Cell, Vol. 99, 133–141, October 15, 1999, Copyright ©1999 by Cell Press

mut-7 of *C. elegans*, Required for Transposon Silencing and RNA Interference, Is a Homolog of Werner Syndrome Helicase and RNaseD

René F. Ketting, Thomas H. A. Haverkamp, Henri G. A. M. van Luenen, and Ronald H. A. Plasterk*
Division of Molecular Biology
The Netherlands Cancer Institute
Center for Biomedical Genetics
Plesmanlaan 121
1066 CX Amsterdam
The Netherlands

Summary

While all known natural isolates of *C. elegans* contain multiple copies of the Tc1 transposon, which are active in the soma, Tc1 transposition is fully silenced in the germline of many strains. We mutagenized one such silenced strain and isolated mutants in which Tc1 had been activated in the germline ("mutators"). Interestingly, many other transposons of unrelated sequence had also become active. Most of these mutants are resistant to RNA interference (RNAi). We found one of the mutated genes, *mut-7*, to encode a protein with homology to RNaseD. This provides support for the notion that RNAi works by dsRNA-directed, enzymatic RNA degradation. We propose a model in which MUT-7, guided by transposon-derived dsRNA, represses transposition by degrading transposon-specific messengers, thus preventing transposase production and transposition.

Introduction

All natural isolates of *C. elegans* known to date contain multiple copies of Tc1 (Emmons et al., 1983); in all isolates, somatic activity of Tc1 is observed (Emmons and Yesner, 1984). In some natural isolates, such as the Bergerac strain, Tc1 jumping also occurs in the germline and is the main cause of spontaneous mutations (Moerman and Waterston, 1984; Eide and Anderson, 1985). In other strains, such as Bristol N2, of which the entire genomic DNA sequence was determined (*C. elegans* Sequencing Consortium, 1998), no jumping of Tc1 or any other transposon is seen in the germline. The difference between Bristol (inactive) and Bergerac (active) was found to be genetic; some determinants of germline activity (*mut* genes) showed Mendelian segregation and were mapped (Mori et al., 1988). From a Bergerac strain, a mutant could be derived by chemical mutagenesis in which transposition was further enhanced (the "high hopper" strain, containing the *mut-2(r459)* mutation [Collins et al., 1987]).

Anecdotal evidence has been obtained that the Bristol N2 strain can turn into a mutator (Babity et al., 1990), suggesting that the absence of transposition could be the result of negative regulation. This is in line with the observation of somatic excision and jumping of Tc1 in the Bristol N2 strain (Emmons and Yesner, 1984; Vos et al., 1993). Apparently, the entire machinery for transposition is present in Bristol N2, and thus the absence of jumping in the germline is presumably a regulatory effect.

Both the excision and the integration reaction of the Tc1 and Tc3 transposons are mediated by the transposase protein, which is encoded by the transposon (van Luenen et al., 1993; Vos et al., 1993). In vitro, transposase is the only protein required for Tc1 excision and integration (Vos et al., 1996). The only gene within the transposon is the transposase gene. We performed biochemical and genetic analysis of the transposase (Vos et al., 1993; van Luenen et al., 1994; Vos and Plasterk, 1994), including X-ray crystallography of the transposase bound to DNA of transposon termini (van Pouderoyen et al., 1997); it was shown that, for example, the Tc1 and Tc3 transposases bind specifically to their own termini and do not activate the other transposon (Vos et al., 1993; Colloms et al., 1994). This raises the conundrum of how one locus can control the activity of multiple transposons, each of which seems to depend on one and only one protein for activity.

As shown by Tabara et al. (1999 [this issue of *Cell*]), the phenomenon of transposon activation by mutator genes is genetically linked to the phenomenon of RNA interference or RNAi. (A description of RNAi is found in Tabara et al. [1999] and references therein.) Findings that are relevant for the work described here are as follows: (1) double-stranded (ds) RNA can silence expression of endogenous *C. elegans* genes; (2) it is essential that the dsRNA corresponds to an exon, not an intron, of that gene, pointing at a postsplicing target for RNAi (Fire et al., 1998); (3) RNAi acts in a nonstoichiometric fashion (Fire et al., 1998), as low amounts of dsRNA can silence many mRNAs; and (4) the effect of RNAi is the removal of mRNA (Montgomery et al., 1998). These latter two points suggest an active process of enzymatic mRNA degradation, targeted by the interfering dsRNA.

In this study, we report the isolation of mutant derivatives of the Bristol N2 strain, in which Tc1 transposition is being activated in the germline. In addition other transposons have been activated (Tc3, Tc4, and Tc5), suggesting that a common transposon silencing mechanism was hit. Following up on the observation that some RNAi-resistant mutants show transposon activation (Tabara et al., 1999), we tested our mutants for RNAi resistance and found that many, but not all, mutants are resistant to RNAi.

We identified one mutator gene, *mut-7*, and found it to be homologous to proteins with 3′-5′ exonuclease domains, such as Werner syndrome protein and RNaseD. This suggests that transposon silencing occurs via RNA interference, possibly via an RNase activity of MUT-7 that is directed to its target by dsRNA. We speculate that the natural function of RNAi may be to silence multicopy sequences (such as viruses and transposons), which give themselves away by producing transcripts of each

*To whom correspondence should be addressed (e-mail: rplas@nki.nl).

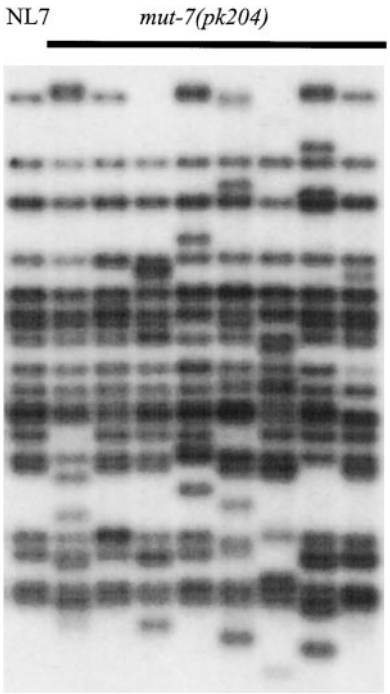

Figure 1. Mobilization of Tc3

Eight *mut-7(pk204)* cultures were grown in parallel for 6 weeks. Their DNA was isolated, cut with EcoRI, run on a Southern blot, and probed with a Tc3-specific probe. Tc3 patterns were compared to the unmutagenized parent strain, NL7. Mobilization of Tc3 is demonstrated by the appearance and disappearance of Tc3 containing restriction fragments.

strand, by a readthrough mechanism. These can form dsRNA, which triggers degradation of the transposase mRNA, preventing the production of transposase protein and transposon jumping.

Results

Mutant Screen

We performed EMS mutagenesis of a predominantly Bristol N2 strain, totally inactive for transposition, and searched for mutants in which Tc1 jumping had been activated. As described in detail in Experimental Procedures, we took a transposon Tc1 allele of a muscle gene, originally isolated in a transpositionally active strain, and substituted its genetic background for that of Bristol N2, by repeated crossing. Hence, this transposon allele shows no reversion. We then cloned out F1 animals after mutagenesis and searched among 7500 of them for cultures that showed reversion of the Tc1 allele in the progeny. These could be isolated at knockout frequency (43 in 15,000 genomes). One of these mutants is *mut-7(pk204)*. We and others have successfully used this strain to isolate Tc1 insertions into various genes, demonstrating complete transposition of the Tc1 element.

Apart from Tc1, other transposons are also found in the genome of *C. elegans*. All of these elements produce their own transposase, needed for activity. We showed previously that the transposases of Tc1 and Tc3 do not cross-activate (Vos et al., 1993). Remarkably, when we made Southern blots of several independently grown *mut-7(pk204)* strains and probed these for Tc3 polymorphisms, these were also seen (Figure 1). We also found that Tc3, Tc4, and Tc5 alleles of *unc-22* are able to revert when *mut-7(pk204)* is present (Table 1). This shows that *mut-7(pk204)* does not specifically activate Tc1 but instead mobilizes different transposons.

How can one mutation activate all these transposons? A hint is provided by data from Kelly and colleagues, who found that silencing of repetitive genes in transgenic DNA is context dependent: multicopy transgenes get shut off in the germline but not in the soma, while these same constructs are expressed in the germline in a less repetitive environment (Kelly et al., 1997). Tabara et al. (1999) now show that *mut-7(pk204)* can desilence a repetitive transgene in the germline. This suggests that a mechanism may exist that silences repetitive transgenic (and endogenous) DNA in the germline. Possibly MUT-7 acts in regulating germline gene expression, including Tc1, Tc3, Tc4, Tc5, etc. transposase genes.

RNAi Resistance

How then can a single mutation, such as *mut-7(pk204)*, affect the expression of multiple unrelated transposons? A recent study (Tabara et al., 1999) shows that mutants of *C. elegans* can be isolated that are resistant to RNAi. RNAi is the experimental silencing of gene expression by a dsRNA of a region from that gene. Apart from *C. elegans* (Fire et al., 1998), this mechanism has also been shown to work in *Drosophila* (Kennerdell and Carthew, 1998), trypanosomes (Ngo et al., 1998), planarians (Alvarado and Newmark, 1999), and plants (Waterhouse et al., 1998). Some (but not all) of the RNAi-resistant mutants isolated by Tabara et al. were found to be mutators, as were *mut-2(r459)* and *mut-7(pk204)*. They also found that the mutator *mut-6*, which spontaneously arose in a Bergerac background (Mori et al., 1988), was not RNAi resistant. We tested our EMS-derived mutants from Bristol N2 and found 22 out of 30 mutants, including *mut-7(pk204)*, *mut-7(pk719)*, and *mut-7(pk720)* to be resistant to RNAi (Figure 2). Thus, there is a significant overlap between these two classes of mutants, suggesting RNAi and transposon silencing are intimately connected.

Pleiotropic Effects of *mut-7(pk204)*

We noticed that the *mut-7(pk204)* mutation results in temperature-sensitive sterility. This is at least in part due to a defect in the sperm, as at elevated temperatures little sperm is detected using DAPI staining, and the sterility can be rescued by male mating (data not shown). We also noticed that the strain shows a *h*igh *i*ncidence of *m*ales (Him) phenotype, indicative of X chromosome loss during meiosis, as described previously for the

Table 1. *mut-7(pk204)*-Dependent Reversion of Transposon Alleles of *unc-22*

	unc-22::Tc1	*unc-22::Tc3*	*unc-22::Tc4*	*unc-22::Tc5*
mut-7(+)	−	−	−	−
mut-7(pk204)	+	+	+	+

A plus indicates reversion with a frequency of 10^{-4} to 10^{-5} per generation; a minus indicates reversion $<10^{-6}$.

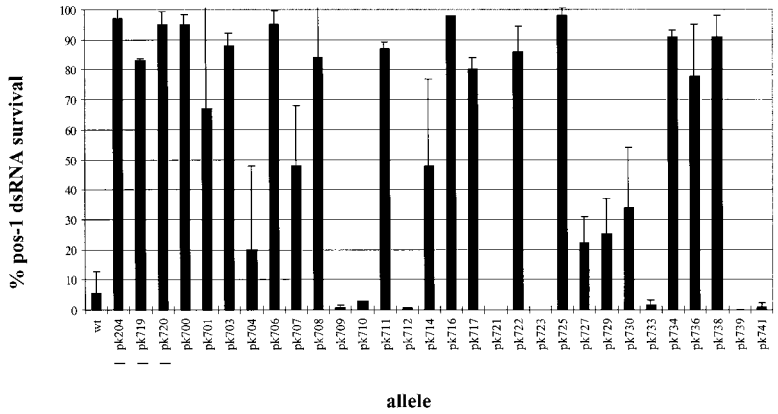

Figure 2. RNAi Resistance of Mutator Mutants

Worms are fed on *E. coli* producing dsRNA of the *pos-1* gene (for details, see Tabara et al., 1999), and their progeny was scored for the percentage of dead eggs. In wild-type worms, feeding on *pos-1* dsRNA containing food leads to around 95% dead eggs. Twenty-two out of 30 mutators tested show resistance to this phenomenon. The three *mut-7* alleles are grouped and underlined.

mut-2 high hopper strain (Collins et al., 1987). We then systematically investigated the temperature dependence of these phenotypes in *mut-7(pk204)*; as shown in Figure 3, transposon jumping, sterility, and the incidence of males all get stronger at elevated temperatures. We tested other mutants from the literature, such as the *mut-2* mutation (Collins et al., 1987) and the Bergerac and *mut-6* strains, and found that they show the same effect (data not shown). It is unlikely that these mutator alleles themselves are temperature sensitive, as the *pk720* allele, which defines a complete knockout of *mut-7* (see later), displays temperature sensitivity as well. It seems that the loss of *mut-7* or other mutators unveils a series of events that get stronger at elevated temperatures.

Parental Effects of *mut-7(pk204)*

Studies on the *mut-5* and *mut-6* mutators show that these genes display a clear parental effect in the first generation after a cross between a mutator and a non-mutator strain (Mori et al., 1990). It was found that introduction of the mutator via the mother resulted in higher transposition frequencies compared to introduction via the father. We checked this for the *mut-7(pk204)* allele and also determined how the phenotypes behave in subsequent generations. Both the RNAi resistance and transposon activation phenotype of *mut-7(pk204)* show a clear parental effect (Figures 4A and 4B, first generation). Heterozygous *mut-7(pk204)* worms are mutator, but only when the mutant allele comes from the mother. When the mutation is introduced via the father, the animal behaves wild type (i.e., no transposition events can be detected and the animals are fully sensitive to RNAi, suggesting that the MUT-7 protein is present in the female germline; see Experimental Procedures for details). These results are in agreement with results obtained by Tabara et al. (1999); they find that *mut-7(pk204)* mainly affects RNAi of maternally expressed genes. The effect of *mut-7(pk204)* on zygotic genes is less profound. This could, for example, be caused by the fact that other genes can take over the function of *mut-7* in the soma, but not in the germline. Similar maternal effects on transposition have also been found in *Drosophila* (Bingham et al., 1982; Rubin et al., 1982; Bryan and Hartl, 1988).

The homozygous self-progeny of both types of heterozygotes (*mut-7(pk204)* introduced either via the father or the mother) show a full *mut-7(pk204)* phenotype, when the RNAi resistance is assayed (Figure 4B). It does not matter whether the original *mut-7(pk204)* allele had been introduced into the heterozygote through the male

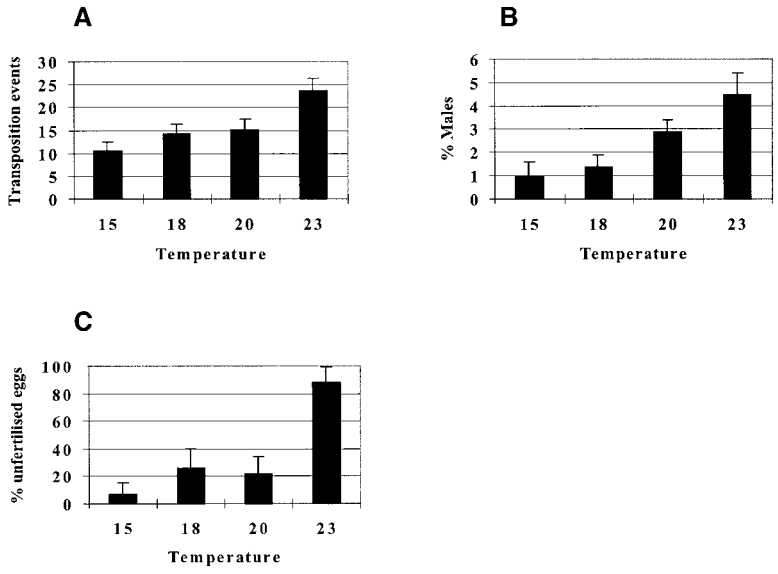

Figure 3. Pleiotropic Effects of *mut-7(pk204)*

Apart from transposon activation, a Him phenotype and sterility are observed. All three phenotypes are temperature sensitive. Transposition activity (A), incidence of males (B), and sterility (C) all become stronger at elevated temperatures.

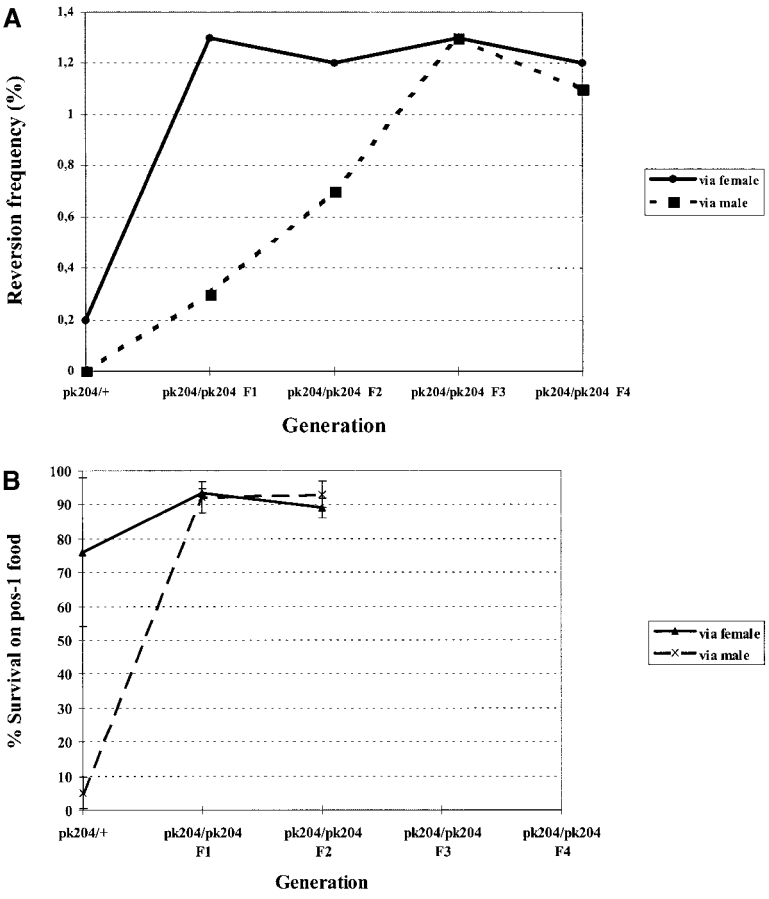

Figure 4. Parental Effect of *mut-7(pk204)*

Transposon activity, as measured by reversion frequency in percent (A) and survival on bacteria producing dsRNA against the *pos-1* gene (B). The mutant phenotype of each generation was determined by scoring its progeny for revertants (A) or survival (B). Indicated on the X axis is the generation whose progeny was scored. The *mut-7(pk204)* allele either came from the father or from the mother, as indicated in the graph. The homozygous animals are obtained from the heterozygotes through self-fertilization (for details, see Experimental Procedures).

or the hermaphrodite. Transposition activation, however, behaves differently: when the mutation is introduced in the F1 through the mother, the homozygous mutant F2 self-progeny again shows maximum transposon activity. Introduction of the mutation via the male, on the other hand, results in a gradual rise of transposition activity over three generations (Figure 4A). So, whereas the RNAi resistance phenotype is already maximal after one generation, transposition activation takes four generations to build up. There are two possible explanations for this observation. First, the RNAi assay may be less sensitive than the transposition assay in discriminating subtle differences in MUT-7 activity in the subsequent generations. Second, this may be the result of two independent epigenetic effects; first, the RNAi resistance phenotype needs to be established in one generation, and then the levels of transposase need to be established over the next two generations. In either case, it will be interesting to further analyze this epigenetic effect that develops over four generations.

The *mut-7* Gene Is a Homolog of RNaseD and Is Nonessential

To better understand the molecular basis for transposon silencing and RNAi, we identified *mut-7*. Since *mut-7* has a role in the germline, it might be difficult to clone the gene by rescue experiments, as transgenes are often silenced in the germline (Kelly et al., 1997). Therefore, we fine mapped the gene using SNPs that we identified in natural isolates of *C. elegans* (R. Koch et al., personal communication) and identified the gene by sequencing candidate genes. As shown in Figure 5, two *mut-7* alleles, *pk204* and *pk719*, have point mutations in the gene ZK1098.8, as identified by the *C. elegans* Sequencing Consortium (1998). These mutations delete 98 and 99 amino acids from the C terminus by introducing amber codons. A third allele, *pk720*, defines a large deletion of 9,710 bp, starting at position 15,765 (in ZK1098.3) and ending at position 25,475 (in ZK1098.9) in ZK1098. In between these endpoints, nucleotides 15,754 to 15,764 are repeated once in a direct orientation. This deletion removes ZK1098.8(*mut-7*) and ZK1098.4(GCN3 homolog) completely. Together, these data show that loss of MUT-7 activity relieves a block on transposition activity. The data also show that a complete loss-of-function mutant of *mut-7* plus the genes ZK1098.3, GCN3, and ZK1098.9 is viable. The phenotype of the point mutants do not seem less severe than that of the deletion allele, arguing that *pk204* and *pk719* are also null alleles.

In addition, we are able to rescue the mutant phenotype of *mut-7(pk204)* by introducing a wild-type copy of ZK1098.8 on an extrachromosomal array (see Experimental Procedures). This shows that the mutations identified in the *mut-7* gene are sufficient to cause the observed phenotypes.

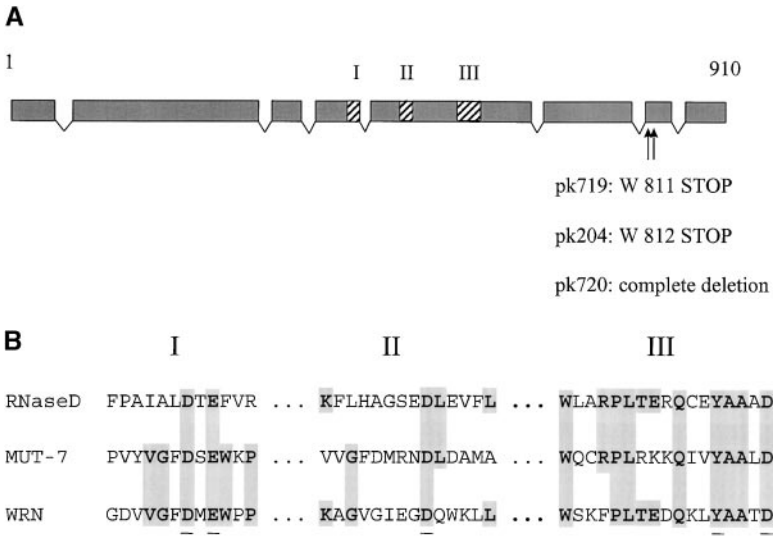

Figure 5. *mut-7* Identity

(A) Identity of the *mut-7* gene. The gene corresponds to ZK1098.8, in the center of chromosome III. The catalytic domains as predicted by homology (Mian, 1997) are indicated by hatched regions. The *pk204* and *pk719* alleles induce amber codons at the indicated positions. *pk720* deletes the complete gene and more (see text).

(B) Alignment of MUT-7, WRN, and RNaseD from *E. coli*, showing the conservation in the catalytic domains I, II, and III (3′-5′ exonuclease activity). The acidic residues involved in Mg^{2+} binding and the tyrosine that coordinates the nucleophilic water molecule, as predicted by homology (Mian, 1997), are underlined.

ZK1098.8 encodes a protein with homology to the catalytic domains of RNaseD (Figure 5B) (Mian, 1997), an enzyme that degrades RNA in a 3′ to 5′ direction (Deutscher, 1993). The protein also shows significant homology with the Werner syndrome helicase, which has exonuclease activity as well (Suzuki et al., 1999). MUT-7, however, does not contain the helicase domains found in the WRN protein.

The identification of *mut-7* as a gene encoding an RNase homolog suggests a speculative model that RNAi acts via enzymatic degradation of mRNAs. It is known that the amounts of dsRNA required for effective silencing of a gene are nonstoichiometric (Fire et al., 1998); it is also known that after RNAi, the resident mRNA is largely lost (Montgomery et al., 1998; Sharp, 1999). We propose that the RNAi machinery, consisting of dsRNA as well as MUT-7 and probably other proteins, is directed to mRNAs by the dsRNA component. This would presumably require some form of base pairing between the dsRNA and the target RNA, possibly after (partial) unwinding of the dsRNA. After successful targeting, the target RNA will be degraded by protein(s) in the complex, such as MUT-7 (Figure 6A). The dsRNA component will not be affected by the exonuclease activity and can subsequently target the complex to a new mRNA molecule. It is likely that other mutants in the set described above will define additional proteins involved in this complex.

Discussion

MUT-7 Is a Homolog of RNaseD

At this time, *mut-7* is the only mutator gene characterized at the molecular level in *C. elegans*. Figure 5B shows an alignment of MUT-7 to RNaseD of *E. coli* and the Werner disease protein. The similarities indicated are in the catalytic domain. All the critical residues for exonuclease activity are conserved. The Werner disease protein shows both RNase and DNase activities (Suzuki et al., 1999). Thus, it seems likely that MUT-7 acts as a DNase or RNase. In the former case, one could consider a model in which the transposon becomes a substrate for MUT-7 DNase activity after it is excised from its location, which would prevent reintegration of the element. However, this scenario does not explain that also transposon excision is repressed by the MUT-7 wild-type protein. As discussed below, we currently prefer a model in which MUT-7 acts as an RNase; the actual test whether MUT-7 is indeed a 3′-5′ RNase awaits the in vitro characterization of the protein.

A Speculative Model for MUT-7 Action in Transposon Silencing and RNA Interference

The natural function of RNAi is obviously not to provide experimentalists with tools to inactivate genes. Possibly one of the natural functions is to silence transposons and viruses in the germline; the one and only thing all these elements have in common is that they are present in several copies at more or less random locations in the genome. Thus, their common Achilles heel is that, once their copy number has reached a certain level, they will be transcribed from both strands: one strand by readthrough of one copy, the other strand by other copies in the genome. In contrast, single-copy genes will usually be transcribed from one strand only (Figure 6B). Thus, all the organism would need to do to protect itself against selfish DNA is to use the presence of dsRNA as a trigger to degrade all corresponding transcripts (Figure 6A).

Alternatively, dsRNA could be generated through a mechanism that is independent of the copy number of a given element. Since many elements (including Tc1, Tc3, Tc4, and Tc5, tested in this study) contain inverted repeats at their termini, RNA from these regions can easily form dsRNA. In this way, a single copy could already be detected and trigger silencing.

Different Types of Mutator Mutants?

It has been recognized in the past that there is a genetic basis for the difference in mutator activity of the natural isolates Bergerac (mutator) and Bristol N2 (nonmutator) (Mori et al., 1988). Mapping experiments showed that the mutator locus was genetically mobile. At the time it seemed reasonable to conclude that the mutator genes

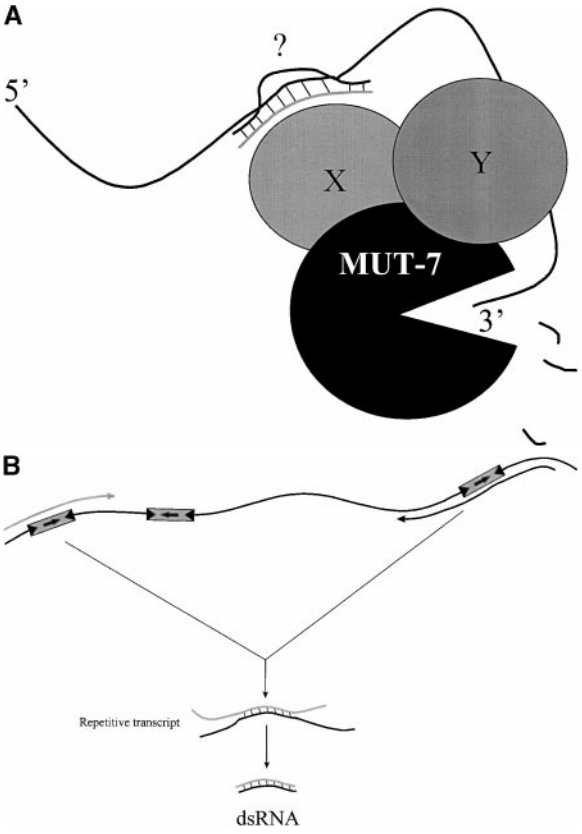

Figure 6. A Model for MUT-7 Action

(A) A speculative model showing the possible mode of action of the MUT-7 protein. A complex of proteins, indicated by X, Y, and MUT-7, binds a dsRNA molecule. The dsRNA specifically targets the complex to homologous mRNA by an as yet unknown mechanism, which needs at some level base pairing between the dsRNA and the target mRNA. This leads to the degradation of the bound mRNA by the RNase function of MUT-7. Because of the homology to RNaseD, this is expected to proceed in a 3′-5′ direction. The dsRNA could survive this process and target the nuclease complex to the next mRNA molecule.

(B) Generation of dsRNA of repetitive sequences. Because of their random distribution over the genome, repetitive sequences (gray) are likely to be transcribed from both strands (gray and black) by readthrough from external initiation sites. The resulting RNA molecules can consequently basepair and form dsRNA. On the other hand, single-copy loci will most likely be transcribed only from one strand. Thus, the presence of gray/black dsRNA indicates repetitive DNA.

(*mut-4*, *mut-5*, and *mut-6*) were themselves mobile elements, comparable to the so-called "autonomous" transposons in plants (for example, Pereira et al., 1986). In this case, these loci possibly represent copies of Tc1 that express their transposase in the germline of the nematode. If so, one would expect that these mutators are transposon specific, in this case for Tc1.

There is a second class of mutators. One was described in 1987 by Collins et al., who derived an EMS-induced mutant from the mutator strain Bergerac that further enhanced transposition (*mut-2(r459)*). In this mutant, many transposons are activated (Collins et al., 1989; Collins and Anderson, 1994). The *mut-2* gene has not yet been identified at the molecular level.

In this study, we describe a similar screen for mutator mutants as was carried out by Collins et al., except that we started with a completely transposition-deficient strain of almost complete Bristol N2 background. We also found a mutant (*mut-7(pk204)*) that activates many transposons. Thus, the *mut-7* and *mut-2* loci are EMS induced and regulate many transposons and as such may differ from *mut-4*, *mut-5*, and *mut-6*. It should be noted that it has not rigorously been tested to what extent these mutator genes are really different from the *mut-2* and *mut-7* type of mutants. We cannot currently exclude that *mut-4* (and thus *mut-5* and *mut-6*) is a transposable element, but there is an alternative interpretation. The mobility of the *mut-4* locus was inferred because after removal of the *mut-4* locus from a strain, there was a new mutator locus that mapped to a different genetic locus. It is possible that these are simple loss-of-function loci, of the *mut-7* kind, caused by insertion of a transposon activated by the original mutator (*mut-4*). This means that the mutagen is a transposon, but it does not mean that the transposon is the mutator; it may have inserted into a mutator gene and possibly inactivated it. This would also explain why the *mut-4*, *mut-5*, and *mut-6* mutator strains show the same kind of temperature-dependent sterility and Him phenotype as the *mut-7* and *mut-2* mutators. A better understanding will require the molecular identification of these loci.

Alternative Models and Other Levels of Regulation

Some RNAi-resistant mutants are mutators (Tabara et al., 1999), and some mutators are RNAi resistant (Tabara et al., 1999, and this paper). In addition, *mut-7* (mutator as well as RNAi resistant) encodes a nuclease, possibly an RNase. Finally, the link between RNAi and transposon silencing seems pretty strong, given the high proportion of mutators that are RNAi resistant (22 out of 30 in Figure 2). These data form the basis for the model presented in Figure 6A, where RNAi acts to detect transposon-encoded mRNAs and to degrade them.

However, not all data fit this model. Not all RNAi-resistant mutants are mutators, and vice versa *mut-6* is not RNAi resistant (Tabara et al., 1999), nor are some of the mutators in Figure 2. One possibility is that the model in Figure 6A is wrong, and that the loss of transposon silencing is an indirect downstream effect of the loss of RNAi (e.g., via an effect of RNAi on an unknown factor X, which is required for silencing transposons).

Such a factor could affect chromatin structure. In plants it has been shown that cosuppression involves RNA molecules (Smyth, 1997; Grant, 1999; Sharp, 1999). However, methylation of the endogenous promoter sequences is also often detected (Wassenegger et al., 1994; Jones et al., 1998). This suggests that the repetitiveness of the DNA targets methylation to specific sequences. In *C. elegans*, there is no methylation, but changes of chromatin structures at dsRNA targeted sites can certainly not be ruled out. In fact, in *Drosophila* it has been shown that cosuppression by transgenes is *polycomb* dependent (Pal-Bhadra et al., 1997). Such changes in chromatin could lead to decreased transcription or to a structure that is less accessible for the transposase protein, thereby causing transposon silencing. Perhaps such changes are responsible for some of the

pleiotropic effects observed in these mutants, such as X chromosome nondisjunction, leading to a Him phenotype.

In this context, it should be noted that strictly speaking we do not even know that the mutator effect is on transposase expression; it could also be on accessibility of the transposon DNA substrate. We do not favor this latter model for several reasons. First, it would seem unlikely that all copies of all different transposons in the Bristol strain would be in regions of the genome that are totally inaccessible to transposase. Also, when studying Tc3 transposition in somatic cells in Bristol N2, we found that simple overexpression of Tc3 transposase resulted in high levels of Tc3 jumping (van Luenen et al., 1993), showing that in that experiment transposase expression, and not transposon DNA accessibility, limited the levels of transposition.

If the model in Figure 6 is in essence correct, then it remains to be explained how some RNAi-resistant mutants could be wild type for the mutator phenotype and vice versa. The former class of mutants could affect steps of RNAi that are upstream of or in parallel to transposon silencing. The observation of Tabara et al. (1999) that the *rde-1* mutant is resistant even to RNAi effects from a transgenic dsRNA producer seems to leave little space for effects upstream of the silencing of transposase expression. Perhaps transposon silencing is more sensitive to low levels of RNAi activity than any of the other experimental readouts used for RNAi.

The other way around, mutators such as *mut-6*, and some of the alleles in Figure 2, which are wild type for RNAi sensitivity, are of particular interest. One possibility is that these are copies of Tc1 elements that express mutant versions of mRNA for Tc1 transposase which are resistant to RNAi. In this case, one would expect that each of these is specific for Tc1 and does not effect other transposons, such as Tc3, Tc4, and Tc5.

Similar Mechanisms in Fungi, Worms, Flies, and Plants?

It is too early to evaluate the generality of the models proposed above. However, it has been shown that the I element in *Drosophila* can be silenced by a cosuppression mechanism, involving an RNA intermediate (Jensen et al., 1999). In plants it has been shown that the cosuppression phenomenon proceeds via RNA molecules (Smyth, 1997; Jorgensen et al., 1998; Grant, 1999; Sharp, 1999, and references therein). Also virus resistance has been implicated in this phenomenon (Brigneti et al., 1998; Jones et al., 1998; Kasschau and Carrington, 1998; Waterhouse et al., 1998).

C. elegans has clear homologs of the RNA-dependent RNA polymerase that was recently found to be required for repeat silencing in *Neurospora* (Cogoni and Macino, 1999). Targeted inactivation of these genes in *C. elegans* and other genes involved in RNA metabolism may be used to probe these genes for their role in RNAi and in transposon silencing.

The identification of additional mutator and RNAi resistance loci will be required to elucidate the complex relationship between RNAi and transposon silencing.

Experimental Procedures

Mutagenesis

We started with Tc1 transposon insertion alleles of the genes *unc-54* and *unc-22*, derived in a Bergerac background. These two alleles (*r323::Tc1* and *st136::Tc1*, respectively) show clear phenotypes (paralysis and twitching), and reversion takes place at a frequency of 10^{-4}–10^{-5}, so that wild-type revertants can be observed in any culture. We crossed these strains multiple times with the Bristol N2 strain. After the first two outcrosses, the strains had already lost the ability to revert; they were outcrossed eight more times with Bristol N2, so that, with the exception of the *unc-54* or *unc-22* marker, the strains should be fully of the Bristol N2 genotype. These strains were mutagenized using 50 mM EMS; 7,500 F1 animals were cloned onto 10 cm plates, and their progeny inspected for the presence of multiple revertants. From positive plates, Unc animals were picked again and tested once more for the ability to revert. Forty-three mutant alleles were recovered—1 per 350 mutagenized genomes. One locus was tested further: *mut-7*. Complementation tests and sequencing identified three alleles of this gene in a subset of 30. Transgenes with the ZK1098.8 gene on cosmid ZK1098 rescue *mut-7*; we find partial rescue of RNAi resistance (data not shown) and sterility at 25°C (*mut-7(pk204)III*; pkEx1529: 28% ± 5% survival, *mut-7(pk204)III* pkEx1530: 23% ± 5% survival at 25°C, versus 1.5% ± 3% for *mut-7(pk204)III*).

Transposon Activity

Activity of the various transposons was determined by the phenotypic reversion of transposon alleles of the *unc-22* gene: *unc-22(st136::Tc1)*, *unc-22(r750::Tc3)*, *unc-22(r765::Tc4)*, and *unc-22(r644::Tc5)*.

The Southern blot was made with EcoRI digested DNA of parallel grown *mut-7(pk204)* lines, probed with a Tc3-specific probe, obtained through PCR with primers 2010 (5′CTGTAAGACGGCAA GAGA3′) and 3610 (5′TCTTGTTCTGAGCATACACG3′).

Quantitative determination of transposon activity in Figure 3A was done by scoring differences in transposon patterns between 15 strains grown in parallel. The transposon pattern of each of these 15 strains was visualized using the transposon display method as described by H. G. A. M . v. L. et al. (unpublished data). Each new fragment and each disappearence of a fragment was scored as a transposition event. The results displayed in Figure 3A represent the cumulative data for Tc1 and Tc3. Also the two transposons separately show a significant temperature-sensitive effect.

Parental Effect Experiments

Transposon Activity

Two different situations were tested. (1) *mut-7(pk204)* introduced via the male: *mut-7(pk204)+/mut-7(pk204)vab-7(e1562) III; unc-22 (e66)dpy-4(e1166)/++ IV* males were crossed with *unc-22(st136:: Tc1)/unc-22(st136::Tc1)IV* hermaphrodites. Heterozygous F1 animals were identified by scoring for Dpy and Vab phenotypes in their progeny. *unc-22(e66)/unc-22(st136)IV* animals were analyzed for reversion frequency of the *st136* allele in their progeny. This heteroallelic situation for *unc-22* provides reversion frequencies in mutator strains that are up to 100 times higher than the reversion frequency in the homoallelic *st136* situation (Plasterk, 1991). Homozygous *mut-7(pk204)vab-7(e1562)III* F2 animals (and further generations) were analyzed further. (2) *mut-7(pk204)* introduced via the hermaphrodite: *unc-22(st136)I+IV* males were crossed with homozygous *mut-7 (pk204)vab-7(e1562)III; unc-22(e66)dpy-4(e1166)IV* hermaphrodites. From there the analysis was as described in (1).

The number of revertants in the progeny of a given generation was used as a measure for the transposition activity in that particular generation.

RNAi Resistance

(1) *mut-7(pk204)* introduced via the hermaphrodite: N2 males were crossed with homozygous *mut-7(pk204)unc-47(e307)III* hermaphrodites. (2) *mut-7(pk204)* introduced via the male: *unc-32(e189)mut-7(pk204)/+mut-7(pk204)III* males were crossed with *unc-22(st136:: Tc1)/unc-22(st136::Tc1)IV* hermaphrodites.

The F1 of these crosses was subjected to RNAi using the assay described by Tabara et al. (1999). The number of F2s they produced

was used as a score for the RNAi resistance of the F1. For scoring subsequent generations, animals were obtained from a parent grown on normal food.

Acknowledgments

We thank Miriam Smits for help in mutant screens, and John Collins and the Caenorhabditis Genetics Centre for strains. We thank Craig Mello, William Kelly, and Andrew Fire for sharing unpublished information and reagents. We thank Piet Borst and Marcel Tijsterman for critical reading of the manuscript. This work was supported by a grant from the Dutch Organization for Scientific Research.

Received July 8, 1999; revised September 3, 1999.

References

Alvarado, A.S., and Newmark, P.A. (1999). Double stranded RNA specifically disrupts gene expression during planarian regeneration. Proc. Natl. Acad. Sci. USA *96*, 5049–5054.

Babity, J.M., Starr, T.V.B., and Rose, A.M. (1990). Tc1 transposition and mutator activity in a Bristol strain of *Caenorhabditis elegans*. Mol. Gen. Genet. *222*, 65–70.

Bingham, P.M., Kidwell, M.G., and Rubin, G.M. (1982). The molecular basis of P-M hybrid dysgenesis: the role of the element, a P-strain-specific transposon family. Cell *29*, 995–1004.

Brigneti, G., Voinnet, O., Li, W.X., Ji, L.H., Ding, S.W., and Baulcombe, D.C. (1998). Viral pathogenicity determinants are suppressors of transgene silencing in Nicotiana benthamiana. EMBO J. *17*, 6739–6746.

Bryan, G.J., and Hartl, D.L. (1988). Maternally inherited transposon excision in *Drosophila simulans*. Science *240*, 215–217.

Cogoni, C., and Macino, G. (1999). Gene silencing in *Neurospora crassa* requires a protein homologous to RNA-dependent RNA polymerase. Nature *399*, 166–169.

Collins, J., and Anderson, P. (1994). The Tc5 family of transposable elements in *Caenorhabditis elegans*. Genetics *137*, 771–781.

Collins, J., Saari, B., and Anderson, P. (1987). Activation of a transposable element in the germ line but not the soma of *Caenorhabditis elegans*. Nature *328*, 726–728.

Collins, J., Forbes, E., and Anderson, P. (1989). The Tc3 family of transposable elements in *Caenorhabditis elegans*. Genetics *121*, 47–55.

Colloms, S.D., van Luenen, H.G.A.M., and Plasterk, R.H.A. (1994). DNA binding activities of the *Caenorhabditis elegans* Tc3 transposase. Nucleic Acids Res. *22*, 5548–5554.

Deutscher, M.P. (1993). Promiscuous exoribonucleases of *Escherichia coli*. J. Bacteriol. *175*, 4577–4583.

Eide, D., and Anderson, P. (1985). Transposition of Tc1 in the nematode *Caenorhabditis elegans*. Proc. Natl. Acad. Sci. USA *82*, 1756–1760.

Emmons, S.W., and Yesner, L. (1984). High-frequency excision of transposable element Tc1 in the nematode *Caenorhabditis elegans* is limited to somatic cells. Cell *36*, 599–605.

Emmons, S.W., Yesner, L., Ruan, K., and Katzenberg, D. (1983). Evidence for a transposon in *Caenorhabditis elegans*. Cell *32*, 55–65.

Fire, A., Xu, S., Montgomery, M.K., Kostas, S.A., Driver, S.E., and Mello, C.C. (1998). Potent and specific genetic interference by double-stranded RNA in *Caenorhabditis elegans*. Nature *391*, 706–811.

Grant, S.R. (1999). Dissecting the mechanisms of posttranscriptional gene silencing: divide and conquer. Cell *96*, 303–306.

Jensen, S., Gassama, M., and Heidmann, T. (1999). Taming of transposable elements by homology-dependent gene silencing. Nat. Genet. *21*, 209–212.

Jones, A.L., Thomas, C.L., and Maule, A.J. (1998). *De novo* methylation and co-suppression induced by a cytoplasmitically replicating plant RNA virus. EMBO J. *17*, 6385–6393.

Jorgensen, R.A., Atkinson, R.G., Forster, R.L.S., and Lucas, W.J. (1998). An RNA-based information superhighway in plants. Science *279*, 1486–1487.

Kasschau, K.D., and Carrington, J.C. (1998). A counterdefensive strategy of plant viruses: suppression of posttranscriptional gene silencing. Cell *95*, 461–470.

Kelly, W.G., Xu, S., Montgomery, M., and Fire, A. (1997). Distinct requirements for somatic and germline expression of a generally expressed *Caenorhabditis elegans* gene. Genetics *146*, 227–238.

Kennerdell, J.R., and Carthew, R.W. (1998). Use of dsRNA-mediated genetic interference to demonstrate that frizzled and frizzled 2 interact in the wingless pathway. Cell *95*, 1017–1026.

Mian, I.S. (1997). Comparative sequence analysis of ribonucleases HII, III, PH and D. Nucleic Acids Res. *25*, 3187–3195.

Moerman, D.G., and Waterston, R.H. (1984). Spontaneous unstable *unc-22 IV* mutations in *C. elegans* var. Bergerac. Genetics *108*, 859–877.

Montgomery, M.K., Xu, S., and Fire, A. (1998). RNA as a target of double-strand RNA-mediated genetic interference in *Caenorhabditis elegans*. Proc. Natl. Acad. Sci. USA *95*, 15502–15507.

Mori, I., Moerman, D.G., and Waterston, R.H. (1988). Analysis of a mutator activity necessary for germline transposition and excision of Tc1 transposable elements in *Caenorhabditis elegans*. Genetics *120*, 397–407.

Mori, I., Moerman, D.G., and Waterston, R.H. (1990). Interstrain crosses enhance excision of Tc1 transposable elements in *Caenorhabditis elegans*. Mol. Gen. Genet. *220*, 251–255.

Ngo, H., Tschudi, C., Gull, K., and Ullu, E. (1998). Double-stranded RNA induces mRNA degradation in *Trypanosoma brucei*. Proc. Natl. Acad. Sci. USA *95*, 14687–14692.

Pal-Bhadra, M., Bhadra, U., and Birchler, J.A. (1997). Cosuppression in *Drosophila*: gene silencing of alcohol dehydrogenase by white-Adh transgenes is polycomb dependent. Cell *90*, 385–387.

Pereira, A., Cuypers, H., Gierl, A., Schwarz-Sommer, Z., and Saedler, H. (1986). Molecular analysis of the En/Spm transposable element system of Zea mays. EMBO J. *5*, 835–841.

Plasterk, R.H.A. (1991). The origin of footprints of the Tc1 transposon of *Caenorhabditis elegans*. EMBO J. *10*, 1919–1925.

Rubin, G.M., Kidwell, M.G., and Bingham, P.M. (1982). The molecular basis of P-M hybrid dysgenesis: the nature of induced mutations. Cell *29*, 987–994.

Sharp, P.A. (1999). RNAi and double-strand RNA. Genes Dev. *13*, 139–141.

Smyth, D.R. (1997). Gene silencing: cosuppression at a distance. Curr. Biol. *7*, 793–795.

Suzuki, N., Shiratori, M., Goto, M., and Furuichi, Y. (1999). Werner syndrome helicase contains a 5′→3′ exonuclease activity that digests DNA and RNA strands in DNA/DNA and RNA/DNA duplexes dependent on unwinding. Nucleic Acids Res. *27*, 2361–2368.

Tabara, H., Sarkissian, M., Kelly, W.G., Fleenor, J., Grishok, A., Timmons, L., Fire, A., and Mello, C.C. (1999). The *rde-1* gene, RNA interference, and transposon silencing in *C. elegans*. Cell *99*, this issue, 123–132.

The *C. elegans* Sequencing Consortium (1998). Genome sequence of the nematode *C. elegans*: a platform for investigating biology. Science *282*, 2012–2018.

van Luenen, H.G.A.M., Colloms, S.D., and Plasterk, R.H.A. (1993). Mobilization of quiet, endogenous Tc3 transposons of *Caenorhabditis elegans* by forced expression of Tc3 transposase. EMBO J. *12*, 2513–2520.

van Luenen, H.G.A.M., Colloms, S.D., and Plasterk, R.H.A. (1994). The mechanism of transposition of Tc3 in *Caenorhabditis elegans*. Cell *79*, 293–301.

van Pouderoyen, G., Ketting, R.F., Perrakis, A., Plasterk, R.H.A., and Sixma, T.K. (1997). Crystal structure of the specific DNA-binding domain of Tc3 transposase of *C. elegans* in complex with transposon DNA. EMBO J. *16*, 6044–6054.

Vos, J.C., and Plasterk, R.H.A. (1994). Tc1 transposase of *Caenorhabditis elegans* is an endonuclease with a bipartite DNA binding domain. EMBO J. *13*, 6125–6132.

Vos, J.C., van Luenen, H.G.A.M., and Plasterk, R.H.A. (1993). Characterization of the *Caenorhabditis elegans* Tc1 transposase in vivo and in vitro. Genes Dev. *7*, 1244–1253.

Vos, J.C., de Baere, I., and Plasterk, R.H.A. (1996). Transposase is the only nematode protein required for in vitro transposition of Tc1. Genes Dev. *10*, 755–761.

Wassenegger, M.S., Heimes, S., Riedel, L., and Sanger, H.L. (1994). RNA-directed de novo methylation of genomic sequences in plants. Cell *76*, 567–576.

Waterhouse, P.M., Graham, M.W., and Wang, M.B. (1998). Virus resistance and gene silencing in plants can be induced by simultaneous expression of sense and antisense RNA. Proc. Natl. Acad. Sci. USA *95*, 13959–13964.