

# RNA interference: Silencing in the cytoplasm and nucleus

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*Although the discovery that double-stranded RNA is able to silence gene expression was only made five years ago, methods for experimentally silencing genes have already been extended into a broad diversity of organisms, including human cells. RNA interference has also been discovered to function in physiological gene silencing. RNA interference works by causing degradation of targeted mRNAs in the cytoplasm. However, recent results suggest that RNA interference may also silence gene activity in the nucleus by remodeling chromatin and repressing the transcription of targeted genes.*

**Keywords** Chromatin, double-stranded RNA, gene silencing, mRNA, RNA interference

## Introduction

RNA interference (RNAi) may be one of the oldest host defense responses known to date [1,2]. RNAi and similar phenomena in other organisms are believed to be parts of an ancient pathway to both combat foreign nucleic acids and prevent the expression of aberrant RNAs [3,4]. Introduction of double-stranded RNA (dsRNA) has been found to result in the degradation of targeted mRNAs in the cytoplasm [5•]. This response effectively silences gene activity through the depletion of mRNAs that are necessary for producing protein. The exact mechanism underlying RNAi remains unclear, however, some details have emerged over the past few years. This review will summarize what is currently known about the mechanism of RNAi and will highlight recent evidence that it may also function in the nucleus to silence transcription.

## Discovery of RNAi

Early evidence that RNA could elicit gene silencing in animal cells came from work by Guo and Kemphues, who used antisense RNA to reduce gene expression in the nematode *Caenorhabditis elegans* (*C. elegans*) and were surprised to find that sense RNA was equally effective [6]. Subsequently, Fire *et al* tested whether a very small amount of dsRNA may be the effective agent in these experiments [5•]. A mixture of both sense and antisense RNA, resulting in dsRNA, gave a vastly more potent effect than either strand alone; so potent that even substoichiometric amounts of dsRNA elicited a penetrant effect. Subsequently, a number of previously known DNA sequence identity-dependent silencing phenomena have been found to share RNAi-like mechanisms, including co-suppression in plants and animals, and

quelling in fungi [7]. RNAi has since become widely used to suppress the function of specific genes. In the age of genomics, RNAi has proved a valuable tool that enables researchers to study a number of important processes such as cell death, development and cancer [1].

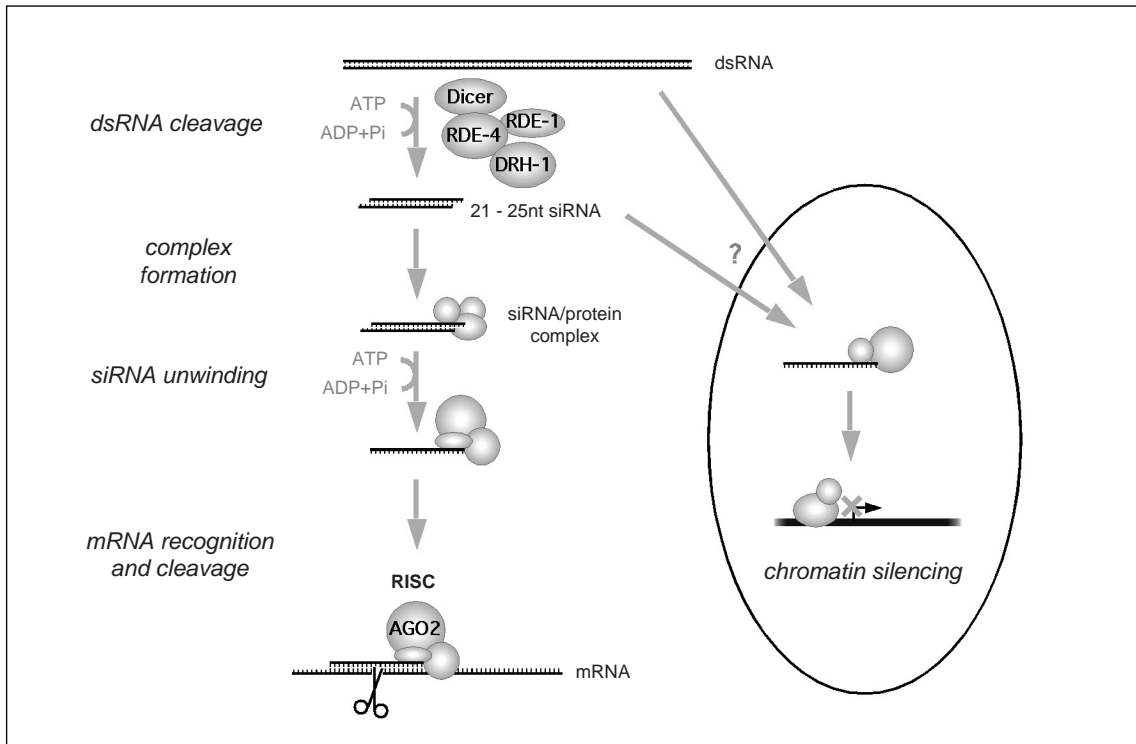
## RNAi in the cytoplasm

Recent genetic and biochemical research in several systems has greatly improved our understanding of how RNAi works (Figure 1). A dsRNA-binding protein recognizes introduced dsRNAs that are typically several hundred nucleotide pairs long [8]. This dsRNA-binding protein associates with Dicer, an RNaseIII-related enzyme, that dices the introduced dsRNA into small duplexes (21 to 25 nucleotides long) [8,9••,10••,11•]. These small duplexes, called small interfering RNAs (siRNAs), then act as guides in association with a large protein complex to target transcripts for degradation. The siRNA/protein complex is termed an RNA-induced silencing complex (RISC) [12,13••,14••] and only becomes competent to target degradation of mRNAs upon ATP-dependent unwinding of the siRNAs [15••]. Concentrations as low as a few molecules of dsRNA per cell can elicit a strong and persistent response; this is likely to be a result of catalytic action by RISC [2,5••]. In some organisms, it may also be a result of an amplification mechanism in which an RNA-dependent RNA polymerase uses the target mRNA as a template to synthesize more dsRNA, using siRNAs as primers for new dsRNA production [16,17•].

Many of the cellular components required for RNAi have been identified [18,19]. Exactly how the majority of these components function in RNAi is not yet clear, and determining what roles they play is an ongoing challenge.

Long dsRNAs cannot be used for gene silencing in all organisms, as some, including mammals, possess dsRNA defense mechanisms. Such mechanisms result in sequence-non-specific effects, thereby limiting the utility of long dsRNAs as a gene-silencing tool. Fortunately, siRNAs alone are able to elicit a potent and effective RNAi response, presumably bypassing the dicing step and directly loading onto RISC without triggering the dsRNA defense mechanisms [20,21••,22••,23•,24].

As RNAi and other sequence identity-dependent silencing phenomena share a remarkable degree of similarity, these diverse gene-silencing events are suspected to be modern versions of an ancient pathway that was used to regulate gene expression [25-30]. Co-suppression in plants and quelling in fungi, for instance, share several features in their RNA-mediated responses [1]. Co-suppression has been found to work, at least in part, post-transcriptionally. Many of the proteins involved in post-transcriptional gene silencing (PTGS) and RNAi are conserved [18,19,31]. Moreover, plants undergoing PTGS have been found to possess small RNAs, approximately 25 nucleotides in length, which were absent in non-silenced control plants [32••]. As in animals, these small RNAs were found to be complementary to both the sense and antisense strands of the silenced gene. Small RNAs have been identified more recently in extracts of *C. elegans* and *Drosophila* [10••,13••,14••].

**Figure 1. Mechanism of RNAi.**

The figure illustrates a working model combining results from various organisms (see text for details and references). Proteins and protein activities are shown as balls; identified proteins or activities are labeled, some speculated proteins and complexes are unlabeled, and ATP-dependent steps are marked. Whether dsRNAs or siRNAs enter the nucleus is not yet clear. Note that Dicer is known to associate with RISC but is not required for RISC activity.

One surprising aspect of RNAi is that the effect can spread between tissues; introducing dsRNA in one part of an organism may lead to silencing of the targeted gene in another [5•,33•]. Proteins specifically required for the spreading effect include a predicted transmembrane protein that may form a channel or act as a receptor for dsRNA, siRNA, or an as yet undiscovered RNAi signal [34•]. Similarly, the ability of suppression to spread is also a feature of PTGS; in plants, tissue from a silenced donor, grafted to an unsilenced plant, can lead to silencing in the host [35].

### RNAi in the nucleus

Recent work has suggested that RNAi in non-plant systems may, in addition to eliminating specific pools of mRNA in the cytoplasm, also work by preventing the production of new mRNA by epigenetic silencing of gene expression in the nucleus.

Epigenetic regulation refers to heritable changes in gene expression, without changes in gene sequence, which usually occur through modifications of chromatin [36]. The eukaryotic nucleus contains large amounts of DNA that must be efficiently packaged into a small space. Mechanisms to remodel chromatin at specific loci, thus rendering the DNA accessible to the transcriptional machinery, have developed in eukaryotic cells. Modifications to histones can regulate the availability of DNA by controlling the condensed state of chromatin; converting chromatin from a closed, heterochromatic

state to an open, euchromatic state. Distinct histone modifications can act sequentially or in combination to form a 'histone code' that dictates whether DNA is either accessible or tightly compacted. These modifications include covalent modifications, including methylation or acetylation, of specific amino acid residues on one or more histone tails [36,37]. In plants, DNA-mediated gene silencing can be epigenetic in nature; it has recently become clear that this can also be true for RNA-mediated gene silencing. RNAi and co-suppression of endogenous genes by high copy number transgenes have both been found to work transcriptionally, as well as post-transcriptionally [38,39]. Upon introduction of high copy number transgenes or dsRNA, PTGS occurs when there is sequence identity within the coding sequence, while transcriptional gene silencing (TGS) occurs when the sequence identity occurs within the promoter [40,41]. TGS in plants requires genes that are implicated in modifying chromatin structure and is also known to involve DNA methylation [42].

DNA methylation does not appear to occur in certain animals, including *C elegans* and *Drosophila*, nor in certain fungi [43]. This has raised the question of whether dsRNA only silences gene activity in the nucleus of plants. An answer to this question has begun to emerge from several studies published over the past year.

### Chromatin modification and RNAi in animals

The possibility that RNAi may also act by modifying chromatin has been suggested since, in animals, a phenotype induced by

RNAi sometimes persists through additional generations and some genes required for RNAi are also required to maintain chromosomal stability [33••,44].

Certain chromatin-modifying proteins have been implicated in RNAi in *C elegans* [45•]. The introduction of dsRNAs that target components of the RNAi machinery itself can, at least in some cases, prevent RNAi from functioning [9••,45•,46,47]. In *C elegans*, this effect has been exploited to identify genes that may play roles in RNAi [45]. Several genes identified by this method encode proteins that are predicted to associate with chromatin, raising the possibility that RNAi may work at the level of chromatin in animal cells, as it does in plants. Mutations in at least three of these genes have confirmed their roles in RNAi; this includes genes that encode proteins homologous to *Drosophila* Polycomb-group proteins, which function to repress gene expression through the modification of chromatin [48].

Recent work using *Drosophila* has implicated a protein called Piwi in the silencing of targeted genes during high copy number transgene-induced silencing [49•]. Piwi is a homolog of the *C elegans* protein RNAi-defective 1 (RDE-1) and belongs to a large family of proteins, many of which are required for RNAi in diverse organisms [50-52]. In *piwi* mutants of *Drosophila*, PTGS is greatly impaired and siRNAs corresponding to the target gene are absent [49•]. High copy number transgenes induce not only post-transcriptional but also transcriptional gene silencing in *Drosophila* [53]. Interestingly, *piwi* mutants are also defective in transcriptional gene silencing.

Since *Drosophila* TGS is dependent on Polycomb-group proteins, it is tempting to speculate that dsRNA might elicit Polycomb-dependent chromatin modifications and gene silencing in flies and worms [49•,53]. To date, however, TGS has only been assayed in flies and only after transgene-induced silencing, not after the introduction of dsRNA. Polycomb-dependent silencing probably does not work by DNA methylation in flies and worms, since little to no DNA methylation can be detected in these organisms [43].

### **Chromatin modification and RNAi in fission yeast**

Recent work has demonstrated that endogenous dsRNAs result in gene silencing in the fission yeast *Schizosaccharomyces pombe* [54•]. Centromeric DNA is normally silenced in *S pombe* and transgenes inserted into centromeric regions are not expressed. Centromeric silencing is dependent on RNAi machinery, as loss of genes homologous to those required for RNAi in animal systems can result in expression of centromeric transgenes. Centromeric DNA is normally flanked by silent, repetitive sequences and loss of the RNAi machinery also results in the abnormal production of transcripts deriving from both strands of the repeat sequences. Wild-type fission yeast do not accumulate such transcripts, and the dependence of the RNAi machinery on their disappearance suggests that dsRNAs normally produced by repetitive sequences are degraded by the RNAi machinery. In concordance with this, others

have detected siRNAs derived from centromere repeat sequences in wild-type *S pombe* [55].

In addition, RNAi in fission yeast functions to repress transcription in the nucleus. Silenced, centromeric sequences have covalent histone H3 modifications (eg, methylation of lysine 9) characteristic of silenced heterochromatin; centromere homologous repeats are sufficient to induce such chromatin modifications elsewhere in the genome [54•,56•]. Loss of the RNAi machinery results in histone H3 modifications characteristic of non-silenced chromatin at centromeric sequences. These results suggest that dsRNAs derived from the repeat sequences in centromeres are processed into siRNAs that serve to target a methyltransferase to a corresponding site in the centromeric region [54•,55,56•]. Recruitment of a methyltransferase could lead to covalent modification of histone H3 and the subsequent formation of silent chromatin. While the loss of RNAi components leads to de-repression of centromeric DNA, the exact molecular mechanism by which this occurs remains to be determined.

Upon chromatin modification directed by the RNAi machinery, other machinery takes over to maintain the repressed state. Histone H3 methylated at lysine 9 can recruit the heterochromatin protein Swi6/HP1 [56•]. After silencing has been established, RNAi components become dispensable for maintenance of the repressed state. Instead, Swi6/HP1 is essential for the silenced state of chromatin to be inherited through mitosis and meiosis [56•].

### **Conclusion**

RNA-mediated gene silencing has become an important tool to analyze gene function in many organisms. As genome sequences become available, RNAi makes determining the function of each and every gene a realistic goal. Moreover, the ability to readily silence specific genes holds promise for designing effective therapies to combat a host of ailments. RNA-mediated gene silencing also plays a critical role in physiological gene silencing in a diverse range of organisms. As a result, there is much interest in understanding the mechanism by which RNA interference results in silencing.

Much research evidence has been produced in the past several years to extend our understanding of how RNAi functions. Until recently, dsRNA had been predicted to act exclusively at the post-transcriptional level to target mRNA stability. Recent work, however, has uncovered an additional role for dsRNA in regulating transcription via remodeling of chromatin. While the exact mechanisms behind chromatin silencing are not yet clear, the notion that RNA may direct chromatin modifications at specific sites along a chromosome is intriguing.

New developments in RNAi are likely to be closely watched, and as the molecular mechanisms of RNAi become fully understood, we will ultimately be better placed to understand genome function.

### **References**

- of outstanding interest
  - of special interest
1. Sharp PA: **RNAi and double-strand RNA**. *Genes Dev* (1999) **13**(2):139-141.

2. Tijsterman M, Ketting RF, Plasterk RH: **The genetics of RNA silencing.** *Annu Rev Genet* (2002) **36**:489-519.
3. Plasterk RH: **RNA silencing: The genome's immune system.** *Science* (2002) **296**(5571):1263-1265.
4. Vazquez Rovere C, del Vas M, Hopp HE: **RNA-mediated virus resistance.** *Curr Opin Biotechnol* (2002) **13**(2):167-172.
5. 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**(6669):806-811.
  - First report that dsRNA could elicit a potent gene silencing effect, even at substoichiometric levels.
6. Guo S, Kemphues KJ: **Par-1, a gene required for establishing polarity in *C. elegans* embryos, encodes a putative Ser/Thr kinase that is asymmetrically distributed.** *Cell* (1995) **81**(4):611-620.
7. Zamore PD: **Ancient pathways programmed by small RNAs.** *Science* (2002) **296**(5571):1265-1269.
8. Tabara H, Yigit E, Siomi H, Mello CC: **The dsRNA binding protein RDE-4 interacts with RDE-1, DCR-1, and a DExH-box helicase to direct RNAi in *C. elegans*.** *Cell* (2002) **109**(7):861-871.
9. Bernstein E, Caudy AA, Hammond SM, Hannon GJ: **Role for a bidentate ribonuclease in the initiation step of RNA interference.** *Nature* (2001) **409**(6818):363-366.
  - Report of the biochemical fractionation and identification of the RNase III enzyme Dicer that can specifically cleave dsRNAs into siRNAs.
10. Ketting RF, Fischer SE, Bernstein E, Sijen T, Hannon GJ, Plasterk RH: **Dicer functions in RNA interference and in synthesis of small RNA involved in developmental timing in *C. elegans*.** *Genes Dev* (2001) **15**(20):2654-2659.
  - Demonstration that the RNase III enzyme Dicer is also involved in regulating small temporal RNAs in *C. elegans*, implicating an RNAi-like mechanism in the regulation of normal development.
11. Knight SW, Bass BL: **A role for the RNase III enzyme DCR-1 in RNA interference and germ line development in *Caenorhabditis elegans*.** *Science* (2001) **293**(5538):2269-2271.
  - Dicer (involved in RNAi) is also involved in normal development of the *C. elegans* germline.
12. Elbashir SM, Lendeckel W, Tuschl T: **RNA interference is mediated by 21- and 22-nucleotide RNAs.** *Genes Dev* (2001) **15**(2):188-200.
13. Hammond SM, Bernstein E, Beach D, Hannon GJ: **An RNA-directed nuclease mediates post-transcriptional gene silencing in *Drosophila* cells.** *Nature* (2000) **404**(6775):293-296.
  - A biochemical approach towards understanding the mechanisms of RNAi identified RISC, a nuclease complex able to cleave targeted mRNAs.
14. 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**(1):25-33.
  - This work added much support to a model that siRNAs act as guides to target homologous transcripts.
15. Nykanen A, Haley B, Zamore PD: **ATP requirements and small interfering RNA structure in the RNA interference pathway.** *Cell* (2001) **107**(3):309-321.
  - Identification of ATP-dependent steps in RNAi, including processing of dsRNAs into siRNAs and unwinding of the siRNA duplex to generate the active RISC complex.
16. 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**(4):169-178.
17. Lipardi C, Wei Q, Paterson BM: **RNAi as random degradative PCR: siRNA primers convert mRNA into dsRNAs that are degraded to generate new siRNAs.** *Cell* (2001) **107**(3):297-307.
  - Demonstration that siRNAs act as primers for an RNA-dependent RNA polymerase in the amplification of the RNAi effect.
18. Hannon GJ: **RNA interference.** *Nature* (2002) **418**(6894):244-251.
19. Hutvagner G, Zamore PD: **RNAi: Nature abhors a double-strand.** *Curr Opin Genet Dev* (2002) **12**(2):225-232.
20. Yang D, Lu H, Erickson JW: **Evidence that processed small dsRNAs may mediate sequence-specific mRNA degradation during RNAi in *Drosophila* embryos.** *Curr Biol* (2000) **10**(19):1191-1200.
21. Elbashir SM, Martinez J, Patkaniowska A, Lendeckel W, Tuschl T: **Functional anatomy of siRNAs for mediating efficient RNAi in *Drosophila melanogaster* embryo lysate.** *EMBO J* (2001) **20**(23):6877-6888.
  - Identification and description of the chemical and structural requirements for mediating efficient RNAi using synthetic siRNAs.
22. Elbashir SM, Harborth J, Lendeckel W, Yalcin A, Weber K, Tuschl T: **Duplexes of 21-nucleotide RNAs mediate RNA interference in cultured mammalian cells.** *Nature* (2001) **411**(6836):494-498.
  - Evidence that siRNAs can mediate gene silencing in mammalian cell culture, bypassing the dsRNA host defense response.
23. Martinez J, Patkaniowska A, Urlaub H, Luhrmann R, Tuschl T: **Single-stranded antisense siRNAs guide target RNA cleavage in RNAi.** *Cell* (2002) **110**(5):563-574.
  - This paper identifies several components of RISC in HeLa cell extracts and demonstrates that RISC contains single-stranded antisense RNA.
24. Shi Y: **Mammalian RNAi for the masses.** *Trends Genet* (2003) **19**(1):9-12.
25. Baulcombe DC: **RNA as a target and an initiator of post-transcriptional gene silencing in transgenic plants.** *Plant Mol Biol* (1996) **32**(1-2):79-88.
26. Cogoni C, Macino G: **Homology-dependent gene silencing in plants and fungi: A number of variations on the same theme.** *Curr Opin Microbiol* (1999) **2**(6):657-662.
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**(25):14687-14692.
28. 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**(7):1017-1026.
29. Wianny F, Zernicka-Goetz M: **Specific interference with gene function by double-stranded RNA in early mouse development.** *Nat Cell Biol* (2000) **2**(2):70-75.
30. Li YX, Farrell MJ, Liu R, Mohanty N, Kirby ML: **Double-stranded RNA injection produces null phenotypes in zebrafish.** *Dev Biol* (2000) **217**(2):394-405.
31. Plasterk RH, Ketting RF: **The silence of the genes.** *Curr Opin Genet Dev* (2000) **10**(5):562-567.
32. Hamilton AJ, Baulcombe DC: **A species of small antisense RNA in post-transcriptional gene silencing in plants.** *Science* (1999) **286**(5441):950-952.
  - Evidence that short RNA species are produced in plants undergoing PTGS, suggesting that these short oligomers mediate silencing.
33. Grishok A, Tabara H, Mello CC: **Genetic requirements for inheritance of RNAi in *C. elegans*.** *Science* (2000) **287**(5462):2494-2497.
  - Demonstration that RNAi can be transmitted through the germline to the next generation and examination of the genetic requirements for inheritance of RNAi.
34. Winston WM, Molodowitch C, Hunter CP: **Systemic RNAi in *C. elegans* requires the putative transmembrane protein SID-1.** *Science* (2002) **295**(5564):2456-2459.
  - Implication that a large multi-pass membrane protein underlies the spread of RNAi between tissues in *C. elegans*.
35. 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**(15):4738-4745.
36. Ahmad K, Henikoff S: **Epigenetic consequences of nucleosome dynamics.** *Cell* (2002) **111**(3):281-284.
37. Strahl BD, Allis CD: **The language of covalent histone modifications.** *Nature* (2000) **403**(6765):41-45.
38. Vaucheret H, Beclin C, Fagard M: **Post-transcriptional gene silencing in plants.** *J Cell Sci* (2001) **114**(Pt 17):3083-3091.
39. Fire A: **RNA-triggered gene silencing.** *Trends Genet* (1999) **15**(9):358-363.
40. 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**(23):13959-13964.

41. Mette MF, Aufsatz W, van der Winden J, Matzke MA, Matzke AJ: **Transcriptional silencing and promoter methylation triggered by double-stranded RNA.** *EMBO J* (2000) **19**(19):5194-5201.
42. Matzke MA, Aufsatz W, Kanno T, Mette MF, Matzke AJ: **Homology-dependent gene silencing and host defense in plants.** *Adv Genet* (2002) **46**:235-275.
43. Paszkowski J, Whitham SA: **Gene silencing and DNA methylation processes.** *Curr Opin Plant Biol* (2001) **4**(2):123-129.
44. 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**(2):133-141.
45. Dudley NR, Labbe JC, Goldstein B: **Using RNA interference to identify genes required for RNA interference.** *Proc Natl Acad Sci USA* (2002) **99**(7):4191-4196.
  - Development of RNAi-based screening methods to identify genes required for RNAi and the first evidence that RNAi in an animal system requires nuclear proteins.
46. Grishok A, Pasquinelli AE, Conte D, Li N, Parrish S, Ha I, Baillie DL, Fire A, Ruvkun G, Mello CC: **Genes and mechanisms related to RNA interference regulate expression of the small temporal RNAs that control *C. elegans* developmental timing.** *Cell* (2001) **106**(1):23-34.
47. Hammond SM, Boettcher S, Caudy AA, Kobayashi R, Hannon GJ: **Argonaute2, a link between genetic and biochemical analyses of RNAi.** *Science* (2001) **293**(5532):1146-1150.
48. Pirrotta V: **Silence in the germ.** *Cell* (2002) **110**(6):661-664.
49. Pal-Bhadra M, Bhadra U, Birchler JA: **RNAi related mechanisms affect both transcriptional and post-transcriptional transgene silencing in *Drosophila*.** *Mol Cell* (2002) **9**(2):315-327.
  - Demonstration of the requirement of *piwi* in transgene-induced TGS and PTGS in flies.
50. Cogoni C, Macino G: **Post-transcriptional gene silencing across kingdoms.** *Curr Opin Genet Dev* (2000) **10**(6):638-643.
51. 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**(2):123-132.
52. Fagard M, Boutet S, Morel JB, Bellini C, Vaucheret H: **AGO1, QDE-2, and RDE-1 are related proteins required for post-transcriptional gene silencing in plants, quelling in fungi, and RNA interference in animals.** *Proc Natl Acad Sci USA* (2000) **97**(21):11650-11654.
53. 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**(3):479-490.
54. Volpe TA, Kidner C, Hall IM, Teng G, Grewal SI, Martienssen RA: **Regulation of heterochromatic silencing and histone H3 lysine-9 methylation by RNAi.** *Science* (2002) **297**(5588):1833-1837.
  - Evidence that proteins involved in RNAi are required in both the initiation and the maintenance of centromeric silencing; further suggesting that RNAi may have a nuclear role.
55. Reinhart BJ, Bartel DP: **Small RNAs correspond to centromere heterochromatic repeats.** *Science* (2002) **297**(5588):1831.
56. Hall IM, Shankaranarayana GD, Noma K, Ayoub N, Cohen A, Grewal SI: **Establishment and maintenance of a heterochromatin domain.** *Science* (2002) **297**(5590):2232-2237.
  - Paper showing that RNAi components are necessary for the initiation of silencing at the yeast mating-type locus and further suggests that RNAi may function in the nucleus.