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RNA Interference and its Mechanisms, Components, Applications and Related Isoforms

Niway Hailemariam, Yosef Deneke and Waktole Yadeta

School of Veterinary Medicine, College of Agriculture and Veterinary Medicine,
Jimma University, P.O. Box: 307, Jimma, Ethiopia

Abstract: RNA interference (RNAi), is an evolutionary conserved silencing pathway in which the double stranded RNA is broken down into small interfering RNA (siRNA) with the help of dicer and RNA inducedsilencing complex (RISC) by series of steps. Two types of small RNA molecules: microRNA (miRNA) and small interfering RNA (siRNA) are central to RNA interference. RNAs are the direct products of genes and these small RNAs can direct enzyme complexes to degrade messenger RNA (mRNA) molecules and thus decrease their activity by preventing translation, via post-transcriptional gene silencing. Moreover, transcription can be inhibited via the pre-transcriptional silencing mechanism of RNA interference, through which an enzyme complex catalyzes DNA methylation at genomic positions complementary to complexed siRNA or miRNA. RNA interference has an important role in defending cells against parasitic nucleotide sequences, viruses and transposons. It also influences development. The two component of DICER, dcr-2/r2d2 an ATP dependent binds to siRNA and helps it to load into RISC by forming RISC loading complex (RLC). RLC recruits their associates to form the effective machinery for gene silencing wherein it may remain binds to the complementary mRNA or may degrade the target. In this way, the activated RISC could potentially target multiple mRNAs and thus function catalytically. The RNA world was given a booster shot with the discovery of RNA interference (RNAi), a compendium of mechanisms involving small RNAs (less than 30 bases long) that regulate the expression of genes in a variety of eukaryotic organisms. Rapid progress in our understanding of RNAi-based mechanisms has led to applications of this powerful process in studies of gene function as well as in therapeutic applications for the treatment of diseases.

Key words: Small Interfering RNA · Rnai · Microrna · RNA-Induced Silencing Complex · Gene Silencing

INTRODUCTION

RNA interference (RNAi) is a phenomenon that primarily can be demonstrated as interference by RNA in the expression of genetic information [1]. It is a phenomenon primarily for the regulation of gene expression; self or non self-depending upon the surrounding factors or conditions, with the help of RNA molecules that are noncoding in nature to control cellular metabolism and help in maintaining genomic integrity by preventing the invasion of viruses and mobile genetic elements [2].

RNA molecules were long believed to serve only as messengers, bearing genetic information from DNA. But in the early 1980s it was revealed in *Escherichia coli* that small RNA molecules (about 100 nucleotides in length) can bind to a complementary sequence in mRNA and inhibit translation. This inactivates mRNA and therefore prevents it from passing on the genetic information [3].

The phenomena of RNAi first came into notice in the year 1990, when plant scientists were trying to intensify the hue of red petunias for commercial benefits. Rich Jorgensen introduced a gene that controls the formation of red pigments in petunia flowers but in some cases it was observed that the colour disappeared altogether. The reason behind such an observation was not clear at that time. The phenomenon was named as co-suppression. Co-suppression is a classical form of eukaryotic post-transcriptional gene silencing. It was first reported in transgenic petunia, where a sense transgene

Corresponding Author: Niway Hailemariam, School of Veterinary Medicine, College of Agriculture and Veterinary Medicine, Jimma University, P.O. Box: 307, Jimma, Ethiopia.

meant to overexpress the host Chalcone Synthase-A (CHS-A) gene caused the degradation of the homologous transcripts and the loss of flower pigmentation. Subsequently, such a phenomenon was also observed in fungi, which was termed as quelling [4, 5].

RNA silencing in animals was first reported when [6], were attempting to use antisense RNA to shut down expression of the par-1 gene in order to assess its function. As expected, injection of the antisense RNA disrupted expression of par-1, but quizzically, injection of the sense-strand control did too used antisense RNA to block par-1 mRNA expression in *Caenorhabditis elegans* but found that the par-1 mRNA itself also repressed par-1.

According to Andrew Fire *et al.* [7] study on the gene function especially on the gene responsible for movement in *C. elegans*. When these nematodes were injected with the mixture of sense and antisense RNA, nematodes were observed with the impaired movement suggesting a defective muscle gene protein. They concluded that the dsRNA injected must match the mature "trimmed" mRNA sequence for the gene and the interference could not be elicited by intron sequences. This implies that interference takes place after transcription, probably in the cytoplasm rather than in the cell nucleus.

The mRNA was shown to be targeted with large complex proteins to form an RNA induced silencing complex, commonly called RISC; in association with si RNA[8]. Subsequently, it has been shown that RISC contains at least one member of the argonaute protein family, which is likely to act as an endonuclease and cut the mRNA (often referred to as the Slicer function). It was also demonstrated that a ribonuclease III-like nuclease, called Dicer, is responsible for the processing of dsRNA to short RNA. In certain systems, in particular plants, worms and fungi, an RNA dependent RNA polymerase (RdRP) plays an important role in generating and/or amplifying siRNA [9].

Since understanding the applications of RNAi is very crucial in biomedical research, health care and technology, this review aimed: to review the recent available literature on RNA interferences and its mechanisms, components, applications and related isoforms.

Definitions of Rna Interference and Studies in Several Species: RNA interference (RNAi) is a highly conserved gene silencing mechanism that uses double-stranded RNA (dsRNA) as a signal to trigger the degradation of homologous mRNA [10]. RNAi represents an evolutionarily conserved cellular defense for controlling the expression of foreign genes in most eukaryotes including humans [11]. The trigger can occur naturally, as in the case of a cellular infection by a dsRNA virus, or by the intentional introduction of dsRNA to induce user-directed degradation of the cognate transcript(s) [12].

RNA interference (RNAi) or PTGS is a biological response to double-stranded RNA that mediates resistance to both endogenous parasitic and exogenous pathogenic nucleic acids and regulates the expression of protein-coding genes. This natural mechanism for sequence-specific gene silencing promises to revolutionize experimental biology and may have important practical applications in functional genomics, therapeutic intervention, agriculture and other areas [13].

Mechanism of Rna Interference: As the various pieces of the RNAi machinery are being discovered, the mechanism of RNAi is emerging more clearly. In the last few years, important insights have been gained in elucidating the mechanism of RNAi[22]. The Mechanism of RNA interferences as understood is that it comes into play when a double stranded RNA is introduced either naturally or artificially in a cell. An endoribonuclease enzyme cleaves the long dsRNA into small pieces of RNA.

The small pieces could be mi RNA or si RNA depending upon the origin of long dsRNA i.e. endogenous or exogenous respectively. A double-stranded RNA may be generated by either RNA dependent RNA polymerase or bidirectional transcription of transposable elements or physically introduced[2].The mechanism of RNA interference(silencing) can be divided into two stages: Initiation and Effector.

Initiation: The Initiation stage is characterized by generation of siRNA fragments about 21 to 25 nucleotides long ends mediated by type III endonuclease Dicer. They contain 5'phosphate and 3'hydroxyl termini and two additional overhanging nucleotides on their 3'end[10]. InDrosophila, Dicer, which is a large multidomain RNase III enzyme has been identified in existence into two forms: Dcr-1/Loquacious (Loqs)-PB (also known as R3D1-L[long]): generate miRNA and Dcr-2/R2D2 generates siRNA [23]. Dcr-1 shares a structural homology with Dcr-2, despite that they display different sets of properties such as ATP requirements and substrate specifications. Dcr-1 is an enzyme that shows ATP independent functions and affinity towards stem-loop form of RNA (precursor of miRNA) [24].

Table 1: RNAi studies in several species

Species	Phylum	References
Caenorhabditis elegans	Nematode	[7]
Danio rerio	Zebrafish	[14]
Trypanosoma brucei	Unicellular	[15]
Hydra magnipapillata	Cnidarian	[16]
Scmidteamediterrania	Planarian	[17]
Escherischia coli	Bacteria	[18]
Neurosporacrassa	Fungus	[5]
Drosophila melanogaster	Fruit-fly	[9]
Mus musculus	Mammals	[19]
Arabidopsis thaliana	Plants	[20]

Source: [21]

It has been identified that Dcr-1 requires a double-stranded RNA binding protein partner. In Drosophila Dicer-1 is seen to interact with the dsRBD protein Loquacious (Loqs). Immunoaffinity purification experiments reveal that Loqs resides in a functional pre-miRNA processing complex and stimulates and directs specific pre-miRNA processing activity. These results support a model in which Loqs mediates miRNA biogenesis and, thereby, the expression of genes regulated by miRNAs [25]. Loquacious protein is supposed to be composed of three ds RNA binding domains, which is encoded by *loqs*gene into two types of proteins PA and PB; isoform to each other, by alternate splicing, among which PB is known to enhance the affinity of Dcr-1 towards pre-miRNA [23].

Dcr-2 shows ATP dependent activity with substrate specificity to double stranded RNA. Structurally homologous to Dcr-1, it also requires a double stranded RNA binding protein, namely R2D2, which functions in association with specific RNase enzyme Dcr-2 forming a heterodimeric complex. R2D2, unlike Loq is supposed to be composed of two dsRNA binding domainsthat interact with long double stranded RNA but do not regulate si RNA generating activity of Dcr-2 [24].

Dcr-2 contains an RNA helicase domain, a DUF283 domain and a PAZ domain at the N terminus as well as tandem RNase III motifs and a dsRBD motif at the C terminus. It is unclear which of these domains physically contact siRNA. Since neither Dcr-2 nor R2D2 bind siRNA alone, it is possible that siRNA is bound at the interface between Dcr-2 and R2D2, which triggers a conformational change in either or both proteins, allowing them to bind siRNA co-operatively [26].

Explanation of Fig. 1: Long dsRNA and miRNA precursors are processed to siRNA/miRNA duplexes by the RNase-III-like enzyme Dicer. These short dsRNAs are subsequently unwound and assembled into effector complexes, RISCs, which can direct RNA cleavage, mediate translational repression or induce chromatin modification. S. pombe, C. elegans and mammals carry only one Dicer gene. 7mG, 7-methyl guanine; AAAA, poly-adenosine tail; Me, methyl group; P, 5' phosphate[27].

Effector: In the effector step of RNAi, the double-stranded siRNAs produced in the first step are believed to bind an RNAi-specific protein complex to form a RISC.This complex might undergo activation in the presence of ATP so that the antisense component of the unwound siRNA becomes exposed and allows the RISC to perform the downstream RNAi reaction [22].

Zamore *et al.* [28], demonstrated that a 250-kDa precursor RISC, found in *Drosophila* embryo extract, was converted into a 100-kDa complex upon being activated by ATP. This activated complex cleaved the substrate. The size and constitution of the precursor as well as the activated RISC might vary depending on the choice of the system [29]. The antisense siRNAs in the activated RISC pair with cognate mRNAs and the complex cuts this mRNA approximately in the middle of the duplex region [22]. The siRNA strand that is complementary to the targeted mRNA is then used as a primer by an RNA-dependent RNApolymerase (RdRP) to convert the cognate mRNA into dsRNA itself. This dsRNA form of mRNA then becomes a substrate for Dicer cleavage activity, which leads to the destruction of the mRNA and formation of new siRNAs.

Single strands of siRNA and miRNA duplexes, referred to as guide strands, are incorporated into RNAsilencing effecter complexes such as the RNA-induced silencing complex (RISC), or the RNA-inducedinitiation of transcriptional gene silencing (RITS) complex.1. RNA Induced Silencing Complex (RISC) cleaves m RNA or represses their translation by homology dependent m-RNA degradation. 2. RNA-induced Initiation of Transcriptional gene Silencing complex (RITS) regulates heterochromatin assembly. The core of these RNA silencing effecter complexes, RISC was found to be composed of PPD Proteins (PAZ PIWI Domain proteins) which are highly conserved super-family. Members of PPD proteins contains centrally located PAZ (100 amino acids) and C-Terminal located PIWI (300 amino acids) [28].

Subsequently, it was revealed that the PPD protein in RISC was Ago proteins. Ago proteins are members of PPD protein super-family. Not all PPD proteins are known to perform the same task in all systems (Table 2),

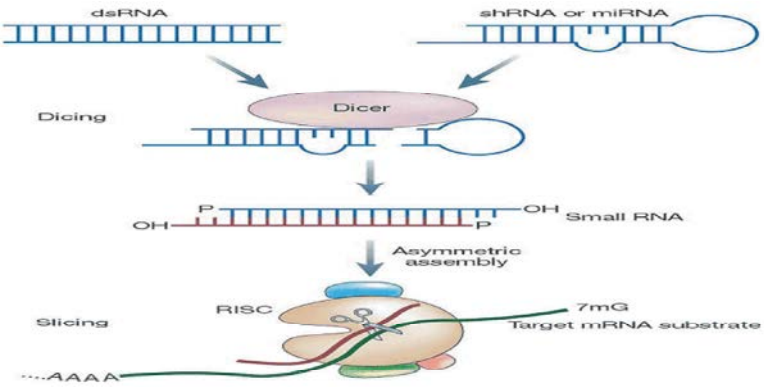

Fig. 1: Mechanism of RNA silencing in different systems

Table 2: Function of PPD Proteins (PAZ PIWI Domain proteins) in variable living system

Type of system	No of PPD proteins exist	Type of PPD Protein involved in siRNA mediated silencing	Function performed
Nemetode (*C. elegans*)	3	PPD, RDE-1, PPW-1	For efficient siRNA mediated m-RNA cleavage
Arthropoda (*D. melanogaster*)	1	Ago-2	For incorporation of siRNA into RISC
Chordata (*Homo sapiens*)	1	hAgo-2	Catalytic activity required for m-RNA cleavage

Source: [30]

but when studies were performed and compared the number of PPD in different systems; Nematodes were found to have the more specific function of PPD than others.

Effectively, this step amplifies the RNAi response and creates a self- perpetuating cycle of "degradative polymerase chain reaction" that will persist until no target mRNAs remain. This basic 'core' pathway defines the RNAi response as one of the most elegant and efficient biochemical mechanisms in nature [30].

Components of Gene Silencing: Genetic evaluations were carried out in the fungus *Neurosporacrassa*, the alga *Chlamydomonasreinhardtii*, the nematode *Caenorhabditiselegans* and the plant *A. thaliana* to search for mutants defective in quelling, RNA interference, or PTGS. Analyses of these mutants led to the identification of host-encoded proteins involved in gene silencing and also revealed that a number of essential enzymes or factors are common to these processes. Some of the components identified o serve as initiators, while others serve as effectors, amplifiers and transmitters of the gene silencing process [22].

Dicer: Bernstein *et al.* [9], identified an RNase III-like enzyme in *Drosophila* extract which was shown to have the ability to produce fragments of 22 nucleotides, similar to the size produced during RNAi. This enzyme is involved in the initiation of RNAi. Owing to its ability to digest dsRNA into uniformly sized small RNAs (siRNA), this enzyme was named Dicer (DCR). These nucleases are evolutionarily conserved in worms, flies, fungi, plants and mammals. It belongs to theRNase III-family and cleaves dsRNA with 3'overhangs of 2 to 3 nucleotides and 5'phosphate and 3'hydroxyl termini [10].

Dicer is ATP-dependent and contains several characteristic domains: an N-terminal helicase domain, a PAZ domain (a domain conservative throughout evolution found in Piwi/Argonaute/Zwille proteins in *Drosophila* and *Arabidopsis* and involved in developmental control), dual RNase III domains and double stranded RNA-binding domain [10, 31].

The predicted *C. elegans* Dicer homologu K12H4.8, was referred to as DCR1 because it was demonstrated to be the functional ortholog of the *Drosophila* Dicer protein [32]. The 8, 165-bp DCR1 protein has a domain structure similar to that of the *Drosophila* Dicer protein. *Dcr1* mutants of *C. elegans* showed defects in RNAi of germ line-expressed genes but noeffect on the RNAi response of somatic genes. These mutantswere found to be sterile, suggesting the important role of thisgene in germline development apart from RNAi[33].CAF1 (carpel factory) has been identified as a Dicer homologsin *A. thaliana*, but it is not involved in PTGS activity. The structure of CAF1 shows the presence of the four distinct domains that were identified in the *Drosophila* Dicer protein [9].

Dicer homologs from many different sources have been identified; some recombinant Dicers have also been examined in vitro and phylogenetic analysis of the known Dicer-like proteins indicates a common ancestry of these proteins [34].

Guide RNAs and RNA-Induced Silencing Complex: The RNA-induced silencing complex, or RISC, is a multiprotein complex, specifically a ribonucleoprotein, which incorporates one strand of a single-stranded RNA (ssRNA) fragment, such as microRNA (miRNA), or double-stranded small interfering RNA (siRNA). 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 [35]. The siRNA is an important part of RISC and was thefirst 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 [36].

The first RISC protein component identified was Agronaute-2, a *C. elegans* RDE-1 homologue 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. There are eight family members in human Argonautes of which only Argonaute 2 is exclusively involved in targeted RNA cleavage in RISC [37].

AGO2 is homologous to RDE1, a protein required for dsRNA-mediated gene silencing in *C. elegans.* AGO2 is a 130-kDa protein containing polyglutamine residues, PAZ and PIWI domains characteristic of members of the Argonaute gene family. The Argonaute family members have been linked both to the gene-silencing phenomenon and to the control of development in diverse species. The first link between Argonaute protein and RNAi was shown by isolation of *rde1* mutants of *C. elegans* in a screen for RNAi deficient mutants. Argonaute family members have beenshown to be involved in RNAi in *Neurosporacrassa* (QDE3) aswell as in *A. thaliana* (AGO1) [38].

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 downstream of the RISC complex. Two major RNA helicase families are involved in RNAi [39]. 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 homolog (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 [40].

RNA-Dependent RNA Polymerase (RdRp): RNA dependent RNA polymerase (RdRp) is an enzyme that catalyzesthe replication ofRNAfrom an RNA template. This is in contrast to a typical DNA-dependent RNA polymerase, which catalyzes the transcription of RNA from a DNA template.RdRP is an essential protein encoded in the genomes of all RNA-containing viruses with no DNA stage i.e. only RNA viruses[41].RdRp catalyzes the amplification and triggering of RNAi, which is usually in small amounts. RdRp catalyzes the siRNA-primed amplification by a polymerase chain reaction to convert mRNA into dsRNAs, a long form that is cleaved to produce new siRNAs. 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 [42]. Some of the RdRps involved in RNAi have been summarized in Table 3.

Various Small Rna Isoforms Related to RNAi

Small Interfering RNAs (siRNAs) Small interfering RNAs are 21–23-nt-long double-stranded RNA molecules with 2–3-ntoverhangs at the 3' termini.SiRNAs are normally generated, by the cleavage of long double-stranded RNAs by RNase III (Dicer) [28]. According to Pinzon *et al.* [44] the (siRNAs) play a central role in the RNA interference (RNAi) response. Usually loaded on a protein of the AGO subfamily of the Argonaute family, they recognize specific target RNAs by sequence complementarity and typically trigger their degradation by the AGO protein.

Table 3: RdRps involved in RNAi

Species	RdRp homolog	Essentiality for RNAi
Arabidopsis	SDE1	Essential for PTGS by transgenes but not by viruses
Neurospora	QDE1	Essential for co-suppression
C. elegans	EGO1	Essential for germline RNAi
	RRF1	Essential for RNAi in soma
	RDE9	Forms complexes with Dicer

Sources: [43]

According to Denli and Hannon [36], siRNAs must be phosphorylated at the 5' termini by endogenous kinases to enter into the RISC complex. The hydroxylated 3' termini are essential for the siRNA-primed amplification step catalyzed by RdRps. In whatever way, Zamore *et al*. showed that non-priming alterations in the 3' hydroxyl group did not adversely affect RNAi-mediated silencing. 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 [40].

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. Thefindings help 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* [45].

Micro RNAs (miRNAs): An important arm of RNAi involves the microRNAs (miRNAs). MicroRNAs (miRNAs) represent a class of noncoding RNA molecules that play pivotal roles in cellular and developmental processes by regulating gene expression at the posttranscriptional level [46]. MiRNAs are endogenous, evolutionarily conserved, small single-stranded non-coding endogenous RNAs that are known to regulate gene expression by multiple mechanisms. These tiny molecules comprising of only 19–25 nucleotides (~22nt) in length control post-transcriptional regulation of genes [47].

MicroRNAs (miRNAs) are ubiquitous, ranging from virus to human tissues and emerge as key post-transcriptional regulators of gene expression by suppressing target messenger RNAs (mRNAs) translation efficiency or degrading target mRNA[48]. MiRNAs are mainly derived from the introns of the pre-mRNA or the genetic interval of the genome through transcription [49]. They have initially expressed as long primary transcripts (pri-miRNAs), which are processed within the nucleus into 60–70 bp hairpins by the Microprocessor complex, consisting of Drosha and DGCR8 into pre-miRNAs. The pre-miRNAs are further processed in the cytoplasm by Dicer and one of the two strands is loaded into RISC, presumably via interaction with one of the Dicer accessory proteins [50].

MiRNAs*lin*-4 and *let*-7 were the first ones to be identified in *C. elegans*. 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 [51]. Multiple miRNAs have been characterized for their physiological roles in cancer and other diseases [43]. Comparisons betweensiRNA and miRNA have been listed in the Table 4.

Applications of RNAi: RNAi technology is applicable for gene silencing in many species.To a great extent RNAi has been used in *C. elegans* for functional genomics. Increase 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* [52]. This strategy has been successfully tried in multiple other model organisms, including humans [53].

RNAi has also been utilized successfully in mammalian cells. Different 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, in single or together) have been used for stable silencing. In addition, 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.

Table 4: Comparative characteristics of siRNA and miRNA

Similarties	
siRNA	MiRNA
1. The siRNAs require processing from long dsRNAs.	1. The miRNAs require processing from stem-loop precursors that are ~70 nt long.
2. An RNase III enzyme Dicer is required for processing.	2. Dicer is required.
3. The siRNAs are usually ~22 nt long.	3. The miRNAs are also ~22 nt long.
Disparities	
SiRNA	MiRNA
1. The siRNAs are double-stranded structures with 2-nt 3' overhangs that are formed during cleavage by Dicer.	1. The miRNAs are single-stranded structures.
2. The siRNA require high homology with the mRNA to bind and cleave.	2. The miRNAs can function even with a few mismatched nucleotides.
3. The siRNAs mediate target mRNA cleavage by RISC.	3. The miRNAs can either block target mRNA translation by binding to it or mediate target mRNA cleavage by RISC.
4. The siRNAs are usually triggered by transgene incorporation, viral infection, or active transposons.	4. The miRNAs are constitutively expressed cellular RNA moieties with potential roles in development and cell proliferation and death.

Source: [43]

Retroviral-vector-ased 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) Once retrovirus-based vectors, such as Moloney murine leukemia virus (MoMuLV) and Murine stemcell 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 [54]. Suitable applications of this technique include inducible and cell typespecific expression patterns.

According to Gupta [55], RNAi is being used for a variety of purposes including biomedical research and health care. It has begun to produce a paradigm shift in the process of drug discovery [27]. In order to address this objective, dsRNA molecules have been designed for the silencing of specific genes in humans and animals. The like silencing RNA molecules are introduced into the cell to forward activation of the RNAi machinery. This method has already become an important research tool in biomedicine. Several recent scholars show successful gene silencing in human cells and experimental animals. As an illustration, a gene causing high blood cholesterol levels was shown to be silenced by treating animals with silencing RNA. The Scenarios also underway to develop silencing RNA as a treatment for cardiovascular diseases, cancer, endocrine disorders and virus infections [55], such as those caused by the hepatitis C virus (HCV) and the human immunodeficiency virus (HIV) [27].

The application of RNAi is not limited to the determination of mammalian gene function and also could be used for treating viral infections and cancer [56]. Smith and his colleagues conducted their work on Viral and human genes that are needed for viral replication to generate viral-resistant host cells or to treat viral infections [57]. According to Shukla *et al.* [58], Oncogenes, which accelerate cancer growth, can be targeted by RNAi. Targeting of molecules important for neovascularization could hinder tumor growth [59].

Control of disease-associated genes makes RNAi an attractive choice for future therapeutics. Basically every human disease caused by activity from one or a few genes should be amenable to RNAi-based intervention [60]. The first clinical applications of RNAi have been directed at the treatment of age-related macular degeneration (AMD), which causes blindness or limited vision in millions of adults annually [61]. The challenge of cell- or tissue-specific delivery of siRNAs is also crucial when investigating the utility of RNAi-based therapies for a given disease; various strategies for nonviral and viral delivery of RNAi triggers have shown to be effective in their respective disease models.

CONCLUSIONS

In general, the development from the initial finding of RNAi to its clinical applications has been staggering. The fundamental biology of RNAi has led to its extensive applications in basic research and later in applications for the therapy of disease. RNAi is an emerging technique in the field of molecular biology to silence genes of human interest. Within the succeeding few years we should expect to more RNAi-mediated regulation of gene expression and will also see RNAi-based drugs approved for use in the treatment of disease. In addition, RNAi has proven to be a sound tool for the study of gene function and has opened new areas of basic investigation. Further studies should continue to unravel the unlimited potential of RNAi to serve humankind.

Abbrevations

AGO	Argonaute proteins
CAF	Carpel factory
CHS-A	Chalcone Synthase-A
Dcr	Dicer
mRNA	Messenger RNA
miRNA	MicroRNA
PAZ	Piwi/Argonaute/Zwille proteins
PTGS	Post transcriptional gene silencing
RNAi	RNA interference
RISC	RNA-induced silencing complex
RLC	RISC loading complex
RdRP	RNA dependent RNA polymerase
RITS	RNA-induced Initiation of Transcriptional gene Silencing complex
siRNA	Small interfering RNA

REFERENCES

1. Chiu, Y.L. and T.M. Rana, 2002. RNAi in human cells: basic structural and functional features of small interfering RNA. Molecular Cell, 10(3): 549-561.
2. Das, S., A. Marwal, D.K. Choudhary, V.K. Gupta and R.K. Gaur, 2011. Mechanism of RNA interference (RNAi): current concept. Int Proc Chem Biol Environ Eng, 9: 240-245.
3. Nordstrom, K. and E.G.H. Wagner, 1994. Kinetic aspects of control of plasmid replication by antisense RNA. Trends in Biochemical Sciences, 19(7): 294-300.
4. Cogoni, C., J.T. Irelan, M. Schumacher, T.J. Schmidhauser, E.U. Selker and G. Macino, 1996. Transgene silencing of the al-1 gene in vegetative cells of Neurospora. The EMBO Journal, 15(12): 3153-3163.
5. Cogoni, C. and G. Macino, 1997. Conservation of transgene-induced post-transcriptional gene silencing in plants and fungi. Trends in Plant Science, 2(11): 438-443.
6. Guo, S. and K.J. Kemphues, 1995. par-1, a gene required for establishing polarity in C. elegansembryos, encodes a putative Ser/Thr kinase that is asymmetrically distributed. Cell, 81(4): 611-620.
7. Andrew Fire, A., S.Q. Xu, M.K. Montgomery, S.A. Kostas, S.E. Driver and C.C. Mello, 1998. Potent and specific genetic interference by double-stranded RNA in *C. elegans*. Nature, 391: 806-810.
8. Montgomery, M.K., S. Xu and A. Fire, 1998. RNA as a target of double-stranded RNA-mediated genetic interference in Caenorhabditis elegans. Proceedings of the National Academy of Sciences, 95(26): 15502-15507.
9. Bernstein, E., A.A. Caudy, S.M. Hammond and G.J. Hannon, 2001. Role for a bidentate ribonuclease in the initiation step of RNA interference. Nature, 409(6818): 363-366.
10. Elbashir, S.M., J. Harborth, K. Weber and T. Tuschl, 2002. Analysis of gene function in somatic mammalian cells using small interfering RNAs. Methods, 26(2): 199-213.
11. Tuschl, T. and A. Borkhardt, 2002. Small interfering RNAs: a revolutionary tool for the analysis of gene function and gene therapy. Molecular Interventions, 2(3): 158.
12. Farrell, R.E. and R.N.A. Methodologies, 1993. A laboratory guide for isolation and characterization. RNA Methodologies, pp: 76-82.
13. Coordinators, N.R., 2017. Database resources of the national center for biotechnology information. Nucleic acids research, 45 (Database issue), pp: D12.
14. Wargelius, A., S. Ellingsen and A. Fjose, 1999. Double–stranded RNA induces specific developmental defects in Zebrafish embryos. Biochemical and Biophysical Research Communications, 263(1): 156-161.
15. Wang, Z., J.C. Morris, M.E. Drew and P.T. Englund, 2000. Inhibition of Trypanosoma brucei gene expression by RNA interference using an integratable vector with opposing T7 promoters. Journal of Biological Chemistry, 275(51): 40174-40179.
16. Lohmann, J.U., I. Endl and T.C. Bosch, 1999. Silencing of developmental genes in Hydra. Developmental Biology, 214(1): 211-214.
17. Alvarado, A.S. and P.A. Newmark, 1999. Double-stranded RNA specifically disrupts gene expression during planarian regeneration. Proceedings of the National Academy of Sciences, 96(9): 5049-5054.
18. Tchurikov, N.A., L.G. Chistyakova, G.B. Zavilgelsky, I.V. Manukhov, B.K. Chernov and Y.B. Golova, 2000. Gene-specific silencing by expression of parallel complementary RNA in Escherichia coli. Journal of Biological Chemistry, 275(34): 26523-26529.
19. Wianny, F. and M. Zernicka-Goetz, 2000. Specific interference with gene function by double-stranded RNA in early mouse development. Nature Cell Biology, 2(2): 70-75.
20. Akashi, H., M. Miyagishi and K. Taira, 2001. Suppression of gene expression by RNA interference in cultured plant cells. Antisense and Nucleic Acid Drug Development, 11(6): 359-367.
21. Thakur, A., 2003. RNA interference revolution. Electronic Journal of Biotechnology, 6(1): 1-2.

22. Agrawal, N., P.V.N. Dasaradhi, A. Mohmmed, P. Malhotra, R.K. Bhatnagar and S.K. Mukherjee, 2003. RNA interference: biology, mechanism and applications. Microbiol. Mol. Biol. Rev., 67(4): 657-685.
23. Saito, K., A. Ishizuka, H. Siomi and M.C. Siomi, 2005. Processing of pre-microRNAs by the Dicer-1-Loquacious complex in Drosophila cells. PLoS Biology, 3(7).
24. Jiang, F., X.Ye, X.Liu, L. Fincher, D. McKearin and Q. Liu, 2005. Dicer-1 and R3D1-L catalyze microRNA maturation in Drosophila. Genes & Development, 19(14): 1674-1679.
25. Forstemann, K., Y. Tomari, T.T. Du, V.V. Vagin, A.M. Denli, D.P. Bratu, C. Klattenhoff, W.E. Theurkauf and P.D. Zamore, 2005. Normal microRNA maturation and germ-line stem cell maintenance requires Loquacious, a double-stranded RNA-binding domain protein. PLoS Biology, 3(7): 1187-1201.
26. Liu, X., F. Jiang, S. Kalidas, D. Smith and Q. Liu, 2006. Dicer-2 and R2D2 coordinately bind siRNA to promote assembly of the siRISC complexes. Rna, 12(8): 1514-1520.
27. Hannon, G.J. and J. Rossi, 2004. Unlocking the potential of the human genome with RNA interference. Nature, 431: 371-378.
28. Zamore, P.D., T. Tuschl, P.A. Sharp and D.P. Bartel, 2000. RNAi: double-stranded RNA directs the ATP-dependent cleavage of mRNA at 21 to 23 nucleotide intervals. Cell, 101(1): 25-33.
29. Hannon, G.J., 2002. RNA interference. Nature, 418(6894): 244-251.
30. Hutvagner, G. and P.D. Zamore, 2002. RNAi: nature abhors a double-strand. Current Opinion in Genetics & Development, 12(2): 225-232.
31. Carmell, M.A. and G.J. Hannon, 2004. RNase III enzymes and the initiation of gene silencing. Nature Structural & Molecular Biology, 11(3): 214-218.
32. Peele, C., C.V. Jordan, N. Muangsan, M. Turnage, E. Egelkrout, P. Eagle, L. Hanley-Bowdoin and D. Robertson, 2001. Silencing of a meristematic gene using geminivirus-derived vectors. The Plant Journal, 27(4): 357-366.
33. Knight, S.W. and B.L. Bass, 2001. A role for the RNase III enzyme DCR-1 in RNA interference and germ line development in Caenorhabditis elegans. Science, 293(5538): 2269-2271.
34. Golden, T.A., S.E. Schauer, J.D. Lang, S. Pien, A.R. Mushegian, U. Grossniklaus, D.W. Meinke and A. Ray, 2002. A Dicer homolog, Plant Physiology, 130(2): 808-822.
35. Nykanen, A., B. Haley and P.D. Zamore, 2001. ATP requirements and small interfering RNA structure in the RNA interference pathway. Cell, 107(3): 309-321.
36. Denli, A.M. and G.J. Hannon, 2003. RNAi: an ever-growing puzzle. Trends in Biochemical Sciences, 28(4): 196-201.
37. Meister, G., M. Landthaler, A. Patkaniowska, Y. Dorsett, G. Teng and T. Tuschl, 2004. Human Argonaute2 mediates RNA cleavage targeted by miRNAs and siRNAs. Molecular Cell, 15(2): 185-197.
38. Fagard, M., S. Boutet, J.B. Morel, C. Bellini and H. Vaucheret, 2000. 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. Proceedings of the National Academy of Sciences, 97(21): 11650-11654.
39. Carmell, M.A., Z. Xuan, M.Q. Zhang and G.J. Hannon, 2002. The Argonaute family: tentacles that reach into RNAi, developmental control, stem cell maintenance and tumorigenesis. Genes & Development, 16(21): 2733-2742.
40. Martinez, J., A. Patkaniowska, H. Urlaub, R. Lührmann and T. Tuschl, 2002. Single-stranded antisense siRNAs guide target RNA cleavage in RNAi. Cell, 110(5): 563-574.
41. Ganeshpurkar, A., G. Gutti and S.K. Singh, 2019. RNA-Dependent RNA Polymerases and Their Emerging Roles in Antiviral Therapy. In Viral Polymerases, pp:1-42. Academic Press.
42. Lipardi, C., Q. Wei and B.M. Paterson, 2001. RNAi as random degradative PCR: siRNA primers convert mRNA into dsRNAs that are degraded to generate new siRNAs. Cell, 107(3): 297-307.
43. Singh, P.K., R.E. Brand and K. Mehla, 2012. MicroRNAs in pancreatic cancer metabolism. Nature Reviews Gastroenterology & Hepatology, 9(6): 334.
44. Pinzon, N., S. Bertrand, L. Subirana, I. Busseau, H. Escrivá and H. Seitz, 2019. Functional lability of RNA-dependent RNA polymerases in animals. PLoS Genetics, 15(2): e1007915.
45. Hamada, M., T. Ohtsuka, R. Kawaida, M. Koizumi, K. Morita, H. Furukawa, T. Imanishi, M. Miyagishi and K. Taira, 2002. Effects on RNA interference in gene expression (RNAi) in cultured mammalian cells of mismatches and the introduction of chemical modifications at the 3'-ends of siRNAs. Antisense and Nucleic Acid Drug Development, 12(5): 301-309.
46. Moghaddam, T. and Z. Neshati, 2019. Role of microRNAs in osteogenesis of stem cells. Journal of Cellular Biochemistry, 120(8): 14136-14155.

47. Bartel, D.P., 2009. MicroRNAs: target recognition and regulatory functions. cell, 136(2): 215-233.
48. Ludwig, N., P. Leidinger, K. Becker, C. Backes, T. Fehlmann, C. Pallasch, S. Rheinheimer, B. Meder, C. Stähler, E. Meese and A. Keller, 2016. Distribution of miRNA expression across human tissues. Nucleic Acids Research, 44(8): 3865-3877.
49. Shomron, N. and C. Levy, 2009. MicroRNA-biogenesis and Pre-mRNA splicing crosstalk. BioMed Research International, 2009.
50. Lee, Y., C. Ahn, J. Han, H. Choi, J. Kim, J. Yim, J. Lee, P. Provost, O. Rådmark, S. Kim and V.N. Kim, 2003. The nuclear RNase III Drosha initiates microRNA processing. Nature, 425(6956): 415-419.
51. Ambros, V., R.C. Lee, A. Lavanway, P.T. Williams and D. Jewell, 2003. MicroRNAs and other tiny endogenous RNAs in *C. elegans*. Curr. Biol., 13(03): 807-818.
52. Fraser, A.G., R.S. Kamath, P. Zipperlen, M. Martinez-Campos, M. Sohrmann and J. Ahringer, 2000. Functional genomic analysis of C. elegans chromosome I by systematic RNA interference. Nature, 408(6810): 325-330.
53. Hu, G., J. Kim, Q. Xu, Y. Leng, S.H. Orkin and S.J. Elledge, 2009. A genome-wide RNAi screen identifies a new transcriptional module required for self-renewal. Genes & Development, 23(7): 837-848.
54. Tiscornia, G., O. Singer, M. Ikawa and I.M. Verma, 2003. A general method for gene knockdown in mice by using lentiviral vectors expressing small interfering RNA. Proceedings of the National Academy of Sciences, 100(4): 1844-1848.
55. Gupta, P.K., 2006. RNA interference–gene silencing by doublestranded RNA: The 2006 Nobel prize for physiology or medicine. Curr. Sci., 91: 1443.
56. Whitehurst, A.W., B.O. Bodemann, J. Cardenas, D. Ferguson, L. Girard, M. Peyton, J.D. Minna, C. Michnoff, W. Hao, M.G. Roth and X.J. Xie, 2007. Synthetic lethal screen identification of chemosensitizer loci in cancer cells. Nature, 446(7137): 815-819.
57. Smith, J.A., E.A. White, M.E. Sowa, M.L. Powell, M. Ottinger, J.W. Harper and P.M. Howley, 2010. Genome-wide siRNA screen identifies SMCX, EP400 and Brd4 as E2-dependent regulators of human papillomavirus oncogene expression. Proceedings of the National Academy of Sciences, 107(8): 3752-3757.
58. Shukla, S.K., V. Gunda, J. Abrego, D. Haridas, A. Mishra, J. Souchek, N.V. Chaika, F. Yu, A.R. Sasson, A.J. Lazenby and S.K. Batra, 2015. MUC16-mediated activation of mTOR and c-Myc reprograms pancreatic cancer metabolism. Oncotarget, 6(22): 19118.
59. Swanton, C., M. Marani, O. Pardo, P.H. Warne, G. Kelly, E. Sahai, F. Elustondo, J. Chang, J. Temple, A.A. Ahmed and J.D. Brenton, 2007. Regulators of mitotic arrest and ceramide metabolism are determinants of sensitivity to paclitaxel and other chemotherapeutic drugs. Cancer Cell, 11(6): 498-512.
60. Kim, D.H. and J.J. Rossi, 2008. RNAi mechanisms and applications. Biotechniques, 44(5): 613-616.
61. Fattal, E. and A. Bochot, 2006. Ocular delivery of nucleic acids: antisense oligonucleotides, aptamers and siRNA. Advanced Drug Delivery Reviews, 58(11): 1203-1223.