

Long-term gene silencing by RNAi

Small RNA molecules participate in a variety of activities in the cell: in a process known as RNA interference (RNAi), double-stranded RNA triggers the degradation of messenger RNA that has a matching sequence; the small RNA intermediates of this process can also modify gene expression in the nucleus¹. Here we show that a single episode of RNAi in the nematode *Caenorhabditis elegans* can induce transcriptional silencing effects that are inherited indefinitely in the absence of the original trigger. Our findings may prove useful in the ongoing development of RNAi to treat disease.

It has been shown that phenotypes induced in *C. elegans* by RNAi can last for two or three generations². Because the generation time of a worm is only three days, however, it is not clear whether this effect can be explained simply by a slow dilution of the silencing factors.

We have therefore investigated the heritability of gene silencing by RNAi over many generations in *C. elegans* and used an RNAi screen to identify genes that may influence this inheritance. (For details of methods, see supplementary information.)

We injected wild-type Bristol N2 worms with a double-stranded RNA that targets the *C. elegans* gene *ceh-13* for one generation. The Ceh-13 phenotype, in which the worm is small and dumpy, persisted in some animals indefinitely. Inheritance was not fully penetrant: only about 30% of the progeny of Ceh-13 worms inherited the phenotype. Wild-type siblings never had progeny with the Ceh-13 phenotype, and crossing worms that had a Ceh-13 phenotype with unaffected males showed that the trait is dominant. A single episode of RNAi can therefore induce heritable silencing that is not fully penetrant and behaves in a dominant fashion.

To show that this is a general phenomenon, we targeted 171 other genes by using a single treatment of RNAi and found 13 that could be heritably silenced (among them were *dpy-6*, *dpy-28* and *unc-73*; data not shown).

We also showed that a single transgenic copy of a gene (*gfp*) expressing green fluorescent protein (GFP) could be silenced, and the silencing inherited. We used animals expressing GFP under the control of a germline-specific promoter and created interference by feeding them bacteria that express double-stranded RNA homologous to *gfp*; progeny that did not express GFP were then transferred to new plates. In all siblings, GFP expression was reduced relative

to wild-type expression (Fig. 1). We detected animals that had reduced GFP expression over 80 generations.

Is RNAi the mechanism behind the initial silencing? There are two features of effective interference in *C. elegans*: it targets exons, not introns, and it depends on the canonical RNAi genes *rde-1* and *rde-4* (ref. 3) (see supplementary information). Tests for both show that RNAi is responsible for the effect, and this is further supported by our observation that genes are more likely to be indefinitely silenced in worms with a mutation in *eri-1* (results not shown), which are hypersensitive to RNAi (ref. 4).

But RNAi probably does not underlie the inheritance mechanism — *rde-1* and *rde-4* are dispensable. To investigate further, we used a candidate RNAi screen to identify genes that affect the maintenance of silencing and found four that abolish inheritance when knocked out: *hda-4* (a class II histone deacetylase), *K03D10.3* (a histone acetyltransferase of the MYST family), *isw-1* (a homologue of the yeast chromatin-remodelling ATPase ISW1) and *mrg-1* (a chromo-domain protein) (see supplementary information).

The fact that these genes are all involved in chromatin remodelling suggests that the inheritance of RNAi-induced phenotypes is due to silencing at the transcriptional level. It may be that this is achieved by modification of specific residues in histone tails, because culturing worms in the presence of the histone deacetylase inhibitor trichostatin A relieves silencing.

Earlier work has revealed a link between RNAi and transcriptional silencing⁵ and inheritance of silencing for one generation in mice⁶. We have shown that RNAi can induce silencing effects that, once established, are inherited indefinitely over generations of sexual reproduction, in the absence of the trigger and of RNAi machinery.

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Figure 1 | RNA interference triggers inheritable silencing of a transgene encoding green fluorescent protein (GFP). **a**, Four degrees of GFP expression in *Caenorhabditis elegans* are revealed using Nomarski optics (top panels) and ultraviolet illumination (bottom panels): ++, very bright, only observed in untreated transgenic NL3630 worms; –, +/– and +, weaker GFP signals from progeny of an RNAi-treated worm that did not express GFP. **b**, Worms were fed on bacteria expressing double-stranded RNA targeting the transgene *gfp*. Ten independent lines were quantified for 20 generations. Shown is the mean percentage of worms with the indicated amount of GFP expression in the progeny of a worm that did not express GFP.

