \textit{Z}-stack (lateral view of a \textit{kpc-1(dz185)} mutant shown in A) in which each optical section (total of 12 sections over 7.2 \textmu m equaling 0.6 \textmu m/section) was color-coded with warmer to colder colors for medial to lateral sections of the animal, respectively. Note that the overlapping tertiary dendrites appear in the same focal plane. Since the width of dendritic branches is approximately 200 nm [35], these data suggest that tertiary dendrites are closely apposed (within 0.6 \textmu m) or directly touching. An asterisk marks an area of the cell body that is saturated due to maximum intensity projection. doi:10.1371/journal.pgen.1004657.g007
escent micrographs of mnr-1(dz175); mnr-1(gof)[dzIs43] were

Quantification of ‘baobab’-like dendritic arbors. Mutant alleles used characteristic ‘baobab’-like dendritic arbors (green arrowheads, upper panel) [16] that are absent upon loss of kpc-1 (lower panel). B: Quantification of ‘baobab’-like dendritic arbors. Mutant alleles used were mnr-1(dz175), dzIs43 (ts[myo-3prom::mnr-1]) and kpc-1(gk8). doi:10.1371/journal.pgen.1004657.g008

Supporting Information

Figure S1 Multiple sequence alignment of KPC-1 splice variants with human and yeast enzymes. Multiple sequence alignment of the KPC-1 furin-like proprotein convertase with human proprotein convertases as indicated and the yeast Kex2 subtilisin-like protease using Multalin (http://multalin.toulouse.inra.fr/multalin/). The alignment was rendered using Boxshade (http://www.ch.embnet.org/software/BOX_form.html). Allelic changes are shown. The deletion allele gk8 deletes exons 3, 4 and part of exon 5, resulting in a frameshift after L185 and a predicted stop-codon after 17 non-homologous residues following the Leucine residue in both isoforms. Accession numbers: KPC-1A: NP_492974, KPC-1B: NP_001021101, furin: NP_002560, PCSK4: NP_060043, PCSK5: NP_006191, PCSK6: NP_002561, and Kex2p: NP_014161.

C. Elegans, Hs: Homo sapiens, Sc: Saccharomyces cerevisiae.

Figure S2 PVD defects occur early during PVD development. A–C Epifluorescent micrograph of PVD in animals at different developmental stages (L3 & L4 larval stage and adult). Scale bars indicate 20 μm. (TIF)

Figure S3 Overexpression of KPC-1 in wild type animals does not result in defects in PVD dendrite arborization. Quantification of secondary branch numbers/100 μm anterior to the PVD cell body in wild type, kpc-1(gk8), and animals that cell specifically overexpress the kpc-1 cDNA under the ser-2prom3 promoter (PVD::kpc-1). Data are represented as mean ± SEM. Statistical comparisons were performed using one-sided ANOVA with the Tukey correction and statistical significance is indicated (****, P ≤ 0.0001, ns: not significant (P > 0.05)). (TIF)

Figure S4 Expression of the kpc-1prom2.8::GFP and kpc-1prom5.8::GFP reporters. A: Schematic of the kpc-1 locus on linkage group (LG) I. The transcriptional kpc-1::GFP reporter that contains 2.8 kb of regulatory sequence upstream of the ATG (kpc-1prom2.8::GFP) is shown. B: Composite epifluorescent micrograph of an adult transgenic animal carrying the kpc-1prom5.8::GFP transgene. Expression is seen in the pharynx (ph) and the nervous system, e.g. the phasmids, ventral cord motor neurons (mn) but not in PVD mechanosensory neurons. Whereas widespread expression in the nervous system is seen, no expression in PVD is observed in the right lateral posterior section of the worm which is indicated by a white arrowhead. C–F: Expression of the kpc-1prom5.8::GFP reporter at different developmental stages as indicated (emb: embryo). C is a brightfield image of C. Scale bars indicate 10 μm for the embryo and 100 μm for all other images. Cells, putatively identified as PVD, are circled in orange. (TIF)

Figure S5 Quantification of aggregate branch length in kpc-1 mutant animals. A–C Quantification of aggregate branch length of secondary, tertiary and quaternary per 100 μm anterior to the PVD cell body. Data are represented as mean ± SEM. Statistical comparisons were performed using one-sided ANOVA with the Tukey correction and statistical significance is indicated (*, P ≤ 0.05; **, P ≤ 0.01; ***, P ≤ 0.001, ****, P ≤ 0.0001; ns: not significant (P > 0.05)). N = 21 animals (1371 dendritic branches) of wild type control; N = 21 animals (1153 dendritic branches) of kpc-1(gk8); N = 21 animals (154 dendritic branches of mnr-1(dz175)); N = 22 animals (1251 dendritic branches of sax-7(nj48)); N = 22 animals (520 dendritic branches of dma-1(tm359)); N = 19 animals (1119 dendritic branches of kpc-1(gk8); mnr-1(dz175)); N = 25 animals (1213 dendritic branches of kpc-1(gk8); sax-7(nj48)); N = 22 animals (319 dendritic branches of kpc-1(gk8); dma-1(tm359)). D: Quantification of total aggregate length of all branches per 100 μm anterior to the PVD cell body. Data are represented as mean ± SEM. Statistical comparisons were performed using one-sided ANOVA with the Tukey correction. Statistical significance is indicated (****, P < 0.0005). Number of animals and dendrites scored as in Figure S5A–C. (TIF)

Figure S6 The defects in kpc-1 mutant animals are specific and not caused by defects in neuropeptide processing. A–C Images of adult animals carrying mutations in other proprotein convertases.
For a control image of animals raised at 20°C see Fig. 1A. Anterior is to the right in panels A–G and ventral down (except for C) and arrowheads indicate the PVD axon. Scale bar: 20 μm. D–G Images of adult animals carrying mutations in genes required for neuropeptide processing (E,F) or secretion (G). D is a control image of animal raised at 25°C to compare with the temperature-sensitive mutation egl-21(n611) 25°C, the non-permissive temperature. H Locus and gene model of the ncv-5/kpc-3 on chromosome I. Indicated is the location of the gk149962 nonsense allele, which results in a premature stop codon after 286 amino acids. The resulting truncated protein lacks 119 amino acids of the conserved 291 protease domain and is thus likely a strong if not complete loss of function allele. I Locus and gene model of the pama-1 on chromosome I which encodes the peptidyl-α-hydroxycarboxy-α-amidating lyase. Indicated is the extent of the ok2681 allele which deletes 519 nucleotides. The predicted mRNA encodes a protein with a frameshift after 140 amino acids resulting in a premature stop codon after 4 non-homologous amino acids. This allele is a strong if not complete loss of function allele. (TIF)

Table S1 Whole genome sequencing statistics. Showed whole genome sequencing statistics of the kpc-1(dz177) and kpc-1(dz182) alleles as indicated. (DOCX)

Table S2 Polymorphisms identified by whole genome sequencing within the mapped region. List of all polymorphisms found in kpc-1(dz177) and kpc-1(dz182) alleles within the mapped region. (DOCX)

Table S3 List of predicted furin cleavage sites in candidate proteins. List of proteins known to be involved in PVD development with sites predicted to be cleaved by proprotein convertases such as furin. (TIF)

Movie S1 PVD development in a wild type animal. Lateral view of the left PVD, showing the establishment of secondary, tertiary (ventral side, bottom) and quaternary branches (dorsal side, top) in an animal at the L3 larval stage. The PVD cell body is the biggest brighter spot on the right. Small bright spots are part of the gut auto-fluorescence. The PVD neuron was visualized with GFP in the wds52 transgenic strain [36]. (MOV)

Movie S2 PVD development in a kpc-1(gk8) mutant animal. Ectopic tertiary PVD branching in kpc-1(gk8) null mutant animal at the L3 larval stage. Shown is the PVD neuron sprouting short secondary branches with frequent ectopic short tertiary branches. The PVD neuron was visualized in the kpc-1(gk8); wds52 strain. (MOV)

Text S1 The Supplementary Text provides details Materials and Methods, specifically on the strains used as well as on the cloning of the mutations identified in the genetic screen. (DOCX)

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Author Contributions

Conceived and designed the experiments: YS NJRS HEB. Performed the experiments: YS NJRS. Analyzed the data: YS NJRS HEB. Contributed to the writing of the manuscript: YS NJRS HEB.

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