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Loss of the Putative RNA-Directed RNA Polymerase RRF-3 Makes *C. elegans* Hypersensitive to RNAi

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Summary

RNA interference (RNAi) is a broadly used reverse genetics method in *C. elegans* [1]. Unfortunately, RNAi does not inhibit all genes [2, 3]. We show that loss of function of a putative RNA-directed RNA polymerase (RdRP) of *C. elegans*, RRF-3, results in a substantial enhancement of sensitivity to RNAi in diverse tissues. This is particularly striking in the nervous system; neurons that are generally refractory to RNAi in a wild-type genetic background can respond effectively to interference in an *rrf-3* mutant background. These data provide the first indication of physiological negative modulation of the RNAi response and implicate an RdRP-related factor in this effect. The *rrf-3* strain can be useful to study genes that, in wild-type, do not show a phenotype after RNAi, and it is probably the strain of choice for genome-wide RNAi screens.

Results and Discussion

A loss-of-function mutation in *rrf-3* (*pk1426*) [4] does not result in any obvious morphological defects but does cause a high incidence of males (7–10 times higher than wild-type) and a temperature-sensitive decrease in brood size; *rrf-3* animals grown at 25°C produce few progeny (10 ± 2 compared to 95 ± 8 for wild-type at 25°C). An independently isolated transposon insertion allele (*pk2042*) [4] displays identical phenotypes. Recent analysis of the *rrf* genes of *C. elegans* (a family of putative RNA-directed RNA polymerases [RdRP]) hinted that mutations in the *rrf-3* gene cause increased sensitivity to RNAi [4]. To investigate this, we first assayed bacteria expressing 80 distinct dsRNAs chosen from a genome-wide library designed to induce RNAi when fed to *C. elegans* ([2] and R.S. Kamath et al., submitted). We primarily selected dsRNA segments that do not produce a phenotype when fed to wild-type *C. elegans. rrf-3* (*pk1426*) and wild-type animals were fed as described by Kamath et al. [5]. We scored the percentage of embryonic lethality and assayed sterility, developmental delay, and postembryonic phenotypes. Of the 80 dsRNAs tested, we found 26 that induced phenotypes in a wild-type genetic background. In an *rrf-3* genetic background, we found phenotypes for an additional 23 dsRNAs. Several dsRNAs induced more than one phenotype in the set tested; in total, we detected 45 phenotypes in a wild-type background and 75 in an *rrf-3* background. A large fraction of the dsRNA segments were chosen to correspond to genes with known mutant phenotypes so that we could compare the RNAi phenotypes to known mutant phenotypes. For *rrf-3*, we detected 15 known phenotypes that we could not detect for wild-type animals (Figure 1A). *unc-73* and *lin-1* are two genes that nicely illustrate the increased sensitivity of *rrf-3* to RNAi (Figure 1B). The other independently derived allele of *rrf-3* (*pk2042*) confirmed the enhanced sensitivity to RNAi and showed that the mutations in *rrf-3* cause the increased sensitivity to RNAi.

Previous results have shown that both endogenous genes and transgenic reporter genes can show partial resistance to RNAi in the nervous system [5, 6]. For a GFP reporter (Figure 1C), we see that a wild-type strain is almost fully resistant to RNAi in the neurons in the head region, while the *rrf-3* strain shows clear loss of GFP expression, indicating an enhancement in neuronal sensitivity of *rrf-3* mutant animals to feeding-induced RNAi. Several endogenous genes that are specifically expressed in neurons show a similar effect. *rrf-3* animals that were fed dsRNA for *unc-30*, *unc-33*, or *unc-86* showed a clear uncoordinated movement phenotype, while no phenotype was detected in wild-type animals. We tested five additional neuronally expressed genes by injection of dsRNA into gonads. As shown in Figure 1D, we see a clear enhancement for four genes. Together, these data show that *rrf-3* animals are more sensitive to RNAi for a broad set of *C. elegans* genes.

The applicability of *rrf-3* mutants for functional analysis in the nervous system requires that the nervous system itself is "normal" in such mutants. No behavioral defects were evident in *rrf-3* animals; the wiring of the nervous system also appeared normal, as visualized by staining with antibodies to synaptic components SNT-1, UNC-10, and UNC-64.

In addition to the effects on RNAi, we have also observed that *rrf-3* animals are more sensitive to transgene silencing. Although transgene silencing in *C. elegans* has been found to occur most dramatically in germline

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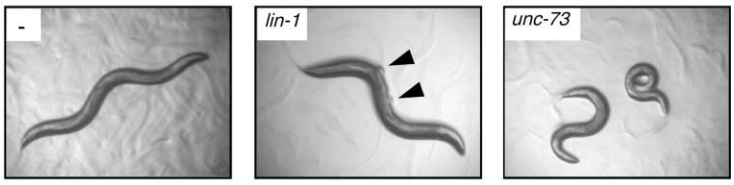
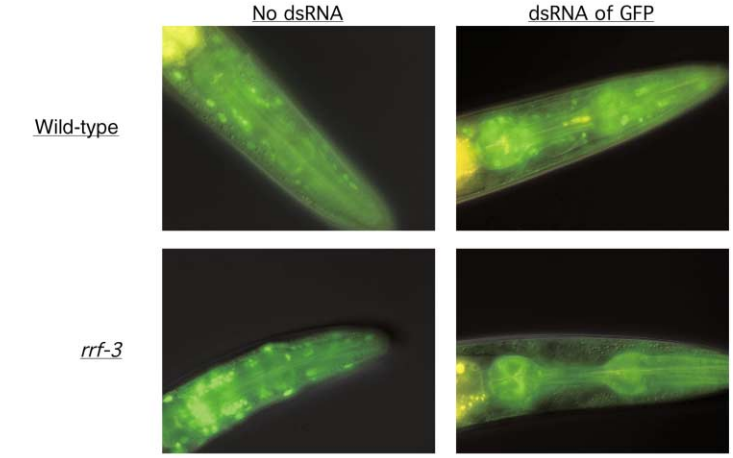
A

Phenotype	Published mutant phenotypes	Published phenotype detected	
		N2	*rrf-3*
All	52	11	26
Emb	10	6	7
Ste	1	0	0
Lvl	7	1	1
Post Emb	37	4	18

B



C



D

Gene	N2	*rrf-3*	RNAi Phenotype
unc-119	0% (372)	82% (324)	very slow curlers
unc-18	0% (128)	68% (374)	slow, thin kinkers
unc-25	0% (180)	46% (168)	spastic response in reverse direction
unc-104	0% (327)	32% (186)	slow, thin kinkers
unc-13	0% (300)	0% (250)	no (wild-type)
gfp	0% (744)	0% (744)	no (wild-type)

Figure 1. RNAi in the *rrf-3* (*pk1426*) Mutant

(A) Detection of published mutant phenotypes by RNAi (Emb, embryonic lethality; Ste, sterility; Lvl, larval lethality; and Post Emb, postembryonic phenotype). We targeted the following genes: *apr-1*, *cel-1*, *clr-1*, *cye-1*, *daf-2*, *dpy-14*, *dpy-18*, *eft-3*, *egl-30*, *etr-1*, *gon-4*, *gpc-2*, *gsa-1*, *him-3*, *hlh-1*, *hmr-1*, *lag-2*, *let-502*, *lin-1*, *lin-31*, *lin-49*, *mom-5*, *par-1*, *pha-1*, *pop-1*, *pos-1*, *ptr-2*, *rec-8*, *ric-19*, *spo-11*, *unc-3*, *unc-4*, *unc-5*, *unc-6*, *unc-11*, *unc-13*, *unc-14*, *unc-15*, *unc-17*, *unc-22*, *unc-29*, *unc-30*, *unc-33*, *unc-36*, *unc-37*, *unc-38*, *unc-40*, *unc-47*, *unc-73*, *unc-76*, *unc-86*, *unc-87*, *unc-89*, *unc-93*, *unc-101*, *unc-104*, *unc-130*, *zyg-1*, C01A2.3, C17E4.9, C32E8.1, C32E8.2, C32E8.3, C32E8.4, C32E8.5, C32E8.6, C32E8.9, C32E8.11, D1081.2, F09F7.4, F20H11.2, F54C4.3, F56F3.1, K04G7.12, PAR2.4, R11A5.1, T23D8.5, Y39A1A.B, Y52B11A.9, and ZK1098.5 (detailed data available upon request).

(B) *rrf-3* animals were fed on food without dsRNA (−) and on food with dsRNA of *lin-1* or *unc-73*. *rrf-3* animals that were fed on *lin-1* dsRNA have multiple protruding vulvae (arrowheads). Animals that were fed on dsRNA of *unc-73* are uncoordinated and dumpyish. These phenotypes are expected based on the described *lin-1* and *unc-73* mutants, but they are not detected for wild-type animals fed on the dsRNAs.

(C) Transgenic wild-type (N2) and *rrf-3* animals that broadly express GFP (*let-858::GFP*) were fed with dsRNA for GFP: (nuclear) expression (small dots) is silenced only in the mutant.

(D) RNAi of neuronally expressed genes by injection of dsRNA into the gonad.

tissue, there have also been examples of silencing in somatic tissue [7, 8]. Transgene arrays carrying the dominant marker gene *rol-6*(*su1006*) cause rolling movement in wild-type; in at least one case, such an array shows wild-type movement in an *rrf-3* mutant background (Figure 2). This failure to display the rolling phenotype depends on the action of the RNAi/mutator genes *mut-7* and *mut-16*, which are also required for cosuppression in the *C. elegans* germline. In contrast, genes that are required specifically for RNAi, i.e., *rde-1* and *rde-4*, are not needed, indicating that the genetic requirements for germline cosuppression in wild-type animals and somatic silencing of this transgene are similar [9, 10].

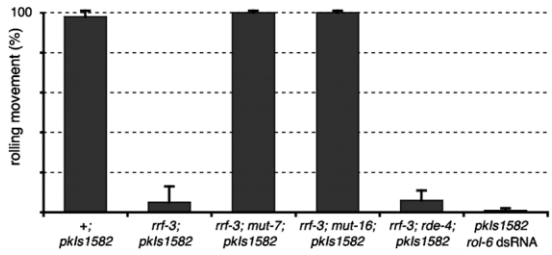


Figure 2. Hyperactive Somatic Transgene Silencing in *rrf-3* Animals

An integrated transgenic array that expresses the dominant *rol-6*(*su1006*) marker is assayed for its ability to induce a rolling phenotype in wild-type (+), *rrf-3*, *rrf-3; mut-7*, *rrf-3; mut-16*, and *rrf-3; rde-4* genetic backgrounds at 20°C. The activity of *rol-6*(*su1006*) can also be silenced by exposing these transgenic animals to *rol-6* dsRNA.

In summary, we here describe that two different loss-of-function alleles of *rrf-3* make *C. elegans* supersensitive to RNAi. This is seen both in the number of genes for which a phenotype is detected as well as the severity and penetrance of some phenotypes. A working hypothesis is that the RRF-3 protein might compete with RRF-1 and EGO-1 for components or intermediates in the RNAi reaction [4, 11]; this indicates that RNAi in wild-type *C. elegans* is under negative regulation.

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