

<<RNA干扰>>

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内容概要

从最开始RNA干扰还只是注射到线虫里的一个人工合成物，到2006年Fire和Mello因此获得诺贝尔奖，再到如今的临床试验，RNA干扰领域的发展可谓日新月异。

RNA干扰：从生物学到临床应用（英文导读版）汇集该领域的多位研究专家，提供了最新的科学知识和实验方案，并将这一生物学分支领域从核酸化学扩展到药理学和信号转导通路的调控。

RNA干扰：从生物学到临床应用（英文导读版）分为三部分，阐述了RNA干扰的生理机制、RNA干扰的实验室研究和siRNA导入、RNA干扰的临床应用。

RNA干扰：从生物学到临床应用（英文导读版）专业权威，通过回顾RNA干扰领域的研究进展、提供具体的实验方案和启发新思路，旨在对该领域起到推动作用。

RNA干扰：从生物学到临床应用（英文导读版）秉承Springer《分子生物学方法》系列丛书的一贯风格，阐述明晰、便于使用，各章包括内容简介，必备材料与试剂的清单，易于操作的实验室方案、疑难问题的注意事项和易犯失误的避免。

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章节摘录

Chapter 1 Endogenous Antiviral Mechanisms of RNA Interference: A Comparative Biology Perspective Abubaker M.E. Sidahmed and Bruce Wilkie Abstract RNA interference (RNAi) is a natural process that occurs in many organisms ranging from plants to mammals. In this process, double-stranded RNA or hairpin RNA is cleaved by a RNaseIII-type enzyme called Dicer into small interfering RNA duplex. This then directs sequence-specific, homology-dependent, posttranscriptional gene silencing by binding to its complementary RNA and triggering its elimination through degradation or by inducing translational inhibition. In plants, worms, and insects, RNAi is a strong antiviral defense mechanism. Although, at present, it is unclear whether RNA silencing naturally restricts viral infection in vertebrates, there are signs that this is certainly the case. In a relatively short period, RNAi has progressed to become an important experimental tool both in vitro and in vivo for the analysis of gene function and target validation in mammalian systems. In addition, RNA silencing has subsequently been found to be involved in translational repression, transcriptional inhibition, and DNA degradation. In this article we review the literature in this field, which may open doors to the many uses to which this important technology is being put, including the potential of RNAi as a therapeutic strategy for gene regulation to modulate host-pathogen interactions. Key words: RNA interference, Dicer, Transposons, siRNA, miRNA, Antiviral, Silencing, Suppressors, Quelling

1. Discovery and Historical Overview RNA silencing is a broad term that has been used to describe RNA interference (RNAi) in animals, posttranscriptional gene silencing in plants, and quelling in fungi, which are all phenotypically different but mechanistically similar forms of RNAi (1). RNAi is a natural process in which double-stranded RNA (dsRNA) or hairpin RNA is cleaved by RNaseIII-type enzyme called Dicer into small interfering RNA (siRNA) duplex of 21-26 nucleotides, which then direct sequence-specific, homology-dependent, posttranscriptional gene silencing by binding to its complementary RNA and triggering its elimination through degradation or by inducing translational inhibition (2, 3). RNA silencing is an evolutionarily ancient RNA surveillance mechanism, conserved among eukaryotes as a natural defense mechanism to protect the genome against invasion by mobile genetic elements, such as viruses, transposons, and possibly other highly repetitive genomic sequence and also to orchestrate the function of developmental programs in eukaryotic organisms (1, 2). Declaration of RNAi in 2002 as a “breakthrough” by the journal *Science* (4) encouraged scientists to revise their vision of cell biology and cell evolution, and the discovery of RNAi resulted in the Nobel Prize for Physiology or Medicine, being awarded to Andrew Fire and Craig Mello in 2006. The discovery of RNAi followed observations in the late 1980s of transcriptional inhibition by antisense RNA expressed in transgenic plants (5), during a search for transgenic petunia flowers that were expected to be a more intense color of purple. In an attempt to alter flower colors in petunias, Jorgensen and colleagues (6) sought to upregulate the activity of the chalcone synthase (chsA) enzyme, which is involved in the production of anthocyanin pigments. They introduced additional copies of this gene. The overexpressed gene was expected to result in darker flowers in transgenic petunia, but instead it produced less pigmented, fully or partially white flowers, demonstrating that the activity of chsA had been significantly decreased. Actually, both the endogenous genes and the transgenes were downregulated in the white flowers. Surprisingly, the loss of cytosolic chsA mRNA was not linked with reduced transcription as tested by run-on transcription assays in isolated nuclei. Further investigation of the phenomenon in plants indicated that the downregulation was due to posttranscriptional inhibition of gene expression by an increased rate of mRNA degradation (6). Jorgensen invented the term “co-suppression of gene expression” to describe the elimination of mRNA of both the endogenous gene and the trans-gene, but the molecular mechanism remained unclear (6). Other laboratories around the same time reported that the introduction of the transcribing sense gene could downregulate the expression of homologous endogenous genes (6, 7). A homology-dependent gene silencing phenomenon termed “quelling” was noted in the fungus *Neurospora crassa* (8). Quelling was recognized during attempts to increase the production of orange pigment expressed by the gene *al1* of *N. crassa* (8). Wild-type *N. crassa* was transformed

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with a plasmid containing a 1.5 kb fragment of the coding region of the *al1* gene. Some transformants were stably quelled and showed albino phenotypes. In these *al1*-quelled fungi, the amount of native mRNA was highly reduced while that of unspliced *al1* mRNA was similar to the wild-type fungi. This indicated that quelling, but not the Endogenous Antiviral Mechanisms of RNA Interference rate of transcription, affected the level of mature mRNA in a homology-dependent manner. Shortly thereafter, plant virologists conducting experiments to improve plant resistance to viral infection made a similar, unexpected observation. While it was documented that plants produced proteins that mediated virus-specific enhancement of tolerance or resistance to viral infection, a surprising finding was that short, noncoding regions of viral RNA sequences carried by plants provided the same degree of protection. It was concluded that viral RNA produced by transgenes could also inhibit viral accumulation (9). Homology-dependent RNA elimination was also noticed to occur during an increase in viral genome of infected plants (10). Ratcliff et al. (11) described a reverse experiment, in which short sequences of plant genes were introduced into viruses and the targeted gene was suppressed in an infected plant. Viruses can be the source, the target, or both for silencing. This phenomenon was named “virus-induced gene silencing” (VIGS), and the whole set of similar phenomena was collectively named posttranscriptional gene silencing (11). Not long after these observations in plants, investigators searched for homology-dependent RNA elimination phenomena in other organisms (12, 13). The phenomenon of RNAi first came to light after the discovery by Andrew Fire et al. in 1998 of a potent gene silencing effect, which occurred after injecting purified dsRNA directly into adult *Caenorhabditis elegans* (2). The injected dsRNA corresponded to a 742 nucleotide segment of the *unc22* gene. This gene encodes nonessential but abundant myofilament muscle protein. The investigators observed that neither mRNA nor antisense RNA injections had an effect on production of this protein, but dsRNA successfully silenced the targeted gene. A decrease in *unc22* activity is associated with severe twitching phenotype, and the injected animal as expected showed a very weak twitching phenotype, whereas the progeny nematodes showed strong twitching. The investigators then showed similar loss-of-function knockouts could be generated in a sequence-specific manner, using dsRNA corresponding to four other *C. elegans* genes, and they coined the term RNAi. The Fire et al. discovery was particularly important because it was the first recognition of the causative agent of what was until then an unexplained phenomenon. RNAi can be initiated in *C. elegans* by injecting dsRNA into the nematodes (2), soaking them in a solution of dsRNA (14), feeding the worms bacteria that express dsRNA (15), and using transgenes that express dsRNA in vivo (16). This very potent method for knocking out genes required only catalytic amounts of dsRNA to silence gene expression. The silencing was not only in gut and other somatic cells, but also spread through the germ line to several subsequent generations (14). Similar silencing was soon confirmed in plants (17), *Sid Ahmed* and *Wilkie* trypanosomes (18), flies (19) and many other invertebrates and vertebrates. In parallel, it was determined that dsRNA molecules could specifically downregulate gene expression in *C. elegans* (2). Subsequent genome screening led to identification of small temporal RNA (stRNA) molecules that were similar to the siRNA in size, but in contrast to the siRNAs, stRNA were single-stranded and paired with genetically defined target mRNA sequences that were only partly complementary to the stRNA (20). Particularly, stRNAs *lin-4* and *let-7* were determined to bind with the 3' noncoding regions of target *lin-14* and *lin-41* mRNAs, respectively, leading to reduction in mRNA-encoded protein accumulation.

These observations encouraged investigators to look for stRNA-like molecules in different organisms, leading to the identification of hundreds of highly conserved RNA molecules with stRNA-like structural properties (21). These small RNAs are termed micro RNAs (miRNAs). They are produced from transcript that folds to stem-loop precursor molecules first in the nucleus by the RNA III enzyme *Dorsha* and then in the cytosol by *Dicer*, and they are present in almost every tissue of every animal investigated (22). Thus, the RNAi pathway guides two distinct RNA classes, double-stranded siRNA and single-stranded miRNA, to the cytosolic RISC complex, which brings them to their target molecules.

2. The Molecular Mechanism of RNA Interference RNAi is a natural process of gene silencing that occurs in many organisms ranging from plants to mammals. RNAi was observed first by a plant scientist in the late 1980s, but the molecular basis of its mechanism remained unknown until the late 1990s, when research using the *C. elegans* nematode showed that RNAi is an evolutionarily conserved gene-silencing

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mechanism (2). Sequence-specific posttranscriptional RNAi gene silencing by double-stranded RNA is conserved in a wide range of organisms: plants (*Neurospora*), insect (*Drosophila*), nematodes (*C. elegans*), and mammals. This process is part of the normal defense mechanism against viruses and the mobilization of transposable genetic elements (2, 3). Although first discovered as a response to experimentally introduced RNA initiator, it is now known that RNAi and related pathways regulate gene expression at both transcriptional and posttranscriptional levels. The key steps in RNAi underlie several gene regulatory mechanisms that include downregulation of the expression of endogenous genes, direct transcriptional gene silencing and alteration of chromatin structure to promote kinetochore function, and chromosome segregation and direct elimination of DNA from somatic nuclei in tetrahymena (23).

3. Intrinsic Antiviral Defense Mechanism of RNAi

3.1. Antiviral RNA Silencing in Mammals

Endogenous Antiviral Mechanisms of RNA Interference

The dsRNAs, generated by replicating viruses, integrated transposons, or one of the recently discovered classes of regulatory noncoding miRNAs, are processed into short dsRNAs (20). These short RNAs generate a flow of molecular and biochemical events involving a cytoplasmic ribonuclease III (RNase III)-like enzyme, known as Dicer, and a multi-subunit ribonucleoprotein complex called RNA-induced silencing complex (RISC). The antisense (guide) strand of the dsRNA directs the endonuclease activity of RISC to the homologous (target) site on the mRNAs, leading to its degradation and posttranscriptional gene silencing. The naturally occurring miRNAs are synthesized in large precursor forms in the nucleus. An RNA III enzyme called Drosha mediates the processing of the primary miRNA transcripts into pre-miRNA (70-80 mers), which are then exported via the exportin-5 receptor to the cytoplasm (24). In the cytoplasm, Dicer cleaves dsRNA, whether derived from endogenous miRNA or from replicating viruses, into small RNA duplexes of 19-25 base pairs (bp). These have characteristic 3' \overline{C} -dinucleotide overhangs that allow them to be recognized by RNAi enzymatic machinery, leading to degradation of target mRNA (25). Dicer works with a small dsRNA-binding protein, R2D2, to pass off the siRNA to the RISC, which has the splicing protein Argonaute 2 (Ago2). Argonaute cleaves the target mRNA between bases 10 and 11 in relation to the 5' \overline{C} -end of the antisense siRNA strand (26). The siRNA duplex is loaded into the RISC, whereupon an ATP-dependent helicase (Ago2) unwinds the duplex, allowing the release of "passenger" strand and leading to an activated form of RISC with a single-stranded "guide" RNA molecule (27, 28). The extent of complementarities between the guide RNA strand and the target mRNA decides whether mRNA silencing is achieved by site-specific cleavage of the mRNA in the region of the siRNA-mRNA duplex (29) or through an miRNA-like mechanism of translational repression (30). For siRNA-mediated silencing, the cleavage products are released and degraded, leaving the disengaged RISC complex to further survey the mRNA pool. To protect themselves from

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