nature REVIEWS GENETICS

RNAi and its applications

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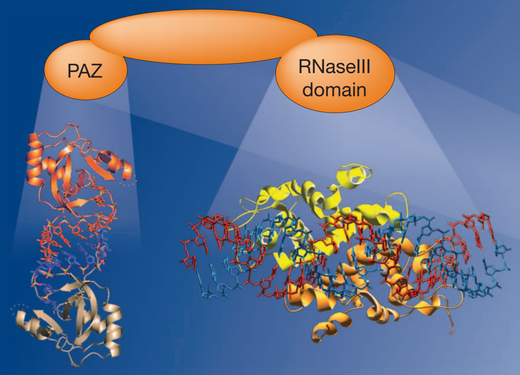
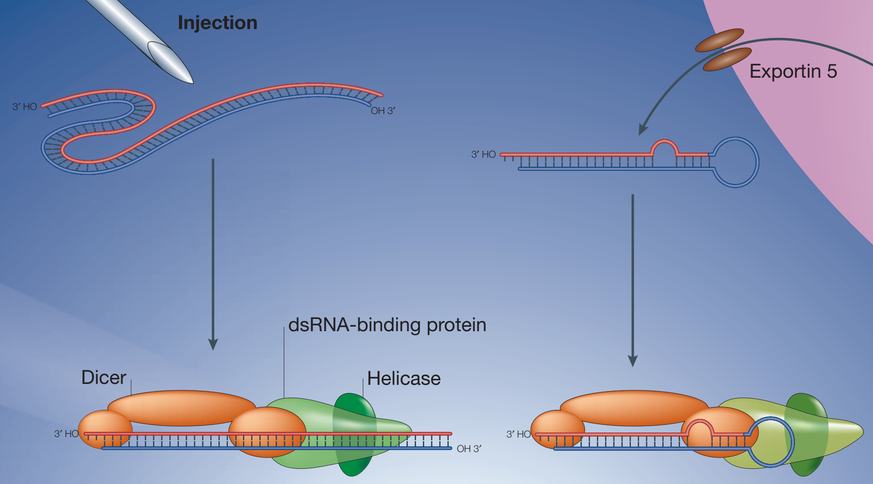
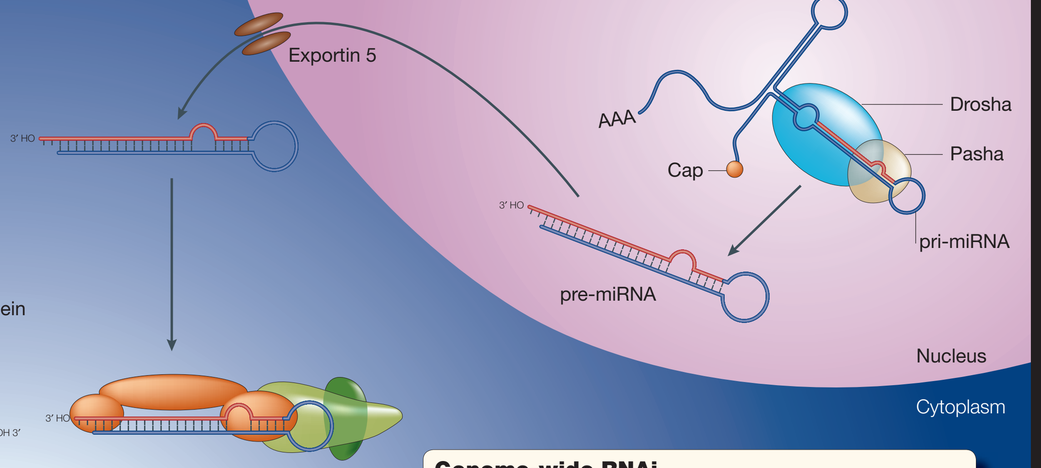
Double-stranded RNA (dsRNA) molecules can be used to induce a potent silencing process, known as RNA interference (RNAi). Key players in this process are two enzymes, Dicer and Argonaute, and a small RNA species, small interfering RNA (siRNA). The siRNA is made by the Dicer enzyme from a longer dsRNA substrate. One of the strands of the duplex siRNA then enters the RNA-induced silencing complex (RISC), a multi-protein complex that contains an Argonaute protein through which siRNAs induce their silencing effect. Because RNAi is both robust and specific it has become an important tool for gene knockdown experiments in the laboratory, and is being developed for use in agriculture and in the clinic.

The means of introduction

- Injection of *in vitro* synthesized dsRNA
- Soaking in dsRNA
- Feeding dsRNA-producing bacteria
- Transfection of synthetic siRNA
- Transgenes that produce dsRNA molecules (hairpins)
- Viruses producing dsRNA molecules

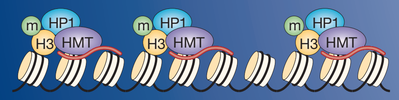




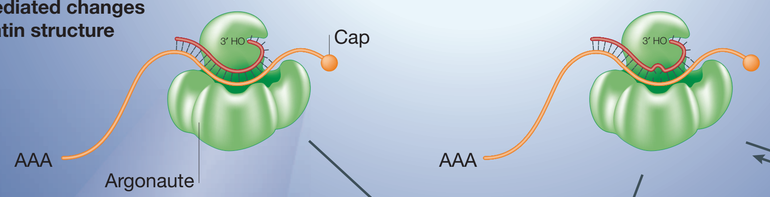


Rules of the game

- Strand selection: the strand with the most loosely base-paired 5′ end in the siRNA duplex will enter the RISC complex
- The 5′ end has to be phosphorylated
- siRNAs need to have a 2-nucleotide 3′ overhang, which is recognized by the PAZ domain, present in Dicer and Argonaute proteins
- The seed sequence of miRNA (nucleotides 2–7) requires perfect base pairing with target to induce silencing



siRNA-mediated changes in chromatin structure



miRNA-mediated translation prevention

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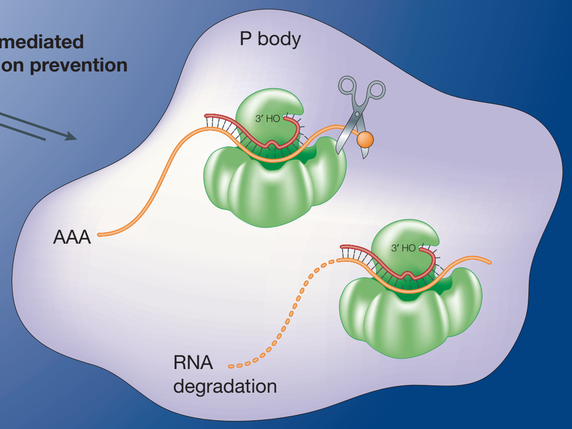
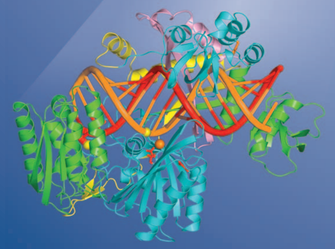
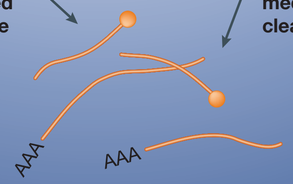




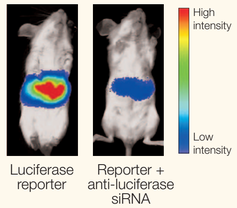
siRNA-mediated cleavage

miRNA-mediated cleavage



Therapy and crop improvement

The specificity and robustness of RNAi have triggered an immense interest in using RNAi as a tool in various settings. For example, RNAi is used to improve crops by providing resistance against parasites, and modified versions of siRNAs that are directed against disease-causing genes are being developed. Some of these therapeutic siRNAs are already being tested in clinical trials. The principle of *in vivo* RNAi is indicated in the figure, where luciferase in the liver is specifically silenced by siRNAs.



Genome-wide RNAi

Libraries have been made that are used to silence genes in a genome-wide approach for several organisms. These libraries are now used for gene discovery as a rapid alternative to classic genetic approaches. Most methods deliver long dsRNA molecules, resulting in gene silencing by a pool of siRNAs. Exceptions to this are the viruses that are used in humans and mice: in these cases siRNAs of only one type are being produced, derived either from custom-made short RNA hairpins or hairpins that are built into larger primary miRNA transcripts.

	Injection	Soaking	Feeding	Virus
Caenorhabditis elegans	+	+	+	
Drosophila melanogaster		+		
Nicotiana benthamiana				+
Planaria			+	
Mouse				+
Human				+

The central component of the RISC complex is a member of the Argonaute family. This protein interacts with Dicer and several other proteins, among which are dsRNA-binding proteins and RNA helicases. One of the two strands of the siRNA duplex, the one with the weakest 5′ end base pairing in the duplex, is selected to enter the RISC complex (this step is called strand selection). The Argonaute protein then binds the siRNA and recruits an homologous mRNA, after which it induces mRNA breakdown by introducing a nick into the mRNA. This cleavage reaction is carried out by the Piwi domain that folds into an RNaseH-like structure. The position of the nick is always halfway along the siRNA–mRNA duplex, opposite the phosphodiester bond between nucleotides 10 and 11 of the siRNA.

In addition to mRNA breakdown, siRNAs can affect the chromatin structure of targeted genes, resulting in transcriptional inhibition. This phenomenon is mediated by another complex known as RITS (RNA-induced transcriptional silencing). siRNAs are thought to guide chromatin modifying enzymes (e.g. histone methyltransferases; HMTs) to specific DNA sequences, resulting in a silent chromatin structure (e.g. in methylated (m) histone H3 on lysine 9).

Shortly after the discovery of RNAi, it became clear that this pathway was used in the cell by a large family of cell-autonomous short RNA — microRNAs (miRNAs). The precursors of these miRNAs, the primary miRNAs (pri-miRNAs), are made in the nucleus where they are processed by Drosha into pre-miRNAs. Similar to siRNAs, pre-miRNAs are then processed by Dicer in the cytoplasm, after which they also are bound by an Argonaute protein. miRNAs can induce silencing through mRNA cleavage, mainly in plants, or inhibition of translation, mainly in animals. The miRNA–RISC complex will specify cleavage if the mRNA has sufficient complementarity to the miRNA; if the mRNA does not have sufficient complementarity to be cleaved, the miRNA–RISC complex will repress mRNA translation. mRNAs that are silenced by miRNAs are recruited to so-called processing bodies (P bodies) where they are either stored or degraded.

References

Fire, A. *et al. Nature* **391**, 806–811 (1998) | Ma, J.-B., Ye, K. & Patel, D. J. *Nature* **429**, 318–322 (2004) | Matzke M. A. & Birchler J. A. *Nature Rev. Genet.* **6**, 24–35 (2005) | McCaffrey, A. P. *et al. Nature* **418**, 38–39 (2002) | Song, J.-J. *et al. Science* **305**, 1434–1437 (2004) | Yuan, Y.-R. *et al. Mol. Cell* **19**, 405–419 (2005) | Zhang, H. *et al. Cell* **118**, 57–68 (2004)

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Image of RNaseIII structure courtesy of Lukasz Jaskiewicz and Witold Filipowicz, FMI, Basel, Switzerland. Image of Argonaute structure courtesy of Yu-Ren Yuan and Dinshaw Patel, MSKCC, New York, USA.
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