

The silence of the genes

Ronald HA Plasterk* and René F Ketting

About two years ago, it was recognized that introduction of double-stranded RNA (dsRNA) had a potent effect on gene expression, in particular on mRNA stability. Since then, this process has been found to occur in many different organisms, and to bear a strong resemblance to a previously recognized process in plants, called cosuppression. Both genetic and biochemical studies have started to unravel the mysteries of RNA interference: genes involved in this process are being identified and *in vitro* studies are giving the first hints of what is happening to both the dsRNA and the affected mRNA molecules after the introduction of the dsRNA.

Addresses

Hubrecht Laboratory, Uppsalalaan 8, 3584 CT Utrecht, The Netherlands

*e-mail: plasterk@niob.knaw.nl

Correspondence: Ronald HA Plasterk

Current Opinion in Genetics & Development 2000, **10**:562–567

0959-437X/00/\$ – see front matter

© 2000 Elsevier Science Ltd. All rights reserved.

Abbreviations

dsRNA double-stranded RNA

PTGS post-transcriptional gene silencing

RNAi RNA interference

Introduction

It is common to find gene orthologs between species as distant as a fungus and an animal but the realization that there is a sophisticated machinery for homology-dependent gene silencing that seems essentially common between algae, fungi, plants and animals came as a big surprise. At least four entirely independent lines of research led to this realization: transgene-dependent gene silencing in plants (or cosuppression), ‘quelling’ in fungi, RNA interference (RNAi) in diverse animals, and the silencing of transposable elements.

Activity in this field is at a peak and a review risks becoming outdated very quickly. This is particularly true when it comes to the mechanism and ‘facterology’: how does it work and which genes (and their products) are involved in which steps? In this review, we describe the phenomenology of the different cases of gene silencing, present the arguments why these cases can all be viewed as manifestations of one mechanism, and provide a provisional picture of this mechanism.

I LOVE YOU

The spring of 2000 witnessed the biggest virus attack on the Internet to date, the ‘I LOVE YOU’ virus (to which the personal computer of the first author of this paper fell victim). In the publicity around this case, the dilemma of virus-protected software on computers was clearly stated: all linked databases are bound to become infected by

viruses therefore how can a protection program anticipate attacks from viruses of which the composition or mode of replication is not yet known? (Of course, the virus definitions need to be updated regularly, but this is always after the fact.)

Genomes are linked databases. They are linked vertically by sexual transmission and horizontally by transfer via viruses and the like. The central issue for the Internet safety regime seems to have been solved by genomes: they found a way to protect themselves against apparently all existing and future viruses and transposons (although these have, of course, developed strategies to evade even that defense; see below). At this point, the reader may close his or her eyes and think ‘how could we ever defend genomes against viruses and transposons of which no sequence is known yet? What do they have in common and how could one begin to get to grips with their silencing?’

Transgene-directed gene silencing in plants

In this review, we do not aim at a historical reconstruction of the newborn field of homology-dependent gene silencing but it is clear that in plants the first observations interpreted as such were those of the Matzke [1], Mol [2] and Jorgensen [3] groups. The latter two groups aimed to raise gene expression levels in *Petunia* by introduction of multiple transgenic copies of a gene and, to their surprise, found gene silencing not only of the transgenic but also of the endogenous gene copy. This was named cosuppression. This area has been reviewed elsewhere [4], but in the context of this review some observations are relevant. One can distinguish pre- and post-transcriptional gene silencing (PTGS). The latter fits best within the universal picture of RNA-mediated gene silencing described below. It should be noted here, however, that it is undisputed that in several test systems there is a clear one-to-one relation between the silencing of a gene and its DNA methylation, where run-on experiments show that the gene silencing occurs via reduction of mRNA synthesis [5,6]. In addition, Polycomb-group genes have been implicated in some cases of cosuppression, indicating transcriptional silencing [7,8]. This is not to say that this silencing may not have been triggered by the same mechanism that acts in PTGS but if it was then there is an additional way the silencing is fed back into the nucleus where the effect is also established and maintained at the DNA level. Experiments that show RNA-directed DNA-methylation in both transcriptional gene silencing and PTGS support this idea [6,9,10].

Too much is known about cosuppression in plants to be summarized in a few lines. For the uninitiated, it is relevant to note that the silencing effect can move within the plant: a silenced transgenic graft on a non-silenced transgenic plant can trigger silencing elsewhere in that

Table 1

Gene products involved in PTGS/RNAi.

	Plants	Fungi	Animals	References
RdRP	SGS2/SDE1	QDE1	EGO-1	[18**,23**,45,53**]
eIF2C	AGO1	QDE2	RDE-1	(a) [40**,44**]
RNaseD	?	?	MUT-7	[39**]
RNA helicase	MUT6	?	?	(b)
Coiled-coil	SGS3	?	?	[18**]

A summary of the proteins that are known to have a role in either PTGS or RNAi. The fact that some genes have clear homologs with similar functions in different organisms strongly supports the idea that

the mechanism of PTGS has been conserved through evolution.

(a) J-B Morel, personal communication. (b) H Cerutti, personal communication.

organism [11]. In addition, locally induced PTGS is capable of spreading through the entire plant [12]. Interesting to note also is the phenomenon of spreading within a transgene: one can silence a gene by introduction of a transgene that corresponds to a part of the transcript only. The silencing effect, however, spreads to the remainder of the transgene, and elegant gene-swap experiments have shown that a transgene, that shows no homology to the suppressing transgene but does have homology to another region in the suppressed gene, is also silenced [12].

Function of PTGS

The natural function of PTGS is most likely in providing resistance to virus infection. It has been found that many viruses are potent inducers of PTGS and viruses encode factors that inhibit this response of the plant [13–15]. In addition, the finding that hosts may have acquired inhibitors of viral PTGS inhibitors suggests an ongoing arms race [16]. In this light, the systemic nature of PTGS becomes even more interesting, as it is an effective way of limiting virus infection to the place of the original virus attack [17]. It will be very interesting to learn how viral proteins can inhibit PTGS, as this will lead to the identification of critical steps in the PTGS mechanism.

Just recently, the implication of PTGS as an anti-virus response was further strengthened by a report from Mourrain *et al.* [18**], who report that *Arabidopsis* mutants impaired in PTGS, in addition have a defective virus response. Furthermore, cosuppression in animals has been linked with the silencing of transposable elements [19,20**,21**].

Quelling in *Neurospora* and other fungi

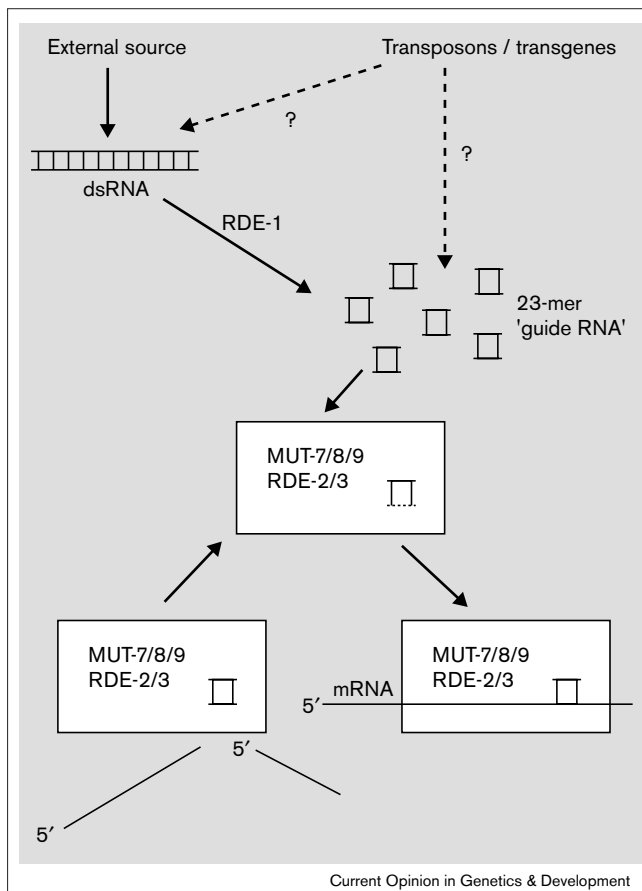
A phenomenon essentially similar to cosuppression in plants was observed in fungi [22]. This led to the first identification of a gene whose wild-type function is required for the effect [23**]. An especially elegant experiment has been performed in *Phytophthora* [24*]. Experiments show internuclear transfer of the silencing signal, which clearly demonstrates an RNA intermediate. Advantage was taken of the ability to create heterokaryons: cells with two distinct

nuclei. It was shown that introduction of a transgenic silenced nucleus into a cell with a normal nucleus resulted in silencing of the endogenous gene in the latter too. When the transgenic nucleus was then removed, the effect persisted in the non-transgenic nucleus. If this nucleus was then paired with a third nucleus, also non-transgenic, then the silencing spread from the silenced nucleus to the other one. These, together with the systemic spreading in plants mentioned earlier, are to date perhaps the strongest arguments that silencing information can be replicated (see below).

RNA interference in *C. elegans* and other organisms

RNAi was originally discovered in the nematode *Caenorhabditis elegans* [25**] and then also demonstrated in *Drosophila* [26], *Trypanosoma* [27], plants [28,29] and vertebrates such as the mouse [30**] and the zebrafish [31,32]. Note that the reproducibility of the effect in the zebrafish has not yet been achieved (R Ho, personal communication). The phenomenon of RNAi involves the silencing of gene expression in response to exogenous double-stranded RNA (dsRNA). In *C. elegans*, the RNA can be injected into the gonad, after which F1 progeny animals show silencing of the interfered gene but can also be introduced by injection into other parts of the body [25**], by soaking of the animals in dsRNA [33], by transcription from a transgene [34], or, most spectacularly, by feeding the animals on *Escherichia coli* that produces both sense and anti-sense strands of a particular gene [35]. Apparently, the dsRNA is somehow able to migrate to the gonad, where it can be passed on the F1 generation. Some properties of RNAi are relevant in this context. First, in general, the dsRNA needs to be directed against an exon, not an intron; one exception to this has been published for an operon [36]. Second, homology is required (threshold ~85% in dsRNA of 200 base pairs: A Fire, personal communication). Third, the targeted mRNA is lost (degraded) after RNAi. Fourth, the effect is non-stoichiometric, so small amounts of dsRNA can wipe out an excess of mRNA (pointing to an enzymatic mechanism). Fifth, ssRNA (sense or antisense) does not work nearly as well as dsRNA.

Figure 1



Possible mechanism for homology dependent gene silencing. The system can be fed by dsRNA or by other molecules, presumably RNA molecules, originating from transposons or transgenes. These molecules are converted to 23–25-nucleotide-long RNA molecules: 'guide RNA'. The RDE-1 protein has been implicated in the step from dsRNA to guide RNA. These guide RNA molecules are within a proteinaceous complex, containing the MUT-7, 8 and 9, and RDE-2 and 3 proteins. It is not known whether the guide RNA within the complex is either single- or double-stranded. This complex is then targeted to homologous RNA molecules. When a target RNA has been bound, it is degraded, possibly by two endonucleolytic breaks. This would result in a cyclic enzymatic reaction that could degrade a large mRNA pool.

Transposon silencing in *C. elegans*

It has been known for almost twenty years that, although all known natural isolates contain multiple copies of transposons Tc1 and Tc3, their jumping is silenced in the germline of some but not all isolates [37,38]. The most studied (and sequenced!) laboratory strain Bristol N2 shows activity of Tc1 in somatic cells but total silencing in the germline. A screen has been performed to isolate mutant derivatives of Bristol N2 that had lost Tc1 silencing in the germline — and thus had gained transposon jumping, excision and insertion [39••]. Surprisingly, in these mutants not only Tc1 but also other unrelated transposons such as Tc3, Tc4 (etc.) had become active. A large subset of these mutants were found to be defective in RNAi. In

addition, transposon-activating mutations were identified in a screen aimed at the isolation of RNAi-defective mutants [40••]. These data show that the two phenomena are mechanistically related.

Cosuppression versus RNAi

It was shown recently that transgene cosuppression works in *C. elegans* as it does in plants [21••]. Transcriptional activity of the transgene is required, indicating that, as shown in plants, the cosuppression proceeds via an RNA intermediate. Experiments in *Petunia* have shown that inverted repeat transgenes are especially prone to cosuppression, strongly suggesting that dsRNA is involved [41]. Testing various *C. elegans* mutant backgrounds, it was found that the ability to perform cosuppression depends to a large extent on the same genes as does RNAi and transposon silencing [21••], suggesting that transgenes feed into the same molecular machinery as dsRNA and transposons do.

The genetics of homology-dependent gene silencing

The first PTGS mutants were isolated in *Neurospora* [23••,42,43,44••] but now mutants are known in *C. elegans* [39••,40••,45], *Drosophila*, *Arabidopsis* [46] and *Chlamydomonas* (H Cerutti, personal communication). Some of the mutated genes have been identified; these are shown in Table 1. Note that the same genes — or at least clear homologs — were found in different organisms, which is one argument that the same mechanism is responsible for all these effects. On the basis of these gene identities, it is not yet possible to derive consistent models for PTGS but obviously the identification of more genes, combined with the biochemistry, will be of great value.

The biochemistry of silencing: 'guide RNA'

As noted, a first indication that the silencing phenomena are related mechanistically comes from genetic studies. A second comes from the biochemistry. The Baulcombe group [47••] have shown that in plants in which a gene is cosuppressed, small RNA molecules of ~21–25 nucleotides can be observed. Both strands are seen, and it is possible (but not certain) that the RNA is double-stranded. Subsequently, the same-size small RNA was seen in *Drosophila* tissue-culture cells in which cosuppression was active [48••]. This small RNA species was then found to co-purify with a complex of ~500 kD (G Hannon, personal communication) that could carry out degradation *in vitro* of a newly added mRNA that was homologous to the small RNA (henceforth referred to as 'guide RNA').

In another series of experiments, the biochemistry was taken a step further in that cell-free extract was incubated with dsRNA and then it could be shown that this resulted in an activity to degrade newly added RNA homologous to the dsRNA trigger [49••]. The extract degrades the trigger RNA into pieces that are also ± 23 nt in length which, in turn, most likely directs mRNA breakdown [50••]. Strikingly, the mRNA is cleaved at 23 nucleotide intervals,

suggesting that the generation of the guide RNA from the dsRNA is not a random process but involves some sequence specificity. The appearance of guide RNA of the same size in *Drosophila* cells and extracts as in cosuppressed plants is another indicator that the core of the silencing machinery is the same across phyla.

The RNA degradation step

It is too early to propose a precise model for the mechanism of RNA-directed gene silencing. On the basis of experiments mentioned above (and others) one can propose a model such as shown in Figure 1. The dsRNA in RNAi is degraded to small guide RNA segments. From the results obtained by Mello and co-workers [51**], this step may well involve the RDE-1 protein. The guide RNA is subsequently bound by a protein core, presumably containing MUT-7 and RDE-2 [51**]. It is likely that the gene products defined by *rde-3* [40**], *mut-8* and *mut-9* [21**] will also be involved at this stage. This complex then finds a homologous target mRNA, and degrades it.

Note that in this stage it is not certain if the guide RNA is double-stranded, and it is also not certain what happens to the sense strand. In Figure 1 it is shown as a dashed line in the complex; it is possible that the antisense strand base pairs to the target mRNA and that after degradation, the mRNA-derived sense strand remains (so that the dashed RNA species is actually not there anymore). Note that the breakdown here is indicated as endonucleolytic, although no direct evidence exists that supports these events (see also review by Bass [52]).

Additional mechanisms

Replication

The spreading effect (see above) and the possible replication that may occur on the basis of the involvement of RNA-dependent RNA polymerases in *Neurospora*, for instance, point to a replicative step in this mechanism. It may be that RdRP in some cases replicates either the trigger RNA or the guide RNA. A recent report by Baulcombe and co-workers [53**] shows that the amount of guide RNA indeed decreases in a RdRP mutant plant, implying a replicative step upstream of the guide RNA formation. However, the spreading effect also suggests that the target RNA can be a template for replication, which would be downstream of the guide RNA formation.

Transport

One of the mysteries in silencing in plants as well as in RNAi in *C. elegans* is that the genetic information required for silencing (not DNA, so presumably RNA) can migrate such a distance — for instance, in *C. elegans*, the dsRNA can be taken up in the gut and apparently can migrate from there to the germline where it presumably acts. It is likely that this transport is not as naked RNA but nothing is known yet from genetics or biochemistry on the mode of transport of the silencing information either in plants or in animals.

Conclusions: a mystery unfolding

This field is still in its romantic phase, but not for much longer. The biochemists are working quietly but steadily in this field and within a year, perhaps two, the biochemistry of the core reaction will be solved. Then we will understand how the guide RNA can find its target and ear-mark it for degradation. Questions will remain. Is the silencing effect replicated, and how? How does the trigger RNA migrate in plants and in animals? How, if at all, does the mechanism feed back into the nucleus to silence gene transcription, or (alternatively) is transcriptional gene silencing an entirely different phenomenon from PTGS? Add also the questions that are asked after every seminar: does this work in human cells? Does it work in stages later than the early embryo? Could it be applied in gene-therapy settings? It is not often that one encounters such an ancient and apparently well-conserved mechanism as the one discussed here. Apparently genomes knew all along what Microsoft is learning now: protection of important data from invading viruses is crucial and it cannot depend on the precise definition of a virus sequence. We do not yet know the details of the silencing mechanism, especially not of the way transposons feed into it. It seems that the copy number of the transposons is important: anything above a certain copy number is bad news. We have proposed [39**] that the dsRNA may be a 'red alert' for multicopy RNA. As soon as multiple copies are found in the genome, there is bound to be transcription of one strand here and of another strand there, allowing formation of dsRNA. Thus, an answer to the riddle posed above, how to spot a new virus, might be that it allows formation of dsRNA of its own sequence. Of course, there are mutants of *C. elegans* that are resistant to exogenous dsRNA but are not permissive for transposition. From this it has been concluded that transposon silencing does not involve dsRNA [54] but in our view this is a *non sequitur*. Admittedly, however, it is not yet clear what the trigger is that silences transposons; genetics as well as biochemistry will need to be performed to resolve this.

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- of special interest
 - of outstanding interest
1. Matzke MA, Primig M, Trnovsky J, Matzke AJM: **Reversible methylation and inactivation of marker genes in sequentially transformed tobacco plants.** *EMBO J* 1989, 8:643-649.
 2. van der Krol AR, Mur LA, Beld M, Mol JN, Stuitje AR: **Flavonoid genes in *Petunia*: addition of a limited number of gene copies may lead to a suppression of gene expression.** *Plant Cell* 1990, 2:291-299.
 3. Napoli C, Lemieux C, Jorgensen RA: **Introduction of a chimeric chalcone synthase gene into *Petunia* results in reversible co-suppression of homologous genes in trans.** *Plant Cell* 1990, 2:279-289.
 4. Kooter JM, Matzke MA, Meyer P: **Listening to the silent genes: transgene silencing, gene regulation and pathogen control.** *Trends Plant Sci* 1999, 4:340-347.
 5. Stam M, Viterbo A, Mol JN, Kooter JM: **Position-dependent methylation and transcriptional silencing of transgenes in inverted T-DNA repeats: implications for posttranscriptional silencing of homologous host genes in plants.** *Mol Cell Biol* 1998, 18:6165-6177.

6. Mette MF, van der Winden J, Matzke MA, Matzke AJ: **Production of aberrant promoter transcripts contributes to methylation and silencing of unlinked homologous promoters in trans.** *EMBO J* 1999, **18**:241-248.
7. Pal-Bhadra M, Bhadra U, Birchler JA: **Cosuppression in *Drosophila*: gene silencing of Alcohol dehydrogenase by white-Adh transgenes is Polycomb dependent.** *Cell* 1997, **90**:479-490.
8. Bingham PM: **Cosuppression comes to the animals.** *Cell* 1997, **90**:385-387.
9. Wassenegger M, Heimes S, Riedel L, Sanger HL: **RNA-directed de novo methylation of genomic sequences in plants.** *Cell* 1994, **76**:567-576.
10. Jones L, Hamilton AJ, Voinnet O, Thomas CL, Maule AJ, Baulcombe DC: **RNA-DNA Interactions and DNA methylation in post-transcriptional gene silencing.** *Plant Cell* 1999, **11**:2291-2301.
11. Palauqui JC, Elmayan T, Pollien JM, Vaucheret H: **Systemic acquired silencing: transgene-specific post-transcriptional silencing is transmitted by grafting from silenced stocks to non-silenced scions.** *EMBO J* 1997, **16**:4738-4745.
12. Voinnet O, Vain P, Angell S, Baulcombe DC: **Systemic spread of sequence-specific transgene RNA degradation in plants is initiated by localized introduction of ectopic promoterless DNA.** *Cell* 1998, **95**:177-187.
13. Anandakshmi R, Pruss GJ, Ge X, Marathe R, Mallory AC, Smith TH, Vance VB: **A viral suppressor of gene silencing in plants.** *Proc Natl Acad Sci USA* 1998, **95**:13079-13084.
14. Jones AL, Thomas CL, Maule AJ: **De novo methylation and co-suppression induced by a cytoplasmically replicating plant RNA virus.** *EMBO J* 1998, **17**:6385-6393.
15. Voinnet O, Pinto YM, Baulcombe DC: **Suppression of gene silencing: a general strategy used by diverse DNA and RNA viruses of plants.** *Proc Natl Acad Sci USA* 1999, **96**:14147-14152.
16. Li HW, Lucy AP, Guo HS, Li WX, Ji LH, Wong SM, Ding SW: **Strong host resistance targeted against a viral suppressor of the plant gene silencing defence mechanism.** *EMBO J* 1999, **18**:2683-2691.
17. Voinnet O, Baulcombe DC: **Systemic signalling in gene silencing.** *Nature* 1997, **389**:553.
18. Mourrain P, Béclin C, Elmayan T, Feuerbach F, Godon C, Morel J-B, Jouette D, Lacombe A-M, Nikic S, Picault N *et al.*: ***Arabidopsis* SGS2 and SGS3 genes are required for posttranscriptional gene silencing and natural virus resistance.** *Cell* 2000, **101**:533-542.
- This paper describes the identification of the first two *Arabidopsis* genes involved in PTGS. One of the two, SGS2, encodes an RdRP, linking PTGS to quelling and RNAi. In addition, the genes are found to be involved in virus resistance. Interestingly, not all RNA viruses are affected by the SGS2 and SGS3 gene products.
19. Jensen S, Gassama MP, Heidmann T: **Cosuppression of I transposon activity in *Drosophila* by I-containing sense and antisense transgenes.** *Genetics* 1999, **153**:1767-1774.
20. Jensen S, Gassama MP, Heidmann T: **Taming of transposable elements by homology-dependent gene silencing.** *Nat Genet* 1999, **21**:209-212.
- This report shows that a phenomenon strongly resembling cosuppression is involved in the silencing of a retro-element: the I-element of *Drosophila*. The authors show that transcription rather than translation is required for the effect.
21. Ketting RF, Plasterk RH: **A genetic link between co-suppression and RNA interference in *C. elegans*.** *Nature* 2000, **404**:296-298.
- Several mutants are analyzed for their ability to support cosuppression in *C. elegans*. It is found that mutants having a defect in both RNAi and transposon silencing have defects in cosuppression, linking these three pathways together.
22. Cogoni C, Irelan JT, Schumacher M, Schmidhauser TJ, Selker EU, Macino G: **Transgene silencing of the al-1 gene in vegetative cells of *Neurospora* is mediated by a cytoplasmic effector and does not depend on DNA-DNA interactions or DNA methylation.** *EMBO J* 1996, **15**:3153-3163.
23. Cogoni C, Macino G: **Gene silencing in *Neurospora crassa* requires a protein homologous to RNA-dependent RNA polymerase.** *Nature* 1999, **399**:166-169.
- The first identification of a gene involved in PTGS in *Neurospora*. The gene is found to encode a protein homologous to tomato RdRP, suggesting that replication of RNA intermediates is involved in PTGS.
24. van West P, Kamoun S, van't Klooster JW, Govers F: **Internuclear gene silencing in *Phytophthora infestans*.** *Mol Cell* 1999, **3**:339-348.
- Demonstration of a cosuppression effect in *Phytophthora*. Elegant experiments show that the silencing signal can diffuse freely between nuclei and most likely is replicated.
25. Fire A, Xu S, Montgomery MK, Kostas SA, Driver SE, Mello CC: **Potent and specific genetic interference by double-stranded RNA in *Caenorhabditis elegans*.** *Nature* 1998, **391**:806-811.
- First report on the potent effects of dsRNA in *C. elegans*. It is demonstrated that the effect is sequence specific, acts on exon sequences only, and is most likely catalytic.
26. Kennerdell JR, Carthew RW: **Use of dsRNA-mediated genetic interference to demonstrate that frizzled and frizzled 2 act in the wingless pathway.** *Cell* 1998, **95**:1017-1026.
27. Ngo H, Tschudi C, Gull K, Ullu E: **Double-stranded RNA induces mRNA degradation in *Trypanosoma brucei*.** *Proc Natl Acad Sci USA* 1998, **95**:14687-14692.
28. Waterhouse PM, Graham MW, Wang MB: **Virus resistance and gene silencing in plants can be induced by simultaneous expression of sense and antisense RNA.** *Proc Natl Acad Sci USA* 1998, **95**:13959-13964.
29. Chuang CF, Meyerowitz EM: **Specific and heritable genetic interference by double-stranded RNA in *Arabidopsis thaliana*.** *Proc Natl Acad Sci USA* 2000, **97**:4985-4990.
30. Wianny F, Zernicka-Goetz M: **Specific interference with gene function by double-stranded RNA in early mouse development.** *Nat Cell Biol* 2000, **2**:70-75.
- First report on RNAi effects in the mouse. dsRNA directed against several genes shows clear interference effects. The effect is posttranscriptional and sequence-specific.
31. Li Y, Farrel MJ, Liu, Mohanty N, Kirby ML: **Double-stranded RNA injection produces null phenotypes in zebrafish.** *Dev Biol* 2000, **217**:394-405.
32. Wargelius A, Ellingsen S, Fjose A: **Double-stranded RNA induces specific developmental defects in zebrafish embryos.** *Biochem Biophys Res Commun* 1999, **263**:156-161.
33. Tabara H, Grishok A, Mello CC: **RNAi in *C. elegans*: soaking in the genome sequence.** *Science* 1998, **282**:430-431.
34. Tavernarakis N, Wang SL, Dorovkov M, Ryazanov A, Driscoll M: **Heritable and inducible genetic interference by double-stranded RNA encoded by transgenes.** *Nat Genet* 2000, **24**:180-183.
35. Timmons L, Fire A: **Specific interference by ingested dsRNA.** *Nature* 1998, **395**:854.
36. Boshier JM, Dufourcq P, Sookhareea S, Labouesse M: **RNA interference can target pre-mRNA: consequences for gene expression in a *Caenorhabditis elegans* operon.** *Genetics* 1999, **153**:1245-1256.
37. Emmons SW, Yesner L: **High-frequency excision of transposable element Tc 1 in the nematode *Caenorhabditis elegans* is limited to somatic cells.** *Cell* 1984, **36**:599-605.
38. Moerman DG, Waterston RH: **Spontaneous unstable unc-22 IV mutations in *C. elegans* var. Bergerac.** *Genetics* 1984, **108**:859-877.
39. Ketting RF, Haverkamp TH, van Luenen HG, Plasterk RH: **Mut-7 of *C. elegans*, required for transposon silencing and RNA interference, is a homolog of Werner syndrome helicase and RNaseD.** *Cell* 1999, **99**:133-141.
- In this report, a screen is described for the isolation of mutants with a defect in transposon silencing. A subset of the mutants is RNAi resistant, linking RNAi to transposon silencing. One identified gene, *mut-7*, is homologous to RNaseD.
40. Tabara H, Sarkissian M, Kelly WG, Fleenor J, Grishok A, Timmons L, Fire A, Mello CC: **The *rde-1* gene, RNA interference, and transposon silencing in *C. elegans*.** *Cell* 1999, **99**:123-132.
- This report describes the isolation of mutants defective in RNAi. A subset of the mutants also has a defect in transposon silencing, linking the two phenomena. One gene, *rde-1*, is identified and found to encode a factor with homology to eIF2C.
41. Stam M, de Bruin R, van Blokland R, van der Hoorn RA, Mol JN, Kooter JM: **Distinct features of post-transcriptional gene silencing by antisense transgenes in single copy and inverted T-DNA repeat loci.** *Plant J* 2000, **21**:27-42.
42. Cogoni C, Macino G: **Isolation of quelling-defective (*qde*) mutants impaired in posttranscriptional transgene-induced gene silencing in *Neurospora crassa*.** *Proc Natl Acad Sci USA* 1997, **94**:10233-10238.

43. Cogoni C, Macino G: **Posttranscriptional gene silencing in *Neurospora* by a RecQ DNA helicase.** *Science* 1999, **286**:2342-2344. Identification of a DNA helicase involved in quelling. A model is proposed in which this helicase would be required for the production of aberrant RNA molecules from transgenes.
44. Catalanotto C, Azzalin G, Macino G, Cogoni C: **Gene silencing in worms and fungi.** *Nature* 2000, **404**:245. Characterization of an RDE-1 homologue in *Neurospora* that is involved in quelling. This gene was found to be mutated in the *qde-2* mutant strain that was isolated earlier by the same lab. This finding strengthens the link between RNAi and quelling.
45. Smardon A, Spoerke JM, Stacey SC, Klein ME, Mackin N, Maine EM: **EGO-1 is related to RNA-directed RNA polymerase and functions in germ-line development and RNA interference in *C. elegans*.** *Curr Biol* 2000, **10**:169-178.
46. Elmayan T, Balzergue S, Beon F, Bourdon V, Daubremet J, Guenet Y, Mourrain P, Palauqui JC, Vernhettes S, Vialle T *et al.*: ***Arabidopsis* mutants impaired in cosuppression.** *Plant Cell* 1998, **10**:1747-1758.
47. Hamilton AJ, Baulcombe DC: **A species of small antisense RNA in posttranscriptional gene silencing in plants.** *Science* 1999, **286**:950-952. First identification of a species of small (anti)-sense RNA molecules as being involved in cosuppression. It is proposed that these molecules could be responsible for the systemic effects of cosuppression.
48. Hammond SM, Bernstein E, Beach D, Hannon GJ: **An RNA-directed nuclease mediates post-transcriptional gene silencing in *Drosophila* cells.** *Nature* 2000, **404**:293-296. Characterization of a ribonuclease from *Drosophila* cells. The nuclease is bound to 25-mer RNA molecules, that direct this nuclease to homologous mRNA molecules.
49. Tuschl T, Zamore PD, Lehmann R, Bartel DP, Sharp PA: **Targeted mRNA degradation by double-stranded RNA *in vitro*.** *Genes Dev* 1999, **13**:3191-3197. First report that describes an *in vitro* system for RNAi, using *Drosophila* extracts. The authors detect partial but sequence-specific RNA degradation in response to dsRNA.
50. Zamore PD, Tuschl T, Sharp PA, Bartel DP: **RNAi: double-stranded RNA directs the ATP-dependent cleavage of mRNA at 21 to 23 nucleotide intervals.** *Cell* 2000, **101**:25-33. This report shows that the small RNA molecules found in RNAi and cosuppression are directly derived from dsRNA through cleavage of the dsRNA. These small RNA molecules subsequently give rise to a characteristic breakdown of homologous mRNA molecules.
51. Grishok A, Tabara H, Mello CC: **Genetic requirements for inheritance of RNAi in *C. elegans*.** *Science* 2000, **287**:2494-2497. The authors conducted elegant genetic experiments designed to classify a number of genes into different steps of the RNAi reaction. They propose that RDE-1 is involved upstream of the generation of small (guide) RNA molecules and that MUT-7 and RDE-2 are downstream.
52. Bass BL: **Double-stranded RNA as a template for gene silencing.** *Cell* 2000, **101**:235-238.
53. Dalmay T, Hamilton A, Rudd S, Angell S, Baulcombe DC: **An RNA dependent RNA polymerase gene in *Arabidopsis* is required for posttranscriptional gene silencing mediated by a transgene but not by a virus.** *Cell* 2000, **101**:543-553. Isolation of a RdRP gene from *Arabidopsis*. This gene is involved in PTGS and the authors show that, in a mutant background, the level of guide RNA molecules is six-fold reduced, implying an RdRP involvement in the generation of these guide RNA molecules.
54. Sharp PA, Zamore PD: **RNA interference.** *Science* 2000, **287**:2431-2433.