RESEARCH COMMUNICATION

Transgene-mediated cosuppression in the *C. elegans* germ line

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Functional silencing of chromosomal loci can be induced by transgenes (cosuppression) or by introduction of double-stranded RNA (RNAi). Here, we demonstrate the generality of and define rules for a transgene-mediated cosuppression phenomenon in the *Caenorhabditis elegans* germ line. Functional repression is not a consequence of persistent physical association between transgenes and endogenous genes or of mutations in affected genes. The cosuppression mechanism likely involves an RNA mediator that defines its target specificity, reminiscent of RNAi. Cosuppression is strongly abrogated in *rde-2* and *mut-7* mutants, but is not blocked in an *rde-1* mutant, indicating that cosuppression and RNAi have overlapping but distinct genetic requirements.

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Functional silencing of specific genes can be deliberately induced in a wide variety of organisms, either by introduction of homologous transgenes (cosuppression or quelling) (Cogoni and Macino 1997; Ruiz et al. 1998; Vaucheret et al. 1998; Jensen et al. 1999) or by delivery of double-stranded RNA molecules homologous to the target locus [RNA interference (RNAi); Guo and Kemphues 1995; Fire et al. 1998; Kennerdell and Carthew 1998; Ngo et al. 1998; Wianny and Zernicka-Goetz 2000]. These observations are exciting because they point to the existence of unexpected biological processes. However, it remains uncertain how many distinct mechanisms may be involved.

Post-transcriptional mechanisms appear to play roles in diverse examples of silencing. For some instances of cosuppression in plants and quelling in *Neurospora*, inhibition of gene function is achieved by elimination of specific transcripts (Cogoni et al. 1996; Vaucheret et al. 1998). Studies in *Caenorhabditis elegans* and trypanosomes indicate that RNAi also involves post-transcriptional removal of targeted transcripts (Montgomery et al. 1998; Ngo et al. 1998). When primed with double-stranded RNA, *Drosophila* extracts that mimic many properties of RNAi will degrade specific RNA substrates, clearly a post-transcriptional event (Tuschl et al. 1999; Hammond et al. 2000; Zamore et al. 2000).

Evidence for additional similarities between RNAi and other post-transcriptional gene silencing (PTGS) phenomena is mounting. Both quelling in *Neurospora* and RNAi in the *C. elegans* germ line require homologs of an RNA-dependent RNA polymerase (Schiebel et al. 1998; Cogoni and Macino 1999; Smardon et al. 2000). Low molecular weight RNAs corresponding to the affected gene are induced in several instances of PTGS in plants (Hamilton and Baulcombe 1999); RNA species of similar size have now been identified in *Drosophila* extracts that have sequence-specific RNA-degradation activity in vitro (Hammond et al. 2000; Zamore et al. 2000).

In *C. elegans,* RNAi can be used to inactivate genes in either the soma or the germ line. However, cosuppression effects are evidently rare for genes required in the *C. elegans* soma. The standard method for generating transgenic nematodes results in a heritable extrachromosomal array containing several hundred copies of each injected DNA (Stinchcomb et al. 1985). For many genes with somatic functions, expression from an array recapitulates the normal expression pattern of the gene with sufficient fidelity to complement loss-of-function mutations and to serve as an expression reporter (Mello and Fire 1995). Thousands of transgenic nematode lines have been generated over the years, but only one case of a somatic cosuppression-like effect has been reported, affecting the *unc-22* gene (Fire et al. 1991).

In contrast, there have been hints that cosuppression may occur more readily in the nematode germ line. Expression from transgene arrays in the germ line has proven notoriously difficult (Mello and Fire 1995). A few investigators have also noted that the presence of a high-copy transgene array in otherwise wild-type worms in some cases mimicked the germ-line phenotype produced by a loss-of-function mutation in the corresponding gene (Jones and Schedl 1995; Gaudet et al. 1996). For the *fem-1* gene, phenocopy required a transgene with an intact promoter (Gaudet et al. 1996). However, both the generality and the implications of these observations were unclear.

Here, we demonstrate that induction of loss-of-function phenocopy by high-copy transgene arrays is not an anomaly, but rather is the reproducible outcome for many germ-line-active genes. Further, we define the basis for target gene specificity and the character of the sequence and genetic requirements for cosuppression, revealing both similarities to and distinctions from RNAi.

Results and Discussion

Transgene arrays induce loss-of-function phenocopy

We first observed induction of phenocopy by transgene arrays during our analysis of *him-14*, a gene required for meiotic crossing over (Zalevsky et al. 1999). A reporter construct designed to express an HIM-14::GFP fusion was coinjected with the *rol-6(su1006)* marker (Mello et

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al. 1991) into wild-type worms, and transgenic lines were produced (Fig. 1, DNA 1). All seven F_1 worms that transmitted the marker transgene to their progeny also exhibited a constellation of phenotypes diagnostic of a defect in meiotic recombination (Dernburg et al. 1998; Zalevsky et al. 1999). They produced many embryos, most of which died; among the few survivors, many were males. Oocyte nuclei in transgenic animals from these lines exhibited a high frequency of achiasmate chromosomes. This *him-14* loss-of-function (Him) phenocopy was reproduced with a PCR product corresponding to the entire unmodified *him-14* gene (Fig. 1, DNA 2), and thus is not specific to the GFP construct. Furthermore, phenocopy is not caused by HIM-14 overexpression, as phenocopy was readily induced with PCR products containing either a *him-14* null allele (Zalevsky et al. 1999) or severely truncated versions of the gene (Fig. 1, DNAs 3, 4, 5, and 7). Similarly, transgenes with incomplete coding regions had induced phenocopy of *unc-22* in the soma (Fire et al. 1991).

A recombination-defective phenotype could also be induced by arrays containing DNA corresponding to *spo-11* or *msh-5*, two other genes required for meiotic recombination (Dernburg et al. 1998; Kelly et al. 2000). Figure 2 shows oocyte nuclei from an affected hermaphrodite carrying a *spo-11*(+) transgene array. Wild-type oocytes contain six bivalents, pairs of homologous chromosomes attached by chiasmata as a consequence of crossover events that occurred at an earlier stage. In contrast, oocytes from animals exhibiting *spo-11* (or *him-14* or *msh-5*) phenocopy have up to 12 univalent chromosomes that lack chiasmata, indicative of a failure in crossing over.

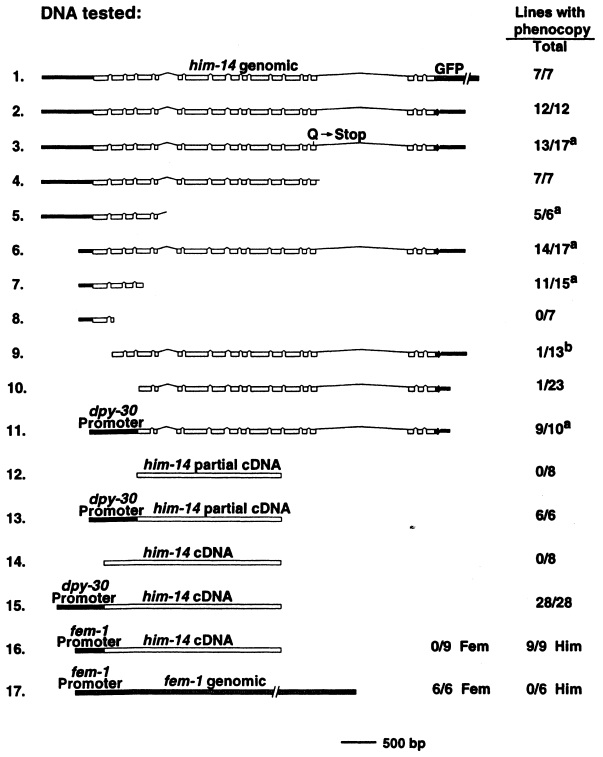

Figure 1. Induction of *him-14* phenocopy by transgene arrays. DNAs tested for their ability to elicit a Him (1–17) or Fem (16–17) phenocopy are shown (see Materials and Methods for details). For each DNA, we indicate the number of transgenic lines that exhibited phenocopy/total number of lines obtained. Except where noted[a], phenocopy was assessed both in the germ lines of the founding transgenic F_1 hermaphrodites and in the germ lines of their progeny. For DNAs that efficiently induced Him phenocopy, most lines (72%–100%) exhibited phenocopy immediately in the germ lines of the F_1; where tested, the remainder showed Him phenocopy in the F_2 generation. [a] Phenocopy was not assessed subsequent to the F_1 generation for these lines. [b] A partial Him phenocopy was observed in this line.

Evidence for involvement of an RNA mediator

Transgene-induced phenocopy is not due to the induction of mutations in the DNA sequence of the chromosomal loci, as loss of the transgene array invariably leads to restoration of the wild-type phenotype. Animals that carry arrays transmit them to some of their progeny, but they also produce progeny lacking the array because of loss during germ-line mitosis or meiosis. For some DNAs, only progeny that inherited the array exhibited phenocopy. For other DNAs, phenocopy sometimes persisted for one or more generations after loss of the array; however, in all cases, the wild-type phenotype was ultimately restored in subsequent generations. Quantitative analysis of this phenomenon (Table 1) hints that persistence of phenocopy after array loss may correlate with overall potency of a transgene in eliciting phenocopy: In the cases where persistence was not observed, not all array-bearing animals exhibited phenocopy. In the case where persistence was observed, all array-bearing animals exhibited phenocopy.

Restoration of the wild-type phenotype differentiates this transgene-induced phenocopy from phenomena such as RIP (repeat-induced point mutation) in *Neurospora*, wherein induction of meiosis in strains carrying a

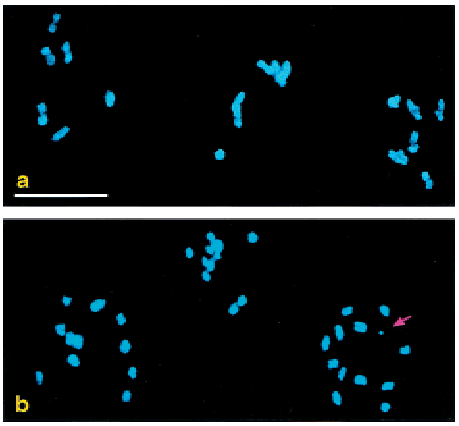

Figure 2. *spo-11* phenocopy induced by a transgene array. (*a*) Three wild-type oocyte nuclei at the end of meiotic prophase. Each contains six bivalents, representing the six pairs of homologous chromosomes linked by chiasmata. (*b*) Same stage from an animal carrying a transgene array with many copies of the *spo-11(+)* gene. Loss of function of this gene has resulted in the absence of chiasmata. In some nuclei, one or more extrachromosomal arrays (arrow) are observed in addition to the 12 univalent chromosomes. Bar, 10 μm.

Table 1. *Phenocopy and inheritance of transgene arrays*

Structure of transgene present in the array[a]	No. of individuals with Him phenocopy/total individuals scored		
	Rol	Non-Rol	Progeny from non-Rol Him
1. *him-14* genomic: C-terminal GFP	51/69	0/39	N.A.
15. *dpy-30* promoter: *him14* cDNA	29/29	11/14	17/19
16. *fem-1* promoter: *him14* cDNA	16/21	0/8	N.A.

The Rol phenotype identified animals carrying the transgene array. Rol and non-Rol progeny from array-bearing parents were plated individually and assessed for Him phenocopy. When non-Rol Him animals were observed, their hermaphrodite progeny were plated to assess persistence of phenocopy. Progeny of animals that were Him at this second generation following array loss were allowed to self-fertilize for 1–2 additional generations, and non-Him animals invariably overtook the population. N.A., Not applicable.

[a]Numbers correspond to Fig. 1. For each DNA, worms from 2–4 independent transgenic lines were tested.

transgene leads to targeted mutagenesis of both the endogene and the transgene (Selker 1990). Furthermore, examples of persistence of phenocopy after array loss suggest the possible involvement of a non-DNA mediator.

How might high-copy transgene arrays repress the function of chromosomal copies of the gene? Previous studies suggest that high-copy transgene arrays may adopt a transcriptionally silent heterochromatin-like state in the nematode germ line (Kelly et al. 1997; Kelly and Fire 1998). In *Drosophila*, physical association between a block of repetitive, heterochromatic DNA and a euchromatic gene can silence expression of the gene, a phenomenon known as *trans*-inactivation (Dernburg et al. 1996). Using FISH, we tested whether cosuppression might be a consequence of physical pairing of the *spo-11* extrachromosomal array with the chromosomal *spo-11* loci (Fig. 3). These experiments failed to detect any pairing between the transgene array and the *spo-11* chromosomal loci, either in premeiotic nuclei or at any stage of meiotic prophase in affected adult hermaphrodites. In contrast, pairing was consistently detected between the two chromosomal *spo-11* loci during meiotic prophase, demonstrating the sensitivity of the cytological assay. Significantly, a substantial fraction of meiotic nuclei did not contain arrays, despite the fact that all oocyte nuclei in the germ line of affected animals display a mutant phenotype (Figs. 2 and 3). These data indicate that physical pairing of the *spo-11* extrachromosomal array with the endogenous *spo-11* loci at the time of expression cannot be responsible for their functional repression. Furthermore, they suggest that cosuppression may involve a diffusible mediator.

We considered the possibility that a transient physical association with the array might have occurred earlier in development and could have imprinted a silent state at the chromosomal locus. However, when we examined germ-line precursor cells at other stages, we saw no evidence of such an association (Fig. 3). Together with our other results (below), these observations favor the idea that germ-line cosuppression is distinct from *trans*-inactivation and does not result from direct contact between the array and the affected chromosomal loci.

Several lines of evidence suggest that the ability to generate transcripts from the array is required either to establish or maintain functional repression of the endogenous genes. First, efficient induction of phenocopy appears to require both a promoter region and sufficient transcribed sequences. Although phenocopy was efficiently induced by a PCR product containing the entire *him-14* coding sequence plus 186 bp upstream of the *trans*-splice acceptor and initiation codon (Fig. 1, DNA 6), PCR products containing the vast majority of the coding sequence but no 5′ upstream region only very rarely (2/34 lines) produced phenocopy (Fig. 1, DNAs 9 and 10). Furthermore, a PCR product consisting of the 186-bp upstream region, the first exon, and the first intron also

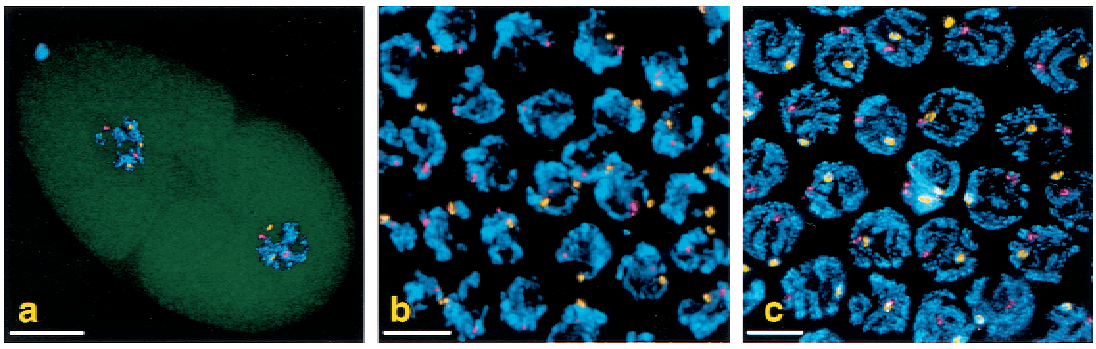

Figure 3. Absence of pairing between a transgene array and the affected chromosomal locus. The subnuclear location of extrachromosomal arrays and the affected chromosomal loci were examined using FISH. (Yellow) Hybridization to the array; (magenta) chromosome region containing the *spo-11* locus. (*a*) A two-cell embryo; a projection through the nuclei is superimposed on an image of green autofluorescence to show the embryo's outline. The condensed nucleus at the anterior pole is the polar body. The posterior P1 cell (*lower right*) gives rise to the adult germ line. In each of the two interphase nuclei, both chromosomal loci are detected, as well as either two or three arrays. (*b*, *c*) Nuclei in early meiotic prophase and mid-prophase (pachytene), respectively. In each nucleus, the two homologous chromosomal loci are paired with each other (resulting in a single focus of hybridization) but are not associated with the extrachromosomal arrays. Due to mitotic instability, some nuclei do not contain arrays (*b*), yet the loss of gene function affects all nuclei. Bar in *a*, 10 μm; in *b* and *c*, 5 μm.

failed to induce phenocopy, indicating that upstream sequences alone are not sufficient (Fig. 1, DNA 8).

Fusion of the ubiquitously expressed *dpy-30* promoter (Hsu et al. 1995) to one of the ineffective 5′-truncated versions of the *him-14* gene restored efficient induction of phenocopy (Fig. 1, DNA 11), strengthening the conclusion that the 5′-truncated gene was ineffective because it lacked a promoter. Moreover, whereas neither a partial cDNA nor a cDNA corresponding to the entire *him-14* coding region was capable of producing phenocopy, either cDNA fused to the heterologous *dpy-30* promoter elicited phenocopy very efficiently (Fig. 1, DNAs 12–15). Thus only *him-14* coding sequences are necessary to specify *him-14* phenocopy.

Experiments using a chimeric DNA in which the promoter region of the *fem-1* gene was fused to the *him-14* cDNA demonstrated further that the specificity of transgene-induced phenocopy resides in the transcript, not in the promoter region. The *fem-1* gene is required for male somatic sexual differentiation and for spermatogenesis in the germ lines of both males and hermaphrodites (Doniach and Hodgkin 1984). Loss of *fem-1* function in the germ line results in the production of only oocytes and no sperm, transforming self-fertilizing hermaphrodites into fertile but obligate-crossing females. Gaudet et al. (1996) reported that high-copy *fem-1* transgenes that were capable of rescuing male somatic sexual development in a *fem-1* mutant paradoxically feminized the germ lines of wild-type animals. As we have observed for the *him-14* phenocopy reported here, this *fem-1* germline phenocopy also required both the 5′ upstream promoter region and *fem-1* transcribed sequences.

fem-1 and *him-14* are required for distinct and independent aspects of germ line function, making it possible to score both loss-of-function phenotypes simultaneously. This allowed us to demonstrate unambiguously that the transcript sequence rather than that of the promoter region dictates the type of phenocopy observed (Fig. 1, DNAs 16 and 17). We found that the *fem-1* promoter region fused to a *him-14* full-length cDNA elicited robust *him-14* phenocopy in 9/9 transgenic lines, but did not cause a *fem-1* phenocopy. In contrast, control *fem-1* transgene arrays efficiently induced *fem-1* phenocopy but did not cause a Him phenotype.

The most economical explanation for all of these results is that transcription from the array is required either to establish or maintain functional repression of the endogenous genes. We favor this interpretation over alternative formal possibilities, such as promoters inducing alterations in surrounding chromatin to facilitate transient physical interaction between transgenes and chromosomal loci. Furthermore, we can rule out one alternative hypothesis, that phenocopy results from regulatory elements titrating specific transcription factors.

Both the apparent requirement for transgene transcription and the dependence of phenotypic specificity on transcript rather than promoter sequences are features shared with cosuppression phenomena previously described in plants (Vaucheret et al. 1998). Involvement of a diffusible mediator is also a property of both cosuppresion in plants and quelling in *Neurospora* (Cogoni et al. 1996; Palauqui et al. 1997). Moreover, the likely involvement of RNA molecules suggests that transgene-mediated cosuppression may be mechanistically related to RNAi.

Overlapping but distinct genetic requirements for transgene-mediated cosuppression and RNAi

Genetic analysis has identified at least nine *C. elegans* genes required for efficient RNAi in the soma and/or germ line (Ketting et al. 1999; Tabara et al. 1999; Smardon et al. 2000). Most fall into two classes: Those required only for dsRNAi, such as *rde-1*, and those also required to suppress germ-line transposon mobilization, including *rde-2* and *mut-7*.

Whereas mutations in *rde-1* profoundly suppress RNAi (Tabara et al. 1999), we found that an *rde-1* mutation does not prevent cosuppression. We observed strong *spo-11* phenocopy in 9/9 transgenic lines generated by introducing wild-type *spo-11* genes into *rde-1* mutant hermaphrodites. Thus, if cosuppression and RNAi do elicit gene silencing by a common mechanism, *rde-1* must function in an upstream event in RNAi that is not required for cosuppression. Grishok et al. (2000) independently concluded that *rde-1* functions at an upstream step in RNAi. They showed that *rde-1* is required for initial formation of a heritable interfering agent, but is not needed for interference thereafter.

Although *rde-1* is not required for cosuppression, *rde-2* and *mut-7* are essential (Table 2). Our findings that *mut-7* is required for cosuppression but *rde-1* is not are in agreement with results from an independent study (Ketting and Plasterk 2000). When arrays were generated in *rde-2* or *mut-7* mutants, we did not observe cosuppression in the founding transgenic animal or in subsequent generations of the resulting transgenic lines. Nevertheless, arrays generated in these mutants are capable of eliciting cosuppression. This capability was demonstrated by crossing array-bearing *rde-2* or *mut-7* hermaphrodites with wild-type males to generate +/*rde-2* or +/*mut-7* F_1 hermaphrodites carrying the array. When arrays contained *spo-11* transgenes, +/*rde-2* or +/*mut-7* array-bearing F_1s consistently exhibited a robust recombination-defective phenotype (Rec^-; Table 2). When arrays contained a truncated *him-14* transgene, only a fraction of +/*rde-2* or +/*mut-7* array-bearing F_1s exhibited a robust Rec^- phenotype; nevertheless, all produced Rec^- F_2 progeny at high frequency (Table 2).

Grishok et al. (2000) showed that *rde-2* and *mut-7* function downstream of *rde-1* in the RNAi pathway. *rde-2* and *mut-7* are dispensable for initial formation of a heritable interfering agent, but are required at a later step to achieve interference. Because *rde-2* and *mut-7* (but not *rde-1*) are also required for transposon silencing (Ketting et al. 1999; Tabara et al. 1999), they proposed a model in which transposons would use an alternative pathway independent of *rde-1* to generate a secondary interfering agent, and then achieve silencing through a common effector pathway that includes *rde-2* and *mut-7*. Our finding that cosuppression also requires *rde-2* and *mut-7* but not *rde-1* accords with this framework of a common

Table 2. rde-2 *and* mut-7 *mutants are deficient for cosuppression*

Genotype	Rec⁻ phenocopy/total	
	Plate assay (produced ≥ 94% dead eggs)	DAPI assay (achiasmate chromosomes in oocyte nuclei)
rde-2; meEx301[spo-11]	0/8	0/13
+/rde-2; meEx301[spo-11]	7/7	11/11
mut-7; meEx300[spo-11]	0/15	0/18
+/mut-7; meEx300[spo-11]	15/15	9/9
rde-2; meEx305[him-14]	0/8	0/6
+/rde-2; meEx305[him-14]	5/12 (in F_1)[a]	4/10 Rec⁻ (in F_1) 4/10 partial Rec⁻ (in F_1)
rde-2; meEx306[him-14]	0/10	0/16
+/rde-2; meEx306[him-14]	2/5 (in F_1)[b]	2/5 (in F_1)
mut-7; meEx307[him-14]	0/10	0/16
+/mut-7; meEx307[him-14]	1/6 (in F_1)[c]	1/6 (in F_1)

Transgene arrays contained either the *spo-11* gene (*meEx301, meEx300*) or a truncated *him-14* gene (DNA #4 from Fig. 1; *meEx305, 306, 307*).
[a]7/7 Rec⁺ F_1s gave Rec⁻ F_2 progeny by DAPI assay (87 Rec⁻/110 total F_2s scored = 79%).
[b]3/3 Rec⁺ F_1s gave Rec⁻ F_2 progeny by DAPI assay (23 Rec⁻/34 total F_2s scored = 68%).
[c]5/5 Rec⁺ F_2s gave Rec⁻ F_2 progeny by DAPI assay (61 Rec⁻/94 total F_2s scored = 65%).

downstream interference mechanism acting in response to a variety of initiating stimuli. However, this model leaves open the questions of the nature of the primary trigger(s) for cosuppression and transposon silencing and how these primary triggers might be converted into a secondary interfering agent. The apparent requirement for transgene transcription to elicit cosuppression suggests that RNA molecules may constitute the primary trigger, but if so, the pertinent transcripts must be aberrant in some way to be recognized as triggers for silencing.

Transgene arrays, normally silent in the germ line, show elevated expression in the germ lines of *mut-7* mutants (Tabara et al. 1999). This phenotype could be explained in part as a consequence of reduced cosuppression, which probably contributes to functional repression of transgenes in addition to silencing endogenous genes. However, we have also observed that arrays appear to be less condensed in *mut-7* and *rde-2* germ lines than in wild-type germ lines. Further, it is more difficult to generate arrays in *rde-2* and *mut-7* strains (although once generated, arrays are transmitted efficiently). These and other pleiotropic effects such as chromosome missegregation (Ketting et al. 1999; Tabara et al. 1999) are difficult to reconcile with a model in which RDE-2 and MUT-7 act solely as downstream effectors of a targeted RNA degradation process.

One possibility is that the chromatin structure of repetitive transgene arrays in the germ line normally promotes the formation of aberrant transcripts that elicit interference. In *mut-7* and *rde-2* mutants, germ line chromatin may be altered in such a way as to prevent the formation of these aberrant transcripts while also contributing to the abnormal behavior of chromosomes. In this scenario, RDE-2 and MUT-7 would function in an early step in the cosuppression process, in the production of the initial triggering molecules. This scenario does not preclude the possibility that RDE-2 and MUT-7 might also function as downstream effectors of cosuppression, as they clearly do for RNAi (Grishok et al. 2000). A role for *rde-2* and *mut-7* in regulating chromatin structure could help to explain our observation that *him-14* transgene arrays were more likely to elicit strong cosuppression in F_2 animals than in F_1 animals generated following a cross between *rde-2* or *mut-7* hermaphrodites and wild-type males (Table 2). The history of the arrays in the *rde-2* or *mut-7* background may have affected the probability of generating sufficient triggering molecules.

A tool for investigating germ-line gene function

Transgene-mediated cosuppression provides a valuable method for inhibiting gene function in the nematode germ line. We have now targeted numerous additional genes, eliciting diverse germ-line phenotypes including failure in spindle assembly (e.g., C41G7.2) and altered meiotic progression (e.g., R06C7.8).

In certain cases, cosuppression provides experimental advantages over RNAi. For some phenotypes, stable cosuppressed transgenic lines can be generated, permitting assessment of phenotype over many generations. Because the effects of cosuppression appear to be largely restricted to the germ line, the cosuppression approach can also reveal germ line-specific functions for essential genes for which mutation or RNAi would result in lethality.

Materials and methods

Generation of transgenic animals

For Fig. 1, DNAs (50 ng/µl) were coinjected with 50 ng/µl plasmid pRF4 (Mello et al. 1991) into wild-type (N2) hermaphrodites. DNA 1 contains the *him-14* coding sequence and upstream region cloned in GFP fusion vector pPD95.77. DNAs 2 and 4–10 were amplified from N2 genomic DNA. All PCR products gave single bands of expected size by gel analysis; those that failed to elicit phenocopy were further verified by restriction digestion. DNA 2 includes the entire *him-14* coding sequence plus 736 bp upstream of the *trans*-splice acceptor site that immediately precedes the initiation codon and 452 bp downstream of the stop codon (including the 3′ UTR, indicated in black); 4–10 include the indicated subsets of this sequence. DNA 3 is a PCR product amplified from *him-14(it21)* genomic DNA. DNAs 11, 13, and 15 contain 5′-truncated *him-14* genomic DNA, a partial *him-14* cDNA starting at codon 155, or a cDNA corresponding to the entire *him-14* coding sequence cloned downstream of the 683-bp *dpy-30* promoter region (Hsu et al. 1995) in expression vector pdh38ΔM, a derivative of pBluescript KS(–). DNAs 12 and 14 contain these two cDNAs cloned in pBluescript SK(–). DNA 17 is a 13-kb PCR product containing the entire *fem-1* gene. DNA 16 is a chimeric PCR product amplified from the ligation products of the insert from 14 and a genomic PCR product corresponding to the 404-bp *fem-1* promoter region (Gaudet et al. 1996). A 3-kb genomic PCR product spanning the *spo-11* locus was used for all the *spo-11* experiments. The coinjection marker was either plasmid pMM016B containing *unc-119*(+) (Maduro and Pilgrim 1995), injected into *unc-119(e2498)* worms (Figs. 2–3), or pRF4, injected into N2 or *rde-1(ne219)*. All DNAs were injectied at 50–100 ng/µl. For experiments in Table 2, *spo-11* DNA or DNA 4 from Fig. 1 were coinjected with pRF4, each at 50 ng/µl, into the gonads of *rde-2(ne221)* or *mut-7(pk204)* hermaphrodites. In all cases, F_1 worms expressing the transformation marker were plated individually, and their broods were scored for transmission of the transgene array to their progeny and for induction of phenocopy.

Cytology

Fixation, DAPI staining, and FISH were carried out as in Dernburg and Sedat (1998) and Dernburg et al. (1998). Arrays were detected with a probe

composed of the *spo-11* and *unc-119* DNAs used to generate the transgenic animals. A probe specific to the chromosomal *spo-11* locus was generated from four cosmids flanking but not including the *spo-11* gene. Probes were digested and 3′-end labeled with FITC-N4-dCTP and Cy3-dUTP, respectively, as in Dernburg and Sedat (1998). Images were acquired with a DeltaVision microscope then deconvolved and displayed with the accompanying software.

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