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RNA interference (RNAi), an evolutionarily conserved mechanism triggered by double-stranded RNA (dsRNA), causes gene silencing in a sequence-specific manner. RNAi evolved naturally to mediate protection from both endogenous and exogenous pathogenic nucleic acids and to modulate gene expression. Multiple technological advancements and precision in gene targeting have allowed a plethora of potential applications, ranging from targeting infections in crop plants to improving health in human patients, which have been reviewed in this chapter.

**Keywords:** RNA interference, miRNA, RNAi mediated gene silencing, RNA-induced silencing complex

### 1. Introduction

Ascribing the structure and function relationship to a gene and modulating its expression to manifest the desired phenotype have been major challenges for scientists. [1] In order to elucidate the phenotype(s) associated with a given gene, various gene-targeting techniques have been tried with mixed success. Gene silencing can be executed at transcriptional gene silencing (TGS) and posttranscriptional gene silencing (PTGS) levels. [2] The TGS involves targeting genes at DNA level by altering promoter and enhancer efficiencies, methylation status of genes, and deleting parts of genes by homologous recombination, transcription activator-like effector nucleases (TALENs), and clustered regularly interspaced short palindromic repeats (CRISPR)/Cas9 systems. [3] The PTGS techniques rely upon the breakdown of mRNA by various technologies, including antisense RNA, ribozymes, DNAzymes, micro-
RNAs, and RNA interference (RNAi). [4] Among all these techniques, RNAi is the most efficient tool for targeted gene silencing. RNAi is now routinely utilized across multiple biological disciplines to determine gene function. RNAi is also being utilized for therapeutic interventions to downregulate the expression of genes involved in disease pathogenesis. The current review is focused on recent advancements in the biology and applications of RNAi.

2. RNAi-mediated gene silencing: A historical perspective across multiple species

2.1. Discovery of RNAi in plants and fungi

R. Jorgensen and his colleagues identified a novel mechanism of post-transcriptional gene silencing in *Petunia*. [5] They were attempting to introduce a chalcone synthase gene under a strong promoter to deepen the purple color of *Petunia* flowers; however, instead of getting a stronger purple color flower they observed that most flowers lost their color. Thus, they observed diminished expression of both the homologous endogenous gene and the exogenously introduced transgenic copy of the gene and termed the phenomenon as co-suppression. [5] Although the exact mechanism of this phenomenon remained undeciphered at that time, the posttranscriptional nature of gene silencing was still appreciated. [5-7] The suppression of endogenous gene expression by transformation of exogenous homologous sequences was later termed as quelling in *Neurospora crassa*. [8, 9]

2.2. RNAi in *C'. elegans*

In 1995, Guo and Kempheus attempted to knock down the expression of PAR-1 gene by antisense RNA in *C. elegans*; they observed a similar loss of gene expression with sense RNA controls as well [10]. At that time, they could not explain the mechanistic basis of such an observation. In 1998, Andrew Fire, Craig C. Mello, and their colleagues demonstrated efficient and specific interference of gene expression by introducing double-stranded RNA in the nematode *C. elegans* [11]. The genetic interference was genetically heritable and was stronger than the antisense strategy. This novel phenomenon was termed as RNA interference or RNAi by Fire and colleagues [11].

Subsequently, Lisa Timmons and Andrew Fire demonstrated that *C. elegans*, when fed on bacteria genetically engineered to express dsRNA for unc-22 and fem-1 genes, showed specific and reversible silencing of unc-22 and fem-1 genes in the worm [12, 13]. High-throughput genetic screens have been developed by either feeding the worms on genetically engineered bacteria expressing dsRNA or soaking or injecting the nematode with dsRNA. Functional genomic analysis of chromosomes I and III in *C. elegans* have been performed by Fraser and Gonczy, respectively, utilizing the RNA interference strategy [14, 15].

2.3. RNAi technology in *Drosophila*

Specific gene silencing has been achieved in the embryo extracts and cultured cells of *Drosophila* flies by utilizing the RNAi tool [16]. Zamore and colleagues utilized *Drosophila melanogaster*
embryo lysates to demonstrate the cleavage of long dsRNA strands into short interfering dsRNA fragments (siRNA) of ~22 nucleotides (nt) [16]. Later Elbashir and colleagues demonstrated that chemically synthesized 21- or 22-nt-long dsRNA carrying 3′ overhangs could induce efficient RNA cleavage in embryo extracts from *Drosophila* [17].

2.4. RNAi in mammalian systems

A global nonspecific inhibition of protein synthesis was observed in mammalian cells by exposing them to dsRNAs that were greater than 30 base pairs (bp) in length [18]. RNA-dependent protein kinase (PKR), and 2′, 5′ oligoadenylate synthetase (2′, 5′-OAS) were responsible for the nonspecific silencing. PKR phosphorylates eIF-2α, a translation initiation factor, to shut down global protein synthesis. A synthesis product of enzyme 2′, 5′-OAS activates RNase L, which induces nonspecific degradation of all mRNAs in a mammalian cell [18]. Long dsRNAs induce interferon response that activates both of these enzymes in mammalian cells [19]. The nonspecific interference pathways represent the mammalian cell response to viral infection or other stress [20]. Tuschl and colleagues demonstrated that RNA interference could be directly mediated by small interference RNA (siRNA) in cultured mammalian cells [21]. However, because siRNA does not integrate into the genome, the RNAi response from siRNA is only transient. In order to induce stable gene suppression in mammalian cells, Hannon and his colleagues utilized RNA Pol III promoter-driven (e.g., U6 or H1) expression of short hairpin RNAs (shRNAs) [22]. Various approaches have since been developed for mammalian cells to obtain successful gene silencing. Some of the successful gene silencing approaches are listed in Table 1.

<table>
<thead>
<tr>
<th>Kingdom</th>
<th>Species</th>
<th>Silencing process</th>
<th>Induction stimulus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fungi</td>
<td>Neurospora</td>
<td>Quelling</td>
<td>Transgene(s)</td>
</tr>
<tr>
<td></td>
<td>Saccharomyces pombe</td>
<td>RNAi</td>
<td>dsRNA</td>
</tr>
<tr>
<td>Plants</td>
<td>Arabidopsis, Coffea canefora, Nicotiana, Petunia</td>
<td>Transcriptional or Post-transcriptional gene silencing, co-suppression</td>
<td>Transgene(s) and viruses</td>
</tr>
<tr>
<td>Invertebrates</td>
<td>Paramecium</td>
<td>Homology-dependent gene silencing</td>
<td>Transgene(s)</td>
</tr>
<tr>
<td></td>
<td>Amblyomma americanum, Anopheles, Brugia malayi, Dugesia japonica, Hydra, Leishmania donovani, Schistosoma mansoni, Tribolium castaneum, Trypanosoma brucei, etc.</td>
<td>RNAi</td>
<td>dsRNA</td>
</tr>
<tr>
<td></td>
<td>C. elegans</td>
<td>RNAi, TGS</td>
<td>dsRNA, Transgene(s)</td>
</tr>
<tr>
<td></td>
<td>D. melanogaster</td>
<td>Co-suppression, RNAi, Transcriptional gene silencing</td>
<td>dsRNA, Transgene(s)</td>
</tr>
<tr>
<td>Vertebrates</td>
<td>Human, Mouse, Zebrafish</td>
<td>RNAi</td>
<td>dsRNA</td>
</tr>
</tbody>
</table>

Table 1. Gene silencing approaches
3. The mechanism of silencing

RNAi-mediated gene silencing is executed by siRNAs. The process of silencing begins with the cleavage of long dsRNAs into 21–25 -nt fragments of siRNAs in cytoplasm [16, 17]. The process is catalyzed by Dicer enzyme [23]. These siRNAs are inserted into multiprotein silencing complex, which is known as RNA-induced silencing complex (RISC). Subsequent unwinding of siRNA duplex, in turn, leads to active confirmation of RISC complex (RISC*). Next, target mRNA (mRNA to be degraded) is recognized by antisense RNA, which signals RISC complex for the endonucleolytic degradation of the homologous mRNA. Tuschl and his colleagues have defined the directionality of dsRNA processing and the target RNA cleavage sites [17]. According to their results, target mRNA is cleaved in the centre of the region that is recognized by complimentary guide siRNA, which is 10–12 -nt away from the 5′ terminus of siRNA [17]. The RNAi process is completed by the last step of siRNA molecule amplification. It is well established that the next generation of siRNAs is derived from the priming on the target mRNA by RNA-dependent RNA polymerase (RdRp) enzyme by existing siRNAs. The second generation of siRNAs is effective in inducing a secondary RNA interference that is defined as transitive RNAi. The transitive RNAi causes a systemic genetic interference in plants and C. elegans. Interestingly, transitive and systemic RNAi is absent in Drosophila and mammals owing to the lack of RdRp in both organisms [24]. An illustration of the function of RNAi is demonstrated in Figure 1.

A multitude of studies suggests a possible link between RNAi and chromatin remodeling [24]. The dsRNA works at TGS and PTGS in plants, where both pathways related and assist in gene silencing. Only TGS is heritable and drives methylation of endogenous sequences. Multiple proteins, including Polycomb in Drosophila and C. elegans [22], and Piwi in Drosophila [25], execute silencing at both TGS and PTGS levels. Volpe and his colleagues documented that RNAi complex proteins, including Dicer, Agronaute, and RdRp, assist in centromeric silencing in Schizosaccharomyces pombe [26]. This suggests that RNAi contributes to the maintenance of genomic stability [26].

4. Enzymes involved in RNAi

4.1. Dicer

Dicer was first characterized and defined in Drosophila by Bernstein et al. [27]. Dicer belongs to the RNase III-class and assists in ATP-dependent siRNA generation from long dsRNAs. Importantly, human Dicer does not require ATP for the cleavage of long dsRNAs [28]. Structurally, Dicer is a large (~220-kDa) multi-modular protein that acts as an antiparallel dimer. Dicer has multiple domains, including an N-terminal putative DEAH/DEAH box RNA helicase/ATPase domain, an evolutionarily conserved PAZ domain, two neighboring domains that resemble RNase III, and a dsRNA-binding domain. PAZ domain in dicer helps in recognizing the end of dsRNA, whereas RNase III domain helps in the cleavage of dsRNA. Function of other domains is not fully known. Dicer orthologs has been defined in many
organisms, including *S. pombe*, *Arabidopsis thaliana* (CARPEL FACTORY [CAF]), *Drosophila* (DCR-1 and DCR-2), *C. elegans* (DCR-1), mouse, and humans. In addition to RNAi, Dicer also assists in the generation of microRNAs in multiple organisms [29].

4.2. RNA-Induced Silencing Complex (RISC)

RISC is a ribonucleoprotein complex that fragments mRNAs through the production of a sequence-specific nuclease. At first, while working on *Drosophila* embryo extracts, Zamore and his colleagues identified ~250 kDa precursor complex, which turns into an activated complex of 100 kDa upon addition of ATP. However, Hannon and his colleagues found a 500-kDa complex from *Drosophila* S2 cells [30, 31]. The siRNA is an important part of RISC and was the first to be identified. It acts as a template and guides RISC to the target mRNA molecule. To date, a number of RISC protein components are known in *Drosophila* and mammalian species. Interestingly, these components are not completely overlapping, which suggests the developmental stage-specific or evolutionarily non-conserved nature of the components of RISC complex [24].
The first RISC protein component identified was Agronaute-2, a *C. elegans* RDE-1 homologue [32]. Argonaute (AGO) proteins are part of an evolutionarily conserved protein family and they play a central role in RNAi, determination of stem cell developmental regulation, and tumorigenesis. AGOs are ~100 kDa highly basic proteins that contain N-terminal PAZ and mid- and C-terminal PIWI domains [33]. The PAZ domain is an RNA-binding module, which is involved in protein–protein interactions, whereas PIWI is essentially required for target cleavage. Some AGO proteins that are involved in RNAi are listed in the Table 2.

<table>
<thead>
<tr>
<th>Species</th>
<th>Argonaute homolog</th>
<th>Essentiality for RNAi</th>
<th>Citations</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Arabidopsis</em></td>
<td>AGO1</td>
<td>Essential for co-suppression and PTGS</td>
<td></td>
</tr>
<tr>
<td></td>
<td>ZWILE</td>
<td>Non-essential</td>
<td></td>
</tr>
<tr>
<td><em>Tetrahymena</em></td>
<td>TWI1</td>
<td>Essential for DNA elimination</td>
<td></td>
</tr>
<tr>
<td><em>Neurospora</em></td>
<td>QDE2</td>
<td>Required component of RISC</td>
<td></td>
</tr>
<tr>
<td><em>C. elegans</em></td>
<td>RDE-1</td>
<td>Forms complex with Dicer</td>
<td></td>
</tr>
<tr>
<td></td>
<td>ALG-1</td>
<td>Nonessential</td>
<td></td>
</tr>
<tr>
<td></td>
<td>ALG-2</td>
<td>Nonessential</td>
<td></td>
</tr>
<tr>
<td></td>
<td>PPW-1</td>
<td>Essential for germline RNAi</td>
<td></td>
</tr>
<tr>
<td></td>
<td>dAgro1</td>
<td>Essential in embryos, acts downstream of RNAi generation</td>
<td></td>
</tr>
<tr>
<td></td>
<td>dAgo2</td>
<td>Required component of RISC</td>
<td></td>
</tr>
<tr>
<td></td>
<td>dAgo3</td>
<td>Prediction based on DNA sequence</td>
<td></td>
</tr>
<tr>
<td></td>
<td>PIWI</td>
<td>Essential for PTGS and TGS</td>
<td></td>
</tr>
<tr>
<td>Mammals (human)</td>
<td>EIF2C2/hAgo2</td>
<td>Part of RISC complex and catalyzes the miRNA–directed cleavage</td>
<td></td>
</tr>
</tbody>
</table>

Table 2. Argonaute homolog proteins in RNAi

Some RISC components are non-AGO proteins, including dFXR and VIG in *Drosophila*, the fragile X mental retardation 1 (FMR1) homolog in *Drosophila*, and germin3/4 in mammals [34].

### 4.3. RNA helicase

RNA helicases cause unwinding of dsRNA. However, Dicer contains its own helicase activity in the N-terminal helicase domain. Hence, the helicase proteins putatively function down-
stream of the RISC complex. Two major RNA helicase families are involved in RNAi [35]. SDE3 from *A. thaliana* and its homologous proteins in mouse, human, and *Drosophila* constitute the first such helicase family. The second family contains Upf1p from yeast and an Upf1 homologue (SMG-2) in *C. elegans*. The Upf1/SMG-2 is characterized by cysteine-rich motif conserved across species and multiple C-terminal Ser-Gln (SQ) doublets. MUT-6, a DEAH-box helicase in *C. elegans* is also putatively involved in transposon suppression. Another RNA helicase Germin3 resides in complex with human AGO protein EIF2C2/hAgo2 [36].

4.4. RNA-dependent RNA polymerase (RdRp)

RdRp catalyzes the amplification and triggering of RNAi, which is usually in small amounts. RdRp catalyzes the siRNA-primed amplification by polymerase chain reaction to convert mRNA into dsRNAs, a long form that is cleaved to produce new siRNAs [37]. Lipardi and his colleagues demonstrated RdRp-like activity in *Drosophila* embryo extracts, but the enzyme responsible for the RdRp activity in the *Drosophila* or human is not known. Some of the RdRps involved in RNAi have been summarized in Table 3.

<table>
<thead>
<tr>
<th>Species</th>
<th>RdRp homolog</th>
<th>Essentiality for RNAi</th>
<th>Citations</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Arabidopsis</em></td>
<td>SDE1</td>
<td>Essential for PTGS by transgenes but not by viruses</td>
<td></td>
</tr>
<tr>
<td><em>Neurospora</em></td>
<td>QDE1</td>
<td>Essential for co-suppression</td>
<td></td>
</tr>
<tr>
<td><em>C. elegans</em></td>
<td>EGO1</td>
<td>Essential for germline RNAi</td>
<td></td>
</tr>
<tr>
<td></td>
<td>RRF1</td>
<td>Essential for RNAi in soma</td>
<td></td>
</tr>
<tr>
<td></td>
<td>RDE9</td>
<td>Forms complexes with Dicer</td>
<td></td>
</tr>
</tbody>
</table>

Table 3. RdRps involved in RNAi

5. Various small RNA isoforms related to RNAi

5.1. Small interfering RNAs (siRNAs)

Small interfering RNAs are 21–23-nt-long double-stranded RNA molecules with 2–3-nt overhangs at the 3′ termini. siRNAs are normally generated, as mentioned in the above sections, by the cleavage of long double-stranded RNAs by RNase III (Dicer) [16]. siRNAs must be phosphorylated at the 5′ termini by endogenous kinases to enter into the RISC complex [31]. It is thought that the hydroxylated 3′ termini are essential for the siRNA-primed amplification step catalyzed by RdRps. However, Zamore et al. showed that non-priming alterations in the 3′ hydroxyl group did not adversely affect RNAi-mediated silencing [38]. They went on to explain that siRNAs operate as guide RNAs for gene repression but not as primers in the human and *Drosophila* RNAi pathways [38]. Conversely, Hamada et al. showed in mammalian cells that modifying the 3′ end of the antisense strand of siRNA abolished the RNAi effect,
while modifying the 3′ end of the sense strand did not affect the RNAi silencing [39]. These findings support the model that each strand of siRNA has different functions in the RNAi process, and the 3′ hydroxylated end of the antisense strand may prime the amplification. Ambros et al. discovered endogenous siRNA in more than 500 genes in wild-type *C. elegans* [39]. This suggests that siRNA may be a globally conserved and common molecule among species.

5.2. Micro RNAs (miRNAs)

miRNAs are 19–25-nt small RNA species produced by Dicer-mediated cleavage of endogenous ~70-nt noncoding stem-loop precursors. The miRNAs, while allowing mismatches, can either repress the target mRNA translation (mostly in mammals) or facilitate mRNA destruction (mostly in plants) [40]. miRNAs *lin-4* and *let-7* were the first ones to be identified in *C. elegans* [40]. So far, about 2000 different miRNAs have been identified in plants, animals, and lower species. While some miRNAs are evolutionarily conserved, others are specific for some developmental stages or are species-specific. Different terminologies are referred to in literature. According to one terminology, the miRNAs with well-characterized functions (e.g., *lin-4* and *let-7*) are referred to as small temporal RNAs (stRNAs), while other similar small RNAs of unknown functions are called miRNAs [40]. Multiple miRNAs have been characterized for their physiological roles in cancer and other diseases [41, 42]. Comparisons between siRNA and miRNA have been listed in the Table 4.

<table>
<thead>
<tr>
<th>Resemblences</th>
<th>miRNA</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. The siRNAs require processing from long dsRNAs.</td>
<td>1. The miRNAs require processing from stem-loop precursors that are ~70 nt long.</td>
</tr>
<tr>
<td>2. An RNase III enzyme Dicer is required for processing.</td>
<td>2. Dicer is required.</td>
</tr>
<tr>
<td>3. The siRNAs are usually ~22 nt long.</td>
<td>3. The miRNAs are also ~22 nt long.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Disparities</th>
<th>miRNA</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. The siRNAs are double-stranded structures with 2-nt 3′ overhangs that are formed during cleavage by Dicer.</td>
<td>1. The miRNAs are single-stranded structures.</td>
</tr>
<tr>
<td>2. The siRNA require high homology with the mRNA to bind and cleave.</td>
<td>2. The miRNAs can function even with a few mismatched nucleotides.</td>
</tr>
<tr>
<td>3. The siRNAs mediate target mRNA cleavage by RISC.</td>
<td>3. The miRNAs can either block target mRNA translation by binding to it or mediate target mRNA cleavage by RISC.</td>
</tr>
<tr>
<td>4. The siRNAs are usually triggered by transgene incorporation, viral infection, or active transposons.</td>
<td>4. The miRNAs are constitutively expressed cellular RNA moieties with potential roles in development, and cell proliferation and death.</td>
</tr>
</tbody>
</table>

Table 4. Comparative characteristics of siRNA and miRNA.
5.3. Tiny noncoding RNAs (tncRNAs)

Ambros and his colleagues discovered the first tncRNAs in *C. elegans*. They identified and characterized 33 new tncRNAs in *C. elegans* by performing cDNA sequencing and comparative genetics [40]. The tncRNAs are very similar to miRNAs with regard to their size, single-stranded structure, and lack of a precise complementarity to a given mRNA. However, they are distinct with regard to their lack of processing from a “miRNA-like hairpin precursor”, and phylogenetic nonconservation. Similarly to miRNA, tncRNAs are transcribed from noncoding sequences. However, their developmental role is not fully understood. According to Ambros and his colleagues, it is plausible that some of the miRNAs might be processed from noncoding mRNAs in the course of RNAi [40].

6. Evolutionary relevance of RNAi in the immunological responses

RNAi may provide a systemic way to immunize an organism against the invasive nucleic acids from viruses and transposons via inducing the RNAi responses. Virus-induced gene silencing (VIGS) in plants is accomplished by RNAi. Multiple genetic links between RNAi and virulence are known. Many plant viruses code for viral suppressors of gene silencing (VSGS). VSGS acts as a virulence determinant, and hence, is required for developing antivirulence response in the host. In response to the virulence, the host can also modify its PTGS/RNAi mechanisms to prevent future infections. RNAi can even target DNA virus amplification in plants [43]. VIGS mechanisms exist not only in plants and nematodes but also in other species; for example, flock house virus (FHV), a virus that infects *Drosophila*, also codes for a potential silencing suppressor (b2) [24]. Nonetheless, the precise function of RNAi in mammalian antiviral defense is not clear.

RNAi also plays a crucial role in the development process of multicellular organisms. When mutated, CARPEL FACTORY, a Dicer homologue in *Arabidopsis*, can cause developmentally defective leaves and induce overproliferation of floral meristems. Inactivation of Dicer by mutations causes developmental problems and sterility in *C. elegans*. Mutations in AGO protein influence normal development in *Drosophila* as well. Hence, components of RNAi pathway play a significant role in normal development, but such components and the affiliated gene products play crucial roles in related but distinct gene regulation pathways [23].

A potential role of RNAi and human disease pathogenesis has been proposed due to association of RNA binding proteins with RISC complex, such as Vasa intronic gene (VIG) and the fragile X mental retardation protein (FMRP) *Drosophila* homologue [36].

7. RNAi as a functional genomics tool and its applications for therapy

RNAi technology is applicable for gene silencing in many species. RNAi has been used extensively in *C. elegans* for functional genomics. High-throughput investigation of most of the
~19,000 genes has been accomplished. Ahringer and his colleagues produced an RNAi library, representing ~86% of the genes of *C. elegans* [15]. This strategy has been successfully attempted in multiple other model organisms, including human [44].

RNAi has also been utilized successfully in mammalian cells [44]. Various methods have been employed for siRNA knockdown of specific genes in mammalian cells. DNA-vector-mediated RNAi silences genes transiently in mammalian cells, while other expression systems are used for stable silencing. The promoters of RNA polymerase (pol) II and III (U6 and H1, alone or together) have been used for stable silencing. Furthermore, tRNA promoter-based systems have been used for this purpose. However, pol III-based short hairpin RNA (shRNA) expression systems (e.g., H1 RNA pol-based pSuper vector) are suitable choices. Retroviral-vector-based delivery of siRNAs has also been utilized for more efficient silencing. Two classes of retrovirus vectors have been employed: (1) HIV-1-derived lentivirus vectors and (2) Oncoretrovirus-based vectors, such as Moloney murine leukemia virus (MoMuLV) and Murine stem cell virus (MSCV). Transgenic mice have been established with germline transmission of a shRNA expression cassette for silencing of genes not targeted by homologous recombination-based approaches [45]. Desirable applications of this technique include inducible and cell type-specific expression patterns.

The use of RNAi is not limited to the determination of mammalian gene function, and also could be used for treating viral infections and cancer [46, 47]. Viral and human genes that are needed for viral replication can be attacked to generate viral-resistant host cells or to treat viral infections [47]. Oncogenes, which accelerate cancer growth, can be targeted by RNAi [48, 49]. Targeting of molecules important for neovascularization could prevent tumor growth [50]. This book presented several chapters with detailed discussions of therapeutic aspects of the RNAi in immune, blood, cancer, and brain diseases. We refer readers to those chapters (by Hu et al.; Gu; and Cho and Kim) rather to continue repeated information here.

### 8. Conclusions

Fast progress in RNAi technology has shown promise for use in reverse genetics and therapy. However, mechanistic complexities of this technology still need to be determined. RNAi has now been established as a revolutionary tool for functional genomics in organisms. Multiple studies have defined the role of RNAi in mammalian and plant defense systems. A plethora of studies have utilized RNAi technology to modulate gene expression. RNAi-based full genomic screens have allowed identification of specific genes, controlling a given trait with high accuracy. Further studies will continue to unravel the unlimited potential of RNAi to serve humankind.
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