Developmental Wiring of Specific Neurons Is Regulated by RET-1/Nogo-A in Caenorhabditis elegans

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ABSTRACT Nogo-A is a membrane-bound protein that functions to inhibit neuronal migration, adhesion, and neurite outgrowth during development. In the mature nervous system, Nogo-A stabilizes neuronal wiring to inhibit neuronal plasticity and regeneration after injury. Here, we show that RET-1, the sole Nogo-A homolog in Caenorhabditis elegans, is required to control developmental wiring of a specific subset of neurons. In ret-1 deletion mutant animals, specific ventral nerve cord axons are misguided where they fail to respect the ventral midline boundary. We found that ret-1 is expressed in multiple neurons during development, and, through mosaic analysis, showed that ret-1 controls axon guidance in a cell-autonomous manner. Finally, as in mammals, ret-1 regulates ephrin expression, and dysregulation of the ephrin ligand VAB-2 is partially responsible for the ret-1 mutant axonal defects. Together, our data present a previously unidentified function for RET-1 in the nervous system of C. elegans.

KEYWORDS axon guidance; Nogo-A; C. elegans; ephrin

The establishment and maintenance of neuronal circuits is driven by the ability of neurons to receive and process cues from other neurons, glia, and the extracellular matrix (ECM) (Tessier-Lavigne and Goodman 1996; Yu and Bargmann 2001). Axon guidance is particularly reliant on the correct organization of molecular cues, since axons often extend projections over long distances with many intermediate targets (Garel and Rubenstein 2004). Axon guidance cues generally function as either repellents or attractants depending on the complement of receptors that are presented on axonal growth cones. There is also a complex milieu of axon guidance cues that extending growth cones encounter during development. This means that precise spatial and temporal regulation of these cues is required to ensure faithful development of the nervous system. Recently, dysregulation of axon guidance proteins has also been implicated in several neurological disorders, such as amyotrophic lateral sclerosis (ALS), Alzheimer’s disease (AD), and Parkinson’s disease (PD) (Nugent et al. 2012; Cissé and Checler 2015; Van Battum et al. 2015).

Reticulons (RTNs) are membrane-bound proteins located on the cell surface and the endoplasmic reticulum (ER), where they are involved in bending and shaping of the ER membrane and in trafficking from the ER to the Golgi apparatus (Yang and Strittmatter 2007; O’Sullivan et al. 2012). RTNs are characterized by a carboxy terminal reticulon homology domain (RHD), which comprises two long hydrophobic regions flanking a hydrophilic loop. Four genes encode RTN proteins in mammals: RTN1, RTN2, RTN3 and RTN4/Nogo (Oertle et al. 2003; Di Sano et al. 2012). Dysregulation of the neurite outgrowth inhibitory molecule RTN4/Nogo is implicated in ALS and multiple sclerosis (MS) (Bros-Facer et al. 2014; Schmandke et al. 2014). ALS patients, and mouse models of ALS (SOD-1G86R), show an upregulation of Nogo in skeletal muscle, and, as such, Nogo is used as a prognostic biomarker, successfully identifying patients progressing from lower motor neuron syndrome to ALS (Dupuis et al. 2002; Pradat et al. 2007). The most widely studied RTN is Nogo-A, a multifunctional protein implicated in numerous developmental processes such as cell migration, central nervous system (CNS) plasticity, and neuronal regeneration. The role of
Nogo-A in spinal cord injury has also been extensively studied, initially due to the identification of Nogo-A as one of the major neurite growth inhibitory components of myelin in the CNS (Caroni and Schwab 1988a,b). Inhibitors of Nogo-A and the Nogo-A receptor, NgR1, were subsequently shown to enhance regenerative sprouting and growth of damaged fibers after spinal cord injury (Freund et al. 2006). Consequently, several clinical studies are currently being performed, by Novartis (ATI 355) and GlaxoSmithKline (Ozanezumab), using such inhibitors to treat spinal cord injury, ALS, and multiple sclerosis. Despite the implication of Nogo-A in neurogenerative disease, only a few reports have studied the role of Nogo-A in neuronal development (Wang et al. 2008, 2010; Pinzón-Olejua et al. 2014).

In this study, we have used the invertebrate model system Caenorhabditis elegans to dissect Nogo-A functions in neuronal development. In C. elegans, there is a single Nogo-A homolog called RET-1. Previous studies have shown that knockdown of ret-1 through RNA-mediated interference (RNAi) interferes with ER formation during mitosis, and it has further been shown to interact with a regulator of endocytic recycling, RME-1 (Iwahashi et al. 2002; Audhya et al. 2007). Here, we show that ret-1 is highly expressed in the left and right ventral nerve cord (VNC), the embryonic motor neurons (eMNs), and the hermaphrodite specific neurons (HSN) in C. elegans. We subsequently performed single neuron resolution analysis of developed axons in ret-1 loss-of-function mutant animals. We found that ret-1 is required for the correct extension of the PVP and PVQ interneurons and the HSN motor neurons—these axons fail to respect the ventral midline, and inappropriately cross over to the contralateral side in ret-1 mutant animals. Ephrin signaling is known to play a prominent role in VNC axon guidance. Interestingly, we found that ret-1 mutant HSN guidance defects are dependent on expression of the ephrin ligand VAB-2. The suppression of the ret-1 mutant HSN axon guidance defects by loss of VAB-2 is dependent on the Eph receptor VAB-1. This suggests that inappropriate spatial or temporal expression of VAB-2 causes defective ephrin signaling leading to axon guidance defects in ret-1 mutant animals. Therefore, our findings indicate a function for this gene family that is conserved, and lays the foundation for further studies on the function of RET-1 in the genetically tractable nematode system.

Materials and Methods

Caenorhabditis elegans maintenance

All C. elegans strains were cultured at 20°C as previously described (Brenner 1974). All strains generated and used in this study are detailed in Supplemental Material, Table S1.

Mosaic analysis

For mosaic analysis, transgenic animals were generated by injecting rgef-1prom::ret-1 isoform g.2 cDNA, tph-1prom::mCherry and myo-2prom::mCherry into ret-1(gk242); zds13 animals. A transgenic line was selected that exhibited complete HSN axon guidance rescue. Transgenic animals from this line were then scored for phenotypic rescue of the HSN axon guidance defects in the presence or absence of the rescuing array in the HSN neurons by detection of mCherry fluorescence.

DNA constructs and transgenic lines

Rescue constructs were injected into the ret-1(gk242) mutant background at 5–15 ng/µl with myo-2prom::mCherry (5 ng/µl) as injection marker. Expression constructs were injected into the N2 background at 50 ng/µl with myo-2prom::mCherry (5 ng/µl) as injection marker. The 9 kb ret-1 rescuing PCR fragment was injected into ret-1(gk242) at 1 ng/µl with myo-2prom::mCherry (5 ng/µl) as injection marker.

Fluorescence microscopy

Neuroanatomy was scored in L4 and young adult hermaphrodites by mounting on 5% agarose on glass slides. Images were taken using an automated fluorescence microscope (Zeiss, AXIO Imager M2) and ZEN software (version 3.1).

qRT-PCR assays

RNA was isolated from a mixed-stage worm population using standard Trizol-based methods (Chomczynski and Sacchi 1987). Total cDNA was obtained using TaqMan Reverse Transcription Reagents (Invitrogen, Cat. No: N8080234). qRT-PCR reactions were performed in triplicate on a LightCycler 480 System (Roche) using the Maxima SYBR/ROX qRT-PCR Master Mix (Fermentas, Cat. No: K0221). Error bars represent the SEM of at least three independent sets of samples. qRT-PCR assays were normalized with two reference genes (cdc-42 and pmp-3).

Statistical analysis

Statistical analysis was performed in GraphPad Prism 6 using one-way ANOVA for comparison followed by Dunnett’s Multiple Comparison Test or Tukey’s Multiple Comparison Test, where applicable. Values are expressed as mean ± SD. Differences with a P value <0.05 were considered significant.

Data availability

The authors state that all data necessary for confirming the conclusions presented in the article are represented fully within the article. Strains are available upon request.

Results

ret-1 is expressed in the nervous system

Nogo-A is reported to function in neurodevelopmental processes, neuroplasticity, and neuronal regeneration, and has been implicated in a range of neurodegenerative diseases (Caroni and Schwab 1988a; Buffo et al. 2000). Recent studies investigated the role of axon guidance proteins in neurodegeneration, and found several guidance cues implicated in the development of these disorders (Engle 2010; Nugent et al. 2012; Van Battum et al. 2015). We therefore hypothesized that Nogo-A could play a role in axon guidance, and investigated this by analyzing RET-1, the C. elegans Nogo-A homolog.
**Figure 1** The *C. elegans* Nogo-A/RTN4 homolog RET-1 is expressed in the nervous system. (A) Alignment of the C-terminus of *C. elegans* RET-1 with the Nogo-A/RTN4 homologs of mouse, rat and human. The RHD is highlighted in gray. Alignment was generated using ClustalW. (B) The ret-1 locus comprises eight isoforms encoding predicted proteins of 204–3303 amino acids. All isoforms harbor an RHD in their C-termini. The deletion alleles (marked by red bars) used in this study (gk242 and tm390) both affect the RHD and produce frameshifts. The DNA sequence that encodes the RHD is marked by a black bar at the C terminus. We used ret-1 isoform g.2 5000 bp promoter used to report expression (ret-1prom::gfp - now written as ret-1prom::gfp) is shown below the locus. (C–G) Expression of the transcriptional ret-1prom::gfp reporter during embryonic and young adult stages. (C–F) At the bean stage, ret-1prom::gfp is diffusely expressed (D);
The \textit{ret-1} locus in \textit{C. elegans} contains eight isoforms, predicted to encode proteins that range from 204 to 3303 amino acids (Figure 1). All protein isoforms harbor an RHD at their carboxy terminus; however, the ModEncode project showed that the shorter isoforms are predominantly expressed in \textit{C. elegans} (Gerstein et al. 2010). We therefore focused our expression and functional analysis on \textit{ret-1} isoform g.2 (Figure 1). First, we examined the \textit{ret-1} expression pattern using a transgenic fluorescent reporter strain in which GFP is driven under the control of a promoter region upstream of isoform g.2 (designated as \textit{prom}) (Figure 1). Fluorescence in the transcriptional \textit{ret-1}::\textit{prom}::GFP reporter strain was diffuse during early embryogenesis, then robustly detected in several head neurons in the twofold stage of the embryo (Figure 1). Postembryonically, \textit{ret-1} is extensively expressed in the nervous system, with prominent expression in the nerve ring and VNC motor neurons, and other VNC neurons, including the HSNs.

**\textit{ret-1} mutants display VNC axon guidance defects**

Since \textit{ret-1} is expressed in the nervous system, and prominently in neurons that navigate the VNC, we investigated if \textit{ret-1} plays a role in axon guidance at the VNC. To analyze the function of \textit{ret-1}, we obtained two independently isolated deletion alleles of the \textit{ret-1} locus, \textit{gk242} and \textit{tm390}, from \textit{C. elegans} knockout consortia. Both deletion alleles are predicted to cause a frameshift mutation resulting in a premature stop codon affecting all \textit{ret-1} proteins (Figure 1). Thus, the resultant mutant \textit{RET-1} proteins are not predicted to have functional RHDs. We initially focused on the development of the HSN neurons, which have been extensively studied, and are known to be regulated by a range of highly conserved axon guidance molecules (Desai et al. 1988; Wightman et al. 1997; Zallen et al. 1998; Boulin et al. 2006; Pocock and Hobert 2008; Pedersen et al. 2013; Torpe and Pocock 2014). The bilateral HSN neurons are born during embryogenesis, and subsequently migrate toward the presumptive midbody region of the embryo (Sulston and Horvitz 1977; Desai et al. 1988). During larval development, the left and right HSNs (HSNL/R) extend their axons toward the vulval precursor cells at the ventral midline of the worm. Next, they extend anteriorly in two separate VNC axon bundles until they reach the head, where they connect with other neurons at the nerve ring (Adler et al. 2006). We found that loss of \textit{ret-1}, using the \textit{gk242} and \textit{tm390} deletion alleles, causes 40–50% penetrant defects in HSN axon guidance, where axons inappropriately cross over to the contralateral side (Figure 2, A and B, and Table 1). In contrast, HSN cell migration occurs normally in \textit{ret-1} mutant animals (Table 1). To confirm that the HSN axon guidance defects are caused by loss of \textit{ret-1}, we transgenically resupplied \textit{ret-1} genomic DNA (isoforms a, b, c, and g.2), driven by its own promoter, in \textit{ret-1}(gk242) mutant animals (Figure 2C). We found that transgenic expression of \textit{ret-1} genomic DNA fully rescues HSN axon guidance defects in the \textit{ret-1} mutant (Figure 2C). To investigate if \textit{ret-1} can act as an instructive signaling cue, we overexpressed \textit{ret-1} genomic DNA in wild-type animals. Introducing extra copies of \textit{ret-1} did not cause axon guidance defects in wild-type worms, suggesting that \textit{ret-1} cannot act as an instructive molecule for HSN axon guidance (Figure 2C). We confirmed that \textit{ret-1} acts in the nervous system to control HSN guidance by driving the cDNA encoding \textit{ret-1} isoform g.2 under the control of the \textit{rgef-1} promoter in \textit{ret-1}(gk242) animals (Figure 2D). We next performed mosaic analysis to confirm whether \textit{ret-1} is required cell-autonomously in the HSNs to control axon guidance. Axon guidance defects were fully rescued when the \textit{ret-1}-rescuing array was present in the HSNs (5/69 animals, 7%, were defective), whereas loss of the array from the HSNs abrogated rescue (6/14 animals, 43%, were defective). These data indicate that \textit{ret-1} isoform g.2 acts cell autonomously to direct axon guidance of the HSNs. In addition, expression of the other \textit{ret-1} isoforms is not required for the control of HSN axon guidance, or any isoform that contains the RHD can function in this regard.

To further examine the role of \textit{ret-1} in the nervous system, we analyzed neuronal development using a panel of neuron-specific \textit{gfp} reporters. We found that axons of other VNC neurons, the PVQ and PVP interneurons, were also affected, albeit at a lower penetrance compared to the HSNs (16 and 17%, respectively) (Figure 3 and Table 1). To ask if the PVQ guidance defects are caused by deficiencies in development or maintenance of neuronal architecture, we analyzed PVQ guidance of freshly hatched L1 animals in \textit{ret-1}(gk242) animals. We observed similar penetrance of PVQ defects in L1 animals to young adults (18% penetrant defect, \textit{n} = 50). This indicates that the PVQ defects are developmental and not maintenance defects. In addition to the VNC midline defects, we discovered that loss of \textit{ret-1} caused defects in the left/right asymmetry of the commissural D-type motor neurons, and in the fasciculation of the motor neuron axons in the VNC (Figure 3 and Table 1). We did not, however, observe defects in the DA and DB motor neurons (Table 1). Interestingly, cell migration was not affected in any of the cell types studied, except for minor anterior displacement of the AVM mechanosensory neuron (Table 1). Additionally, the structure of the hypodermal tissue, which acts as a substratum for multiple axon guidance events, was intact (Figure 3D). Furthermore, other types of neurons were not affected by the loss of \textit{ret-1} (Table 1), indicating that the \textit{ret-1} axon guidance defects are specific to certain neuronal subtypes.

**HSN defects of \textit{ret-1} mutants are caused by dysregulated expression of the ephrin ligand VAB-2**

During embryogenesis, VNC axons are separated by embryonic motor neurons that provide repulsive cues to extending...
VNC axons to ensure left/right fascicle separation (Boulin et al. 2006). During postembryonic development, a hypodermal ridge forms and presents a physical barrier between left and right axon fascicles (White 1976; Boulin et al. 2006). During both these phases of development, multiple redundant pathways are known to act in parallel to drive axon guidance. To examine the functional relationship between ret-1 and these pathways, we created double mutants carrying the ret-1 (gk242) allele, and mutations in genes that act in axon guidance pathways (Netrin, Slit-Robo and Ephrin), and measured the penetrance of HSN axon guidance defects. As shown in Table 2, we found that ret-1 genetically interacts with mutations in genes of the Netrin and Slit-Robo pathways.

We did, however, find that two null alleles of the ephrin ligand VAB-2, but not the EFN-2 and EFN-3, suppress the HSN axon guidance defects of ret-1 mutant animals (Figure 4 and data not shown). Previous work found that multiple axon guidance molecules, including ephrins, are upregulated in Nogo-A knockout adult mice, which affects neurite outgrowth (Kempf et al. 2013). We therefore postulated that the HSN defects in the ret-1 mutant depend on overexpression...
of vab-2, as removal of vab-2 ameliorates the ret-1 HSN axon guidance defects. We therefore analyzed the transcript level of vab-2, and found that its expression was increased in ret-1 mutant animals (Figure 4A). These data suggest that dysregulation of VAB-2 expression causes, at least in part, the HSN axon guidance defects in ret-1 mutant animals. We next asked whether the suppression of the HSN axon guidance defects of ret-1 mutant animals by vab-2 loss is dependent on the VAB-1 Eph receptor, and we found this to be the case (data not shown). Loss of VAB-1 itself is also unable to suppress the axon guidance defects of ret-1 mutant animals (Figure 4B). These data suggest that ret-1 and vab-1 act in a similar pathway, and that overexpression of vab-2 in ret-1 mutant animals causes dysregulation of ephrin signaling.

**Discussion**

Recent studies have identified several axon guidance cues to function in neurodegenerative disease, neuronal development, and plasticity (Engle 2010; Nugent et al. 2012; Van Battum et al. 2015). One protein that has been intensively studied for its role in neuronal regeneration and plasticity is the neurite inhibitory protein Nogo-A; however, little is known about the role of Nogo-A in axon guidance. Here, we show that the Nogo-A homolog, ret-1, is required for the correct guidance of specific axons along the midline of C. elegans, and that the axon guidance defects in ret-1 loss of function animals are caused by dysregulation of ephrin expression. We investigated the expression pattern of ret-1 using a transgenic ret-1prom::gfp strain, which showed that ret-1 is highly expressed in the nervous system, particularly in VNC motor neurons and neurons that navigate the VNC. Thus, ret-1 is expressed in relevant cells that control axon guidance in the VNC. Using a panel of gfp reporters that label individual cell types, we found that ret-1 is required for the correct axon guidance of a specific subset of neurons.

The neuron subtypes that require RET-1 for their development (HSN, PVQ, PVP, and motor neurons) are all regulated by ephrin signaling in C. elegans (Boulin et al. 2006). In mammals, humans have been shown, that in the adult nervous system, loss of Nogo-A causes upregulation of developmental guidance cues, such as ephrins, at the mRNA level (Kempf et al. 2013). We found that this regulatory relationship between Nogo-A and ephrin is conserved in C. elegans. Loss of ret-1 causes increased mRNA expression of the ephrin vab-2.

To ask whether increased ephrin expression causes the axon guidance defects observed in ret-1 mutant animals, we performed double mutant analysis. First, we found that loss of the sole Eph receptor (VAB-1) in the ret-1 mutant did not increase the HSN defects, which suggests that vab-1 and ret-1 act in the same genetic pathway, whereas, loss of the ephrin ligand VAB-2 suppresses the ret-1 HSN axon guidance defects, suggesting that dysregulation of vab-2 results in defective axon guidance in ret-1 mutant animals. We found that the suppressive effect of vab-2 loss is dependent on the presence of the VAB-1 Eph receptor. These data suggest that VAB-2 is overexpressed in ret-1 mutant animals, perhaps in a temporally or spatially inappropriate manner, and interferes with VAB-1-regulated signaling. A previous study demonstrated
the importance of the correct expression level, and thereby concentration, of ephrin signaling for correct function of the axon guidance signal; this study showed that a change in ephrin concentration from low to high could change the signaling cue from being perceived as a growth promoting to a growth inhibiting signaling cue (Hansen et al. 2004). It is possible that a similar mechanism can cause the increase in ephrin expression we observed in the ret-1 double mutants would be possible to detect. Such suppression of ret-1 mutant defects was observed in two alleles of the VAB-2 ephrin ligand. Data are expressed as mean ± SD, and statistical significance was assessed by ANOVA followed by Tukey’s multiple-comparison test. n > 50, n.s., not significant.

In conclusion, our study shows that ret-1 is required for correct axon guidance of a specific subset of neurons that extend from, and along, the ventral midline in C. elegans. Neurons extending their axons in the left and right VNC are normally repelled by an interaction between an EphR, expressed on the extending axon, and ephrins expressed in motor neurons (Boulin et al. 2006; Pocock and Hobert 2008). In ret-1 deletion mutants, however, axons fail to respect the midline, and cross over to the contralateral side. We find that HSN axon guidance defects are caused by dysregulation of ephrin expression in ret-1 mutant animals. Deletion of the Eph receptor VAB-1, and thereby all canonical ephrin signaling, however, does not suppress HSN defects in ret-1 mutants. This suggests inappropriate signaling through ephrin (VAB-2) induction, as shown in a previous study (Pocock and Hobert 2008), causes neurodevelopmental defects in ret-1 mutant animals. To conclude, we have shown that the regulatory relationship between RTN genes and axon guidance cues is conserved in C. elegans. Therefore, this work indicates that the nematode may be of further use to help decipher RTN/Nogo-A-regulated pathways, which will be of potential benefit in the battle against neurological disorders.

Table 2 Double mutant analysis between ret-1(gk242) and known axon guidance regulators

| HSN Guidance Defects (%) | P Value |
|--------------------------|---------|
| Wild type (zdls13)       | 8       |
| ret-1(gk242)             | 41      |
| unc-6(ev400)             | 97      |
| unc-6(ev400); ret-1(gk242) | 95 | n.s. |
| unc-40(e1430)            | 91      |
| unc-40(e1430); ret-1(gk242) | 100 | n.s. |
| sax-3(ky123)             | 61      |
| sax-3(ky123); ret-1(gk242) | 72 | n.s. |
| slt-1(eh15)              | 21      |
| slt-1(eh15); ret-1(gk242) | 42 | n.s. |
| vab-1(dx31)              | 44      |
| vab-1(dx31); ret-1(gk242) | 49 | n.s. |
| vab-1(e2)                | 26      |
| vab-2(mu1)               | 36      |
| vab-2(mu1); ret-1(gk242) | 23      |
| vab-2(e96)               | 17      |
| vab-2(e96); ret-1(gk242) | 20      |

Quantification of HSN axonal cross-over defects in the indicated strains. VNC defects of HSN axons in ret-1(gk242) animals are not suppressed by mutations in conserved axon guidance pathways: unc-6 and unc-40 (netrin); slt-1 and sax-3 (Slit-Robo). The penetrance of the unc-6 and unc-40 single mutants is high; therefore, only suppression of these defects in the ret-1 double mutants would be possible to detect. Such suppression of ret-1 mutant defects was observed in two alleles of the VAB-2 ephrin ligand. Data are expressed as mean ± SD, and statistical significance was assessed by ANOVA followed by Tukey’s multiple-comparison test. n > 50, n.s., not significant.

Figure 4 VAB-2/ephrin expression is altered in ret-1 mutant animals, and causes HSN axon guidance defects. (A) qRT-PCR showing the expression levels of vab-2 in wild-type and ret-1(gk242) mutant animals. mRNA expression of vab-2 is increased in ret-1 mutants compared to wild type. Statistical significance was assessed by ANOVA followed by Tukey’s multiple-comparison test. **P < 0.0001. (B) HSN axon guidance defects of ret-1 mutant animals are suppressed by mutations in the ephrin ligand VAB-2 but not by the Eph receptor VAB-1. Statistical significance was assessed by ANOVA followed by Dunnett’s multiple-comparison test. n > 50, ****P < 0.0001, n.s., not significant.

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The following *C. elegans* strains were used in this study:

- RJP1691: `rpEx718[ret-1^prom::gfp]`
- RJP133: `Is[tpg-1^prom::gfp]`
- RJP1219: `ret-1(gk242); Is[tpg-1^prom::gfp]`
- RJP1214: `ret-1(tm390); Is[tpg-1^prom::gfp]`
- RJP1192: `rpEx567 Ex[ret-1 isoform g]; ret-1(gk242); Is[tpg-1^prom::gfp]`
- RJP1343: `rpEx601 Ex[ret-1 isoform g]; ret-1(gk242); Is[tpg-1^prom::gfp]`
- RJP1470: `rpEx645 Ex[ret-1 isoform g]; ret-1(gk242); Is[tpg-1^prom::gfp]`
- RJP1467: `rpEx642 Ex[ret-1 isoform g]; Is[tpg-1^prom::gfp]`
- RJP3149: `ret-1(gk242); rpEx1545[prgef-1::ret-1cDNA]; Is[tpg-1^prom::gfp]`
- RJP3150: `ret-1(gk242); rpEx1546[prgef-1::ret-1cDNA]; Is[tpg-1^prom::gfp]`
- VH648: `Is[odr-2^prom::CFP::sra-6^prom::DsRed2]`
- RJP1951: `Is[unc-47^prom::gfp]`
- RJP1188: `ret-1(gk242); Is[unc-47^prom::gfp]`
- RJP1423: `Is[ajm-1^prom::gfp]`
- RJP1237: `ret-1(gk242); Is[ajm-1^prom::gfp]`
- RJP1591: `vab-1(dx31); Is[tpg-1^prom::gfp]`
- RJP1270: `vab-1(dx31); ret-1(gk242); Is[tpg-1^prom::gfp]`
- RJP1595: `vab-2(ju1); Is[tpg-1^prom::gfp]`
- RJP1189: `vab-2(ju1); ret-1(gk242); Is[tpg-1^prom::gfp]`
- RJP1926: `vab-2(e96); Is[tpg-1^prom::gfp]`
- RJP1342: `vab-2(e96); ret-1(gk242); Is[tpg-1^prom::gfp]`
- OH7178: `vab-1(e2); zdis13`
- RJP436: `ret-1(gk242); zdis13`
- RJP3188: `vab-1(e2); ret-1(gk242); zdis13`
- RJP3313: `unc-6(e400); zdis13`
- RJP3318: `unc-6(e400); ret-1(gk242); zdis13`
- RJP3314: `unc-40(e1430); zdis13`
- RJP3315: `unc-40(e1430); ret-1(gk242); zdis13`
- RJP3316: `sax-3(ky123); zdis13`
- RJP3317: `ret-1(gk242); sax-3(ky123); zdis13`
- RJP466: `slt-1(eh15); zdis13; oyls14`
- RJP3320: `ret-1(gk242); slt-1(eh15); zdis13`
- RJP3312: `ret-1(gk242); zdis13; rpEx1586[rgef-1^prom::ret-1gcDNA + tph-1^prom::mCherry + myo-2^prom::mCherry]`
- RJP3184: `ret-1(gk242); hdis26; rpEx1557[sra-6^prom::GFP]`
- VH648: `Is[odr-2^prom::CFP::sra-6^prom::DsRed2]`
- RJP1951: `Is[unc-47^prom::CFP::sra-6^prom::DsRed2]`
- EG1306: `Is[unc-47^prom::gfp]`
- RJP1188: `ret-1(gk242); Is[unc-47^prom::gfp]`
- NW1100: `Is[unc-124^prom::gfp]`
- RJP1218: `ret-1(gk242); Is[unc-124^prom::gfp]`
- RJP122: `Is[mec-4^prom::gfp]`
- RJP1217: `ret-1(gk242); Is[mec-4^prom::gfp]`