Supplementary information

Methods

Worm strains and culturing

Worms were cultured on NGM plates seeded with OP50 bacteria. All experiments were performed at 20ºC. The following strains were used: Bristol N2, GR1373 *eri-1*(*mg366*) and NL3630 *ruIs32*(*pie-1::gfp::H2B*)[1]; *eri-1*(*mg366*). For mutant analysis, single mutants and doubles with *eri-1* were used: NL3301 *rde-1(pk3301)* and NL3631 *rde-1(pk3301)*; *eri-1*(*mg366*), WM49 *rde-4(ne301)* and NL3633 *rde-4(ne301)*; *eri-1*(*mg366*), NL917 *mut-7(pk204)* and NL3634 *mut-7(pk204)*; *eri-1*(*mg366*), NL1800 *mut-16(pk700)* and NL3636 *mut-16(pk700)*; *eri-1*(*mg366*), NL2098 *rrf-1(pk1417)* and NL3638 *rrf-1(pk1417)*; *eri-1*(*mg366*), NL2041 *rrf-2*(pk2040) and NL3639 *rrf-2(pk2040)*; *eri-1*(*mg366*), NL4256 *rrf-3*(*pk1426*). The histone deacetylase inhibitor TSA was added to the medium in a final concentration of 4µM.

RNAi by feeding

HT115 bacteria containing L4440 with inserts of *C. elegans* genes were constructed by and obtained from the Ahringer[2] and Vidal[3] groups or cloned by us. Bacteria were cultured on LB/Amp/Tet plates and a single colony was inoculated into LB/Amp overnight. These cultures were used for seeding NGM/Amp/IPTG plates. Single L4 worms were put on each plate and their progeny was scored for phenotypes.

Analysis of inheritable silencing

To analyze inheritable silencing, we either fed worms with bacteria expressing dsRNA or injected worms with dsRNA. We then typically picked ten worms with the expected phenotype and analyzed these for inheritance. In case of *gfp* silencing, all 10 worms keep segregating worms in which GFP expression is reduced when compared to wildtype levels. In parallel, we determined the rate at which we observe spontaneous silencing of GFP expression in worms fed on bacteria containing an empty vector. We analyzed the level of GFP expression in 2,000 unfed NL3630 worms and never observed spontaneous silencing of GFP expression. Thus, spontaneous silencing is orders of magnitude lower than the rate at which RNAi induced phenotypes are inherited.

Analysis of the genetic requirements for initiation and maintenance of silencing

First, we analyzed the role of genes implicated in RNAi (i.e. *rde-1, rde-4, mut-7, mut-16, rrf-1, rrf-2* and *rrf-3*) for their role in initiation and maintenance of silencing of *ceh-13* and *dpy-28* (for *rrf-2*, maintenance of silencing was only tested for *dpy-28* silencing; for *rrf-3*, maintenance was only tested for *ceh-13*). For *dpy-28*, we constructed double-mutant combinations containing *eri-1* and each of these seven mutants listed.

To identify genes that are required for maintenance of inheritable silencing, we performed an RNAi screen. We tested RNAi feeding clones for 164 genes known to be involved in RNA silencing processes by previous studies[4-7], as well as candidate genes with the potential to be involved in (transcriptional) gene silencing, including *C. elegans* Argonauts, histone (de)acetylases and other genes implicated in chromatin remodeling: AC3.4 (*pqn-2*), B0035.12, B0302.5, B0336.3, B0379.3 (*mut-16*), B0414.7 (*mtk-1*), C01G5.2 (*prg-2*), C04F12.1, C04G4.5 (*mes-6*), C06A1.4, C08B11.2 (*hda-2*), C08F8.2,

C10E2.3 (*hda-4*), C12D8.1, C14B1.7 (*ppw-3*), C14C11.6 (*mut-14*), C16C10.3, C18E3.2, C18E3.7, C18G1.7, C24F3.4, C26C6.1, C26F1.3, C26H9A.1 (*vha-7*), C27B7.4, C28A5.1, C28A5.2, C35A5.9, C37A2.7, C41G7.1 (*smn-1*), C50E10.4 (*sop-2*), C52B9.8, D2030.6 (*prg-1*), D2096.8, DY3.2 (*lmn-1*), F01G4.1 (*psa-4*), F02E9.4 (*pqn-28*), F10E9.8 (*sas-4*), F14H3.12, F15B10.2 (*drh-1*), F16D3.2 (*rsd-6*), F20B6.5, F20D12.1 (*ppw-4*), F21C3.4 (*mut-8*), F22D6.6, F26A3.3 (*ego-1*), F26H11.1, F33A8.1 (*let-858*), F35G12.10 (*asb-1*), F35H8.3, F37A4.8 (*isw-1*), T01C3.8 (*mut-15*), T04A8.14 (*emb-5*), T04C12.2 (*srh-75*), T05E8.3, T07A9.8, T07D3.7 (*alg-2*), T07D4.3 (*rha-1*), T09B4.9, T09E8.1, T12D8.1 (*tag-359*), T13H2.4 (*pqn-65*), T19B10.7 (*ima-1*), T19B4.5, T19C4.5, T20G5.11 (*rde-4*), T22B3.2, T22B7.2 (*egl-13*), T22D1.3, T22H9.3, T23B12.1, T23B5.3, T23D8.7, T24C4.1, T24H7.1 (*phb-2*), W01B11.3, W02A11.4 (*uba-2*), W02D9.3, W05H7.4, Y106G6H.2 (*pab-1*), Y110A7A.16, F37B12.4, F37C12.4 (*rpl-36*), F38A5.10, F39C12.2 (*add-1*), F43E2.1, F43G6.4, F43G9.1, F43G9.5, F45E4.10 (*gfi-4*), F45E4.9 (*hmg-5*), F48F7.1 (*alg-1*), F54C1.3 (*mes-3*), F54F2.2 (*zfp-1*), F54H12.1 (*aco-2*), F55A12.1, F55A3.3 (*phi-6*), F55A4.4, F55F8.3, F56A6.1, F58G1.1, H06I04.3, H19N07.2, H25K10.6, K03D10.3, K04F10.6 (*rde-3*), K07A1.12 (*lin-53*), K07A12.3 (*asg-1*), K07C5.4, K07H8.10, K08D10.4 (*rnp-2*), K08F4.2, K08H10.7 (*rde-1*), K10D12.3, K10D2.3, K12B6.1, K12H4.8 (*dcr-1*), M01F1.3, M03C11.8, M03D4.6, M03F8.3, M04B2.3 (*gfl-1*), R03D7.4, R04A9.2, R06A4.7 (*mes-2*), R06C1.1 (*hda-3*), R06C7.1, R07E5.3, R09A1.1, R13F6.1, Y110A7A.18 (*ppw-2*), Y113G7B.14, Y113G7B.23 (*psa-1*), Y12A6A.1, Y2H9A.1 (*mes-4*), Y37D8A.9 (*mrg-1*), Y38A10A.6, Y39A3CR.8, Y40B10A.6, Y43F8C.8, Y48B6A.3 (*xrn-1*), Y49F6A.1, Y49F6B.4 (*smu-2*), Y51H1A.5 (*hda-6*), Y54E5A.4 (*npp-4*), Y56A3A.17 (*npp-16*), Y71H2AM.23, Y77E11A.7, ZC449.3, ZK1098.8 (*mut-7*), ZK112.2, ZK1127.3, ZK1127.9, ZK1128.5, ZK1248.7, ZK218.8, ZK381.4 (*pgl-1*), ZK593.7 (*lsm-7*), ZK757.3 and ZK858.7. The initial screen was done *in duplo*: worms displaying the Dpy-6 phenotype were put on the RNAi plates and their progeny was scored for the absence of Dpy worms. In the initial screen, only four plates scored positive (*i.e.* no Dumpy worms were present in the progeny). This was confirmed at least in five-fold. For *hda-4*, K03D10.3 and *mrg-1*, these data are also confirmed by relieve of the maintained silencing of GFP expression. Knock-down of *isw-1* results in sterile progeny, making it impossible to look for re-expression of GFP in the germline. Once inheritance is relieved (either by RNAi or TSA), it does not recur when the worms are taken of the RNAi or TSA plates. This was tested for both *dpy-6* and *gfp* inheritable silencing.

Microscopy

Young adults were categorized after inspection with the Zeiss M^2BIO at 660x magnification. AxioVision was used for capturing images (400x) of live worms put on 2% agarose pads; the same exposure time (~400ms) was used for capturing images to be compared in the same experiment.

Supplementary Table 1. Genes required for initiation or maintenance of inheritable silencing.

Gene	Name	Allele	Domain/function	Initiation	Inheritance
Genes implicated in RNAi					
K08H10.7	*rde-1*	*pk3301*	PAZ/PIWI domain	-	+
T20G5.11	*rde-4*	*ne301*	dsRNA binding	-	+
ZK1098.8	*mut-7*	*pk204*	3'-5' exonuclease domain	+	+
B0379.3	*mut-16*	*pk700*	Glutamine/Asparagine-rich domain	+	+
F26A3.8	*rrf-1*	*pk1417*	RdRP	+	+
M01G12.12	*rrf-2*	*pk2040*	RdRP	+	+
F10B5.7	*rrf-3*	*pk1426*	RdRP	+	+
Genes with a chromatin-related function					
C10E2.3	*hda-4*	RNAi	Histone Deacetylase	nd	-
K03D10.3	-	RNAi	Acetyltransferase	nd	-
F37A4.8	*isw-1*	RNAi	Chromatin Remodeling	nd	-
Y37D8A.9	*mrg-1*	RNAi	Chromatin Remodeling	nd	-

Indicated are the gene tested, its canonical name, the allele tested (or RNAi knockout), a short description of its domain or function, its requirement for initiation and its requirement for inheritance (+ not (fully) required, - dispensable).

References

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