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A Genome-Wide Screen Identifies 27 Genes Involved in Transposon Silencing in *C. elegans*

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Summary

Transposon jumps are a major cause of genome instability. In the *C. elegans* strain Bristol N2, transposons are active in somatic cells, but they are silenced in the germline [1], presumably to protect the germline from mutations. Interestingly, the transposon-silencing mechanism shares factors with the RNAi machinery [2]. To better understand the mechanism of transposon silencing, we performed a genome-wide RNAi screen for genes that, when silenced, cause transposition of Tc1 in the *C. elegans* germline. We identified 27 such genes, among which are *mut-16*, a mutator that was previously found but not identified at the molecular level, *ppw-2*, a member of the argonaute family, and several factors that indicate a role for chromatin structure in the regulation of transposition. Some of the newly identified genes are also required for cosuppression and therefore represent the shared components of the two pathways. Since most of the newly identified genes have clear homologs in other species, and since transposons are found from protozoa to human, it seems likely that they also protect other genomes against transposon activity in the germline.

Results and Discussion

The *C. elegans* genome contains 32 copies of the Tc1 transposon [3, 4]. Strikingly, Tc1 elements are active in somatic cells, whereas they do not jump in the germline [1]. The somatic activity suggests that the lack of activity in the germline is a regulatory effect rather than a lack of mechanistic potential. The elements themselves are indeed fully functional in *cis* [5, 6]. In addition to natural mutator loci, EMS-induced mutants ("mutators") have been isolated in which Tc1 transposition is activated in the germline of the Bristol N2 strain [2, 7]. Interestingly, half of the mutator mutants are also defective in the gene-silencing process RNAi [2]. These include alleles of the four *mut* genes that have been identified so far: *mut-7*, a gene with homology to *E. coli* RNaseD [2], *mut-8* (Tops et al., submitted), *mut-14*, a DEAD box helicase [8], and *mut-15* (Ketting et al., personal communication). This partial overlap between the processes of RNAi and transposon silencing led to the notion that a natural function of RNAi is to protect the genome from transposition in the germline [9].

Identification of 27 Genes Required for Transposon Silencing

We used the recently developed tool of genome-wide RNAi screens [10–13] to gain more insight into the mechanism of transposon silencing. To monitor transposition, we used a strain with a visible twitching phenotype caused by a Tc1 insertion in the muscle gene *unc-22*. This strain was fed with bacteria that express dsRNA homologous to (part of) a *C. elegans* gene in order to knockdown that gene by RNAi. We inspected the progeny for wild-type moving worms, i.e., worms in which the transposon had jumped out of *unc-22*, restoring its function. Initially, we screened 14,387 of the currently predicted 19,427 genes in *C. elegans* by using the RNAi feeding library [13]. All positives were retested 5-fold. Table 1 shows the 27 genes that scored positive at least three times in the latter experiment.

The genes were identified by the criterion that their silencing induces reversion of a Tc1 allele of the muscle gene *unc-22*. To verify that the reversion is indeed the result of transposon excision, we performed a transposon insertion display [14] for three of the genes identified. Figure 1 indeed shows that homozygous revertants obtained after RNAi knockdown of three genes (*mut-16*, F10G8.3, and *asg-1*) have lost the Tc1 element in *unc-22*. In addition, the release of transposon silencing induces novel insertions. Finally, we tested *mut-16* for its ability to revert a Tc5 allele. In our previous analysis of mutator mutants, we found that loss of *mut-7* function results in loss of silencing of Tc1, but also of other transposons such as Tc3, Tc4, and Tc5 [2]. We find that knocking down *mut-16* by RNAi also results in transposition of Tc5. We therefore conclude that *mut-16* is a general mutator; it silences transposition of DNA transposons of different types in the germline of *C. elegans*. We assume, but have not tested, that this applies to other genes listed in Table 1.

Molecular Identification of *mut-16*

We previously identified a set of 43 genetic mutants defective in transposon silencing [2]. Here, we sampled this collection of mutants by DNA sequence analysis and found that five *mut-16* mutants, as well as an *rde-6 (ne322)* mutant, which was previously found to be allelic to *mut-16* (F. Simmer and R.H.A.P., unpublished data; H.

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Table 1. 27 Genes Involved in Transposon Silencing

Gene	Name	Pheno	Cosup	*C.b.*	*S.c.*	*D.m.*	*A.t.*	*H.s.*	Short Function
A. Genes with a Mitochondrial Function									
C08F8.2		Nonv		+	SUV3	CG9791	At5g39840	SUPV3L1	ATP-dependent RNA helicase
F35G12.10	*asb-1*	Nonv		+	ATP4	ATPsyn-b, CG8189	–	ATP5F1	ATP synthase subunit
F43G9.1		Nonv	nd	+	IDH2	CG12233	At3g09805	IDH3A	NAD+-isocitrate dehydrogenase
F54H12.1		Nonv	nd	+	ACO1	Acon, CG9244	At4g35830	ACO2	aconitate hydratase
K07A12.3	*asg-1*	Nonv		+	ATP20	l(2)06225, CG6105	–	ATP5L	ATP synthase subunit
M01F1.3		Nonv		+	LIP5	Las, CG5231	At5g08415	LIAS	lipoic acid synthase
T24C4.1		Wt		+	MAS1, QCR2, MAS2	CG4169	At1g51980	UQCRC2	ubiquinol-cyt. c red. complex prot.
T24H7.1		Nonv	nd	+	PHB2	l(2)03709, CG15081	At4g28510	REA	prohibitin
T09B4.9		Nonv	nd	+	TIM44	*CG18304*	At2g20510	TIM44	translocase
Y71H2AM.23		Nonv		+	TUF1	EfTuM, CG6050	At4g02930, At4g20360	TUFM	translation elongation factor Tu
B. Genes Involved in Ribosome Biogenesis and Translation									
C06B8.8	*rpl-38*	Nonv	nd	+	RPL38	CG18001	At2g43460; At2g43460	RPL38	ribosomal protein L38
F17C11.9		Nonv	nd	+	CAM1	Ef1g , CG11901	At1g09640	EEF1G	translation elongation factor EF-1 gamma
F37C12.4	*rpl-36*	Nonv	nd	+	RPL36B, RPL36A	RpL36, CG7622	At2g37600	RPL36	ribosomal protein L36
F55F8.3		Gro	nd	+	PWP2, UTP1	CG12325	At1g15440	PWP2H	pre-rRNA processing
H06I04.3		Gro		+	SPB1	CG8939	At4g25730	FTSJ3	rRNA methyltransferase
K07C5.4		Nonv	nd	+	SIK1 = NOP56	Nop56, CG13849	At1g56110	NOL5A	snoRNP protein
W01B11.3		Nonv	nd	+	NOP58 = NOP5	nop5, CG10206	At5g27120	NOP5/NOP58	snoRNP protein
ZK858.7		Wt	nd	+	GCD10	CG9596	At2g45730	CGI-09	translation initiation factor eIF3 RNA binding subunit
C. Group of Diverse Genes									
B0379.3	*mut-16*	Wt	cde	part. seq[a]	–	–	–	–	unknown
C28A5.1[b]		Wt	cde	+	–	–	–	–	unknown
C28A5.2[b]		Wt	cde	+	–	–	–	–	unknown
D2096.8		Nonv		+	NAP1	Nap1, CG5330	At4g26110; At5g56950	NAP1L1	nucleosome assembly protein
F10G8.3		Nonv		+	GLE2	CG9862	At1g80670	RAE1	nuclear pore complex
Y54E5A.4	*npp-4*	Wt		+	NUP100, NUP116	CG8831, CG10198	At1g59660	NUP98	nuclear pore complex
Y106G6H.2	*pab-1*	Wt	nd	+	PAB1	pAbp, CG5119	At1g49760	PABPC1	poly(A) binding protein
Y110A7A.18	*ppw-2*	Wt	cde	+	–	AGO1, CG6671	At2g27880	EIF2C1	Argonaute-like
Y77E11A.7		Gro		+	–	–	–	–	unknown

The 27 genes that are found to be involved in transposon silencing are divided into three categories. (A) Ten genes encoding mitochondrial proteins, (B) eight genes encoding proteins involved in ribosome biogenesis and protein translation, and (C) nine remaining genes. All genes are shown with their name; phenotype (Pheno) as described by Kamath et al. [13]; involvement in cosuppression (Cosup); predicted homologs in *Caenorhabditis briggsae* (*C.b.*), *Saccharomyces cerevisiae* (*S.c.*), *Drosophila melanogaster* (*D.m.*), *Arabidopsis thaliana* (*A.t.*), and *Homo sapiens* (*H.s.*); and a short description of their (putative) function. Nonv, nonviable; Gro, growth defective; Wt, wild-type; cde, cosuppression deficient; nd, not determined because of sterility or a growth defective phenotype.

[a] The *C. briggsae* sequence of the *mut-16* gene contains unsequenced gaps.

[b] C28A5.1 and C28A5.2 are too similar to target specifically; cross-interference cannot be excluded.

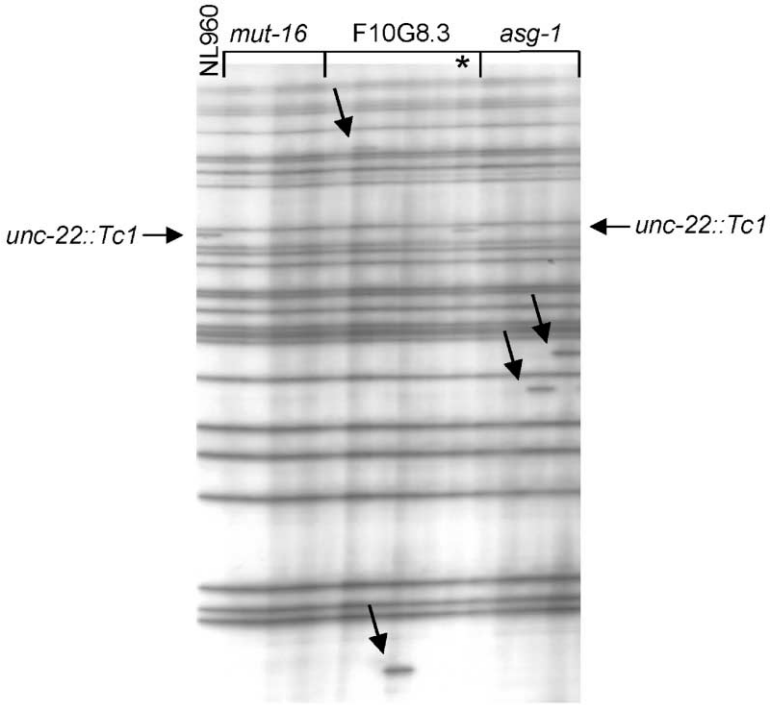
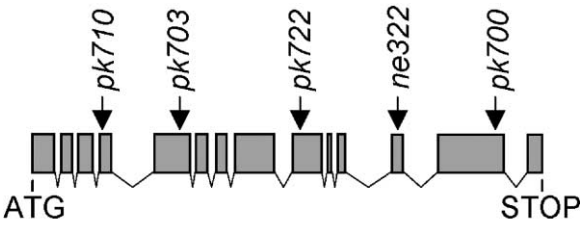


Figure 1. Tc1 Excision and Reintegration after Knocking Down Mutator Genes

NL960 (*unc-22* [*st136::Tc1*]) worms were grown on *E. coli* expressing dsRNA homologous to either B0379.3 (*mut-16*), F10G8.3, or K07A12.3 (*asg-1*). Revertants were analyzed by a transposon insertion display [14]. The bands represent Tc1 elements present in the genome. The *unc-22::Tc1* element present in the starting strain NL960 is indicated; the Tc1 element at this position is lost in all homozygous revertants, but not in a heterozygous revertant (indicated by an asterisk). The arrows indicate reintegration of Tc1 elements at new locations. For B0379.3, new insertions were visible in other display experiments (data not shown).

Tabara and C. Mello, personal communication), contain mutations that cause an early stop in B0379.3 (Figure 2). These mutations affect both transposon silencing and RNAi. The identification of *mut-16* shows that in this screen, we can identify genes that are involved in the RNAi mechanism. It might be expected that RNAi could not inactivate this class of genes, since this requires that genes essential for RNAi can themselves be inactivated via RNAi. However, the success of this approach probably depends on the precise timing and relative stability of mRNA and protein levels. Indeed, several previous reports describe the successful knockdown of genes involved in RNAi by RNAi [15–18]. Assays to detect RNAi defects for the other 26 identified genes failed (data not shown), and we are currently searching for genetic nulls to further address this question.



pk710 : W189 STOP
pk703 : W306 STOP
pk722 : R 575 STOP
ne322 : Q 732 STOP
pk700 : Q 930 STOP

Figure 2. Gene Structure and Genetic Mutants of *mut-16*

We sampled our collection of 43 genetic mutants defective in transposon silencing [2] by DNA sequence analysis, and in five *mut-16* mutants, as well as an *rde-6 (ne322)* mutant (which was previously found to be allelic to *mut-16*), we found a mutation in B0379.3, a gene that we identified in our screen. *mut-16* (B0379.3) encodes a protein that has proline-rich and glutamine/asparagine-rich regions. The gene structure is based on EST data from Y. Kohara (WormBase, http://www.wormbase.org, WS104) and additional cDNA sequencing. Note that two *mut-16* mutants have the same mutation (*pk700* = *pk701*).

Four Novel Mutator Genes Are also Involved in Cosuppression

The transposon silencing and RNAi pathways not only share components with each other, but they also share key components with the pathway controlling transgene-induced cosuppression [19–21]. Transgene-induced cosuppression is the silencing of a transgene and a homologous (endogenous) gene. We tested the entire set of genes for a role in transgene-induced cosuppression in the germline. We created a strain in which germline expression of GFP is cosuppressed by a second transgene that contains a truncated GFP gene (Figure 3B). We then targeted the genes found in the screen by RNAi. Knocking down *ppw-2* (Figure 3C), *mut-16* (Figure 3D), C28A5.1, and C28A5.2 (data not shown) by RNAi results in reexpression of GFP in the nuclei of the germline, i.e., knocking down these genes results in a cosuppression-deficient phenotype, indicated with "cde" in Table 1. It should be noted that, as always in RNAi experiments, negative results are not necessarily meaningful, and one may not conclude that the other genes are not required for cosuppression.

Transposon Silencing, Cosuppression, and RNAi

We here identified 27 genes involved in transposon silencing, and this is certainly an underestimation of the

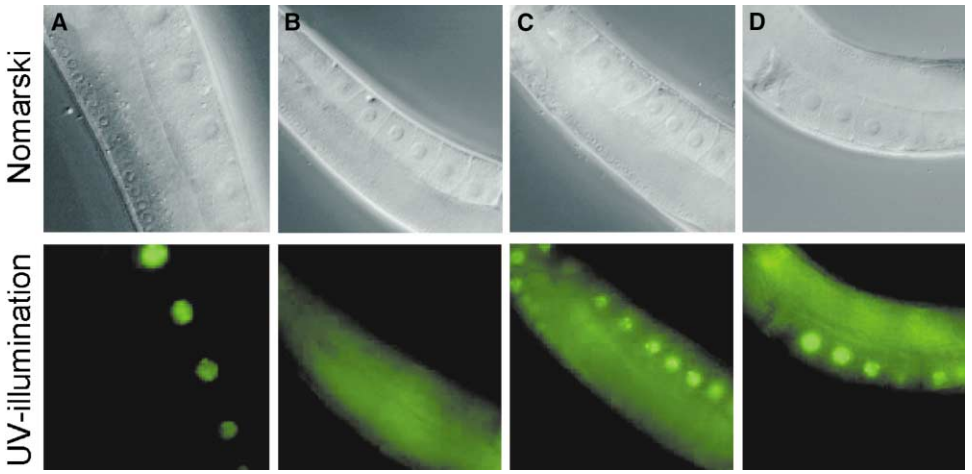


Figure 3. Reexpression of GFP after Targeting *ppw-2* and *mut-16* by RNAi

(A) Gonad arm of an AZ212 nematode in which GFP is expressed from a single-copy transgene in the nuclei of oocytes.

(B) Gonad arm of an NL3847 nematode in which *gfp* is cosuppressed by an array containing multiple copies of a truncated form of the *gfp* gene.

(C) Gonad arm of an NL3847 nematode after targeting *ppw-2*; cosuppression is impaired, and GFP is reexpressed.

(D) Gonad arm of an NL3847 animal after RNAi against *mut-16*; cosuppression is impaired, and GFP is reexpressed.

complete set, since some genes may have such weak effects that they are not detected in this assay and other genes might not be silenced by RNAi to levels that result in the known null phenotype (we were not able to obtain a mutator phenotype by RNAi for *mut-7, mut-8*, *mut-14*, and *mut-15* [data not shown]). Moreover, 18 of the 27 genes are required for fertility or viability: their silencing clearly results in partial embryonic lethality and growth defects (Nonv and Gro in Table 1). The revertants that were obtained after targeting these genes by RNAi are obviously escapers from this lethal phenotype. Apparently, targeting these genes by RNAi results in expression levels that do not induce lethality but do establish transposition. This suggests that we might have missed essential genes that have a more severe nonviable phenotype upon RNAi.

Based on sequence comparisons, we categorized the 27 predicted proteins involved in transposon silencing in three groups. Table 1A shows a diverse group of genes with a mitochondrial function. It is conceivable that transposon silencing is one of the first things to stop when there is shortage of energy, and therefore (some of) these genes may not be genuine "mutators" involved in the mechanism of silencing. The identification of these genes does show that the silencing of transposons requires a healthy energy metabolism.

Table 1B shows a group of genes that are involved in protein translation, including ribosomal proteins, ribosome biogenesis factors present in the nucleolus, and translation factors. It has been shown previously that ribosomal proteins cofractionate with small interfering RNAs (siRNAs) in *Drosophila* [22] and micro RNAs (miRNAs) in *C. elegans* (Ketting et al., personal communication). siRNAs are derived from the dsRNA that triggers RNAi and are thought to confer sequence specificity to the RNA destruction complex RISC (RNAi-induced silencing complex) in *Drosophila* [23], Neurospora [24], and human [25]. Their suggested association with ribosomal proteins might be important for stabilization and/or guidance to the mRNA. A role for siRNAs in the silencing of Tc1 in the germline of *C. elegans* is supported by the detection of siRNAs derived from Tc1 sequences ([26] and T. Sijen, in preparation). These siRNAs could direct destruction of the transposase mRNA, thereby preventing transposition. If so, the ribosomal proteins, ribosome biogenesis factors, and translation factors that we find might play a role in stabilizing and guiding the siRNAs involved in transposon silencing and RNAi or specifically in transposon silencing. We cannot exclude, however, that the function of this class of proteins in transposon silencing reflects an indirect role.

Table 1C shows the remaining genes. This group includes *mut-16*, a mutator that was previously found in genetic screens, but not identified molecularly. MUT-16 is involved in transposon silencing, RNAi, and cosuppression. The predicted protein has proline- and glutamine/asparagines-rich regions and has no apparent orthologs in other systems, except *C. briggsae*. A transgene containing *mut-16* upstream sequences and the *mut-16* open reading frame, fused to *gfp* coding sequences, shows broad expression, both in the cytoplasm and in nuclei (data not shown). Interestingly, we also identified *ppw-2* as a mutator. PPW-2 is a protein containing a piwi and a PAZ domain and is a homolog of the *C. elegans* proteins RDE-1 [21], ALG-1 and -2 [17], PPW-1 [27], and of several proteins in other systems, together forming the argonaute family. The argonaute family has been implicated in several gene-silencing processes (recently reviewed by Carmell et al. [28]). Members have been identified in complex with DICER (the enzyme that generates siRNAs from dsRNA) and also as components of RISC, which also contains siRNAs [23–25]. PPW-2 is the first member of the argonaute family shown to play a role in transposon silencing and cosuppression in *C. elegans*. We also identified three nuclear proteins as mutators that could imply

a role for chromatin structure in the silencing of transposons. The nucleosome assembly factor D2096.8 could be directly involved in structuring chromatin. The role of the nuclear pore complex proteins F10G8.3 and Y54E5A.4, however, could be more indirect since it has been shown recently that in yeast, nuclear complexes are linked to the boundaries of heterochromatin domains [29], linking the nuclear envelope with chromatin. All three could imply a role for chromatin structure in the silencing of transposons. In plants [30, 31], Chlamydomonas [32], and yeast [33], transcriptional gene silencing plays an important role in silencing (retro-) transposons. Moreover, in *C. elegans*, transgene silencing requires polycomb group proteins [34] and an isoform of histone H1 [35]. The identification of D2096.8 and the nuclear pore complex proteins as mutators suggests that chromatin structure might also play a role in transposon silencing and possibly RNAi in *C. elegans*.

Supplemental Data

Supplemental Data including the Experimental Procedures are available at http://www.current-biology.com/cgi/content/full/13/15/1311/DC1/.

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