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RNAi: An innate gene knockdown mechanism

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ABSTRACT

RNA interference (RNAi) is an evolutionarily conserved mechanism in eukaryotic cells, It is a gene down-regulatory process occurring in both nucleus and cytoplasm at different stages of cell cycles (cell proliferation, developmental stage and cell death). In this mechanism, dsRNA derived small RNAs (19-28 nt in length) act as molecular scissor which the homologues mRNA has been degraded with the help of Argonaute protein, Dicer (RNase III like enzyme) and other cofactors these effectors-protein complex named as RNA-induced silencing complex (RISC).

KEYWORDS

RNAi;
Types of small RNA;
Gene silencing;
Methylation.

INTRODUCTION

Discovery of RNAi

Before the discovery of RNAi, homology dependent gene silencing was observed in petunia. For example, in 1990 the post transcriptional gene silencing was first observed in petunia. Initially, this phenomenon was referred as co-suppression. A similar mechanism was observed in Neurospora crassa, but was termed quelling. When chalcone synthase gene was introduced in petunia, its expression was suppressed by both transgene and homologous gene[1,2]. This phenomenon suggests that an increased copy of expressed gene leads to silencing by dsRNA either by mRNA degradation (PTGS) or by DNA methylation (TGS)[3]. Some transgenes involved in gene silencing mechanism were first observed in certain plant species. Subsequently, many eukaryotes such as nematodes, fungi, insects and protozoans also were known to exhibit such gene silencing[4].

It was Andrew Fire and Craig Mello who first reported the dsRNA mediated gene silencing in Caenorhabditis elegans. Mello coined the term RNA interference. They chose sense, anti-sense and dsRNA and injected into C.elegans. By injecting sense and antisense strand, they found no obvious change in the worm. But they observed some phenotypic effects when injected with dsRNA. From their experiments, Fire and Mello concluded that gene silencing was triggered efficiently by injected dsRNA, but weakly by sense or antisense ssRNA, silencing only on homologous of dsRNA, other mRNA were not affected, dsRNA had to directly complimentary to the mature mRNA neither intron nor promoter sequence triggered a response, The target mRNA disappeared suggesting that it was degraded, only a few dsRNA molecules per cell were sufficient to accomplish full silencing. This indicated that the dsRNA

was amplified. It could be spread between tissues and even to the progeny[5].

Classes of small RNA

Small non-coding RNAs are 19-28ntd long that can be found in diverse organisms. On the basis of its origin and biogenesis it has been broadly classified into two types, namely, siRNA and miRNA. The siRNA are 22nt long, which are derived from a long double standard RNA. Whereas, miRNAs are single stranded RNA (ssRNA) of 19-25nt long, which are derived from hairpin shaped dsRNA. The reaction is catalyzed by Dicer enzyme. TasiRNA is a 21nt long sequence, which is generated from an intronic region of the gene in Arabidopsis. Recently the TasiRNA has been identified in nematode worms. RasiRNAs are found in plants, Trypanosoma brucei, Drosophila melanogater and fission yeast. The function of rasiRNA is to form heterochromatin by arrangement of repetitive elements of the sense and anti-sense strands in the genome. During conjugation of ciliated protozoans (*Tetrahymena thermophila*) 30ntd long small scan RNA (scnRNA) has been identified that originate as an internally eliminated segmented sequence (ranges from 0.5kb to 20kb)[6,7].

(1) **TasiRNA:** Trans-acting siRNA, RasiRNA: Repeat assosciated siRNA, ScnRNA: Small scan RNA.

(2) **tncRNA:** Tiny non-coding RNA, smRNA: Small modulatory RNA, piRNA: Piwi-interacting RNA.

Recently tiny non-coding RNA (tncRNA) and small modulatory RNA (smRNA) are also identified in small RNA class. These are identified through various cloning experiments. The tncRNAs are shorter than the miRNA and is similar to miRNA that is found in *C.elegans*. The smRNA is identified from adult hyppo campal neural stem cell. The smRNA is expressed at the early stages of neural differentiation and it functions as a transcriptional modulator. However, its biogenesis is still unclear[6]. The piRNA genes are identified in mouse testes and are abundant in chromosomes 2,4,5 and 17 but lesser in intergenic regions. The piRNA sequences are frequently found in retrotransposons majority of which are clustered in short genomic loci (below 1kb to above 100kb). Its function is believed to be engaged in spermatogenesis by possibly regulating meiosis and/or suppressing tetrotransposons[7].

Mechanism of RNAi

(1) Key proteins involved in RNAi

Dicer is an enzyme highly conserved in all eukaryotic organisms. Human dicer homologues are multi domain protein of ~200kDa, 1,922 amino acids in length. It consists of two RNase III domains (RIIIDa and RIIIDb) and double stranded RNA binding domain (dsRBD). In addition, it has a long N terminal segment that consists of a PAZ domain, DEAD-BOX RNA HELICASE DOMAIN and DUF283 domain. The PAZ domain binds to the 3' ends of small RNAs, the DEAD-box RNA helicase domain hydrolyzes ATP and unwind an RNA duplex. In total, there is one Ago family protein in *S.pombe*, more than 20 in *C.elegans*, five in *Drosophila*, eight in human and about ten in *Arabidopsis*. Ago protein is about ~100kDa in size that contains two domains namely PAZ and PIWI[6,8]. PAZ domain is ~130 amino acids which located at the center of the protein. It interacts with the 3' overhang of dsRNA. The PIWI domain has ~300 amino acids and is structurally homologous to RNase H.

Human drosha enzyme is classified under RNase III family protein. It is a large protein of ~160kDa containing 1,374 amino acids, two RIIIDs domain, dsRBD, proline rich region (P-rich) and arginine and serine rich residues (RS-rich). Drosha binds with cofactor, the DGCR8 protein for processing of pri-miRNA that results to form mature miRNA the entire process occurs in nucleus. The human homologues DGCR8 (DiGeorge syndrome critical region gene8) protein is also known as Pasha in *D.melanogaster* and *C.elegans*. It is a ~120kDa protein of 773 amino acids that contains two dsRBD (dsRNA binding protein). The biochemical pathway of these proteins is still unclear[8].

(2) Transcriptional gene silencing

In mammals and plants, formation of heterochromatin (transcriptional inactive form) occurs by hypermethylation of cytosine in DNA and h methlylation in histone H3 of lysine K9 (H3K9) that is directed by small non-coding RNA . This methylation process is carried out by DNA methyltrasferase (DNMT) and Histone methyltrasferase enzyme (HMT)[9]. The functions of RNA dependent DNA and/or histone methyla-

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TABLE 1 : Classes and sub classes of small RNAs and functions

No.	Classes	Sub classes	Organism	Function
1	miRNA (19-25nt)	Not yet identified	C.elegans, D.melanogaster	Diverse functions, degradation of mRNA repression of translation
		Tasi RNA (21nt)	Arabidopsis	Regulatory endogenous cellular function.
2	siRNA (22nt)	Rasi RNA (24-26nt)	T. brucei	Heterochromatin in repetitive element of the sense, anti-sense orientation of genome.
		Scn RNA (28nt)	Tetrahymena thermophila	Methylation of H3K9
3	tncRNA (~22nt)	Not yet identified	C.elegans	Unknown
4	smRNA (~20nt)	Not yet identified	Hippo campus	Neural differentiation and its function as transcriptional modulator.
5	piRNA (26-31nt)	Not yet identified	Mouse testes	Spermatogenesis.

tion are to control gene expression and act as an epigenetic marker[10]. RNA directed DNA methylation (RdDM) process was first discovered in viral infected plants (Tobacco virus, cytoplasmic RNA viruses)[1]. Plants produce 24nt siRNA to form RdDM complex and methylation of cytosine residue at symmetrical and asymmetrical sites (CpGp and CpHpHpG H= A, C, or T) that dependupon HEN1 protein and domain rearrangement methyltransferase2 (DRD2)[10]. In nucleus, dsRNA triggered gene silencing is initiated by an aberrant transgene, inverted repeat sequence of dsRNA, or secondary siRNA produced by RNA dependent RNA polymerase (RdRP).Normally,mammals lack RdRP based production of dsRNA[9,11]. RNA dependent DNA polymerase has to synthesis secondary siRNA from an aberrant primary siRNA as a template catalyzed by dicer, these secondary 24nt siRNAs is linked to sequence specific cytosine methylation that potentially triggers transcriptional gene silencing (TGS)[11].

(3) Posttranscriptional gene silencing

The miRNA and siRNA posttranscriptional gene silencing (PTGS) process is slightly different on the basis of biogenesis and assembly of RISC complex. These differences have been identified in some eukaryotes. For example, humans and *Caenorhabditis elegans* have only one dicer enzyme, *Drosophila* has two Dicer enzymes (Dicer-1, and Dicer-2), in which short interference RNA production is associated with Dicer-2, but not Dicer-1. In Drosophila at embryo stage, maturation of miRNAs and siRNAs are required respectively by Ago1 and Ago2 for the assembly of RICS complex[12]. *Arabidopsis thaliana* contains four Dicer enzymes, in which Dicer-2, 3 and Dicer-4 involve in the production of different sizes of siRNAs (21, 22, 24 nucleotide in length), whereas Dicer-1 produces variable sizes of miRNA[11].

MicroRNAs belong to family of large endogenous RNA. A short single strandard miRNAs are formed in two phases. In nucleus, miRNA transcripts (~60-70nt) are synthesized by RNA polymerase II, which is recognized by Drosha-DGCR8 complex (microprocessor). The primary RNA (pri-miRNA contains hairpin shape indicates the stem loop, cap structure and poly A tail structure[13]. Exportin5, a nuclear membrane protein, functions in the export of priRNA into cytoplasm[14]. The Dicer (RNase III) cleaves the pri-miRNA to form short 22nt miRNA with 2nt 3' overhang. The mature miRNA is recognized by Argonaute protein and Dicer and finally forms RNA induced silencing complex (RISC), which results from the cleavage of a complementary mRNA known as PTGS[15]. In plants, miRNA target interaction is more complementary and involves within the coding regions. But animal miRNA targets are interrupted by gaps, mismatches and 3'UTR of mRNA. Some miRNAs are mostly responsible for translational repressor[16].

Long dsRNA can be synthesized from various sources such as transgenes, transposons, converge promoters by means of bidirectional transcription[14,17]. Double standard RNA can be formed by pairing of the sense RNAs and anti-sense RNAs that result from aberrant transcription of the their genes[18]. RNA-dependent RNA polymerase (RdRP) possibly involves in producing dsRNA, which triggers PTGS[4]. RdRP was present in wide varieties of organisms like plants, worms, fungi and fission yeast. This enzyme helps in the conversion of primary and aberrant transcripts into dsRNA[14]. Short interfering RNAs are generated by Dicer from exogenous or endogenous long dsRNA.In addition, a short siRNA is incorporated into a ribo-

nucleoprotein (argonaute protein), which forms a RICS complex. As a final RICS complex containing single-stranded RNA molecule, more often it involves in eliminating 2nt 3' overhang RNA. Other end of the strand is eliminated by endonucleolytical cleavage, resulting in total degradation of target mRNA[19,20]. The 3' overhangs are more efficiently processed than blunt ended RNA molecules[19]. The initial RICS containing siRNA is inactive and transforms into active form by loss of one of the strands[15].

CONCLUSION

The small RNA classes and sub classes are found in many eukaryotes, but their biogenesis, pathways and functions are quite different from one another. For example, in plants the major role of RNAi is to act as an anti-viral agent. And in mammals they are found in large numbers in germ cells, (spermatogenesis). The RNAi is believed to control the gene expression at all stages i.e., transcriptional, post transcriptional, and translational level. Its function is more advanced than ribozyme and anti-sense RNA. These results suggest that eukaryotic genome has gene down regulatory mechanisms during cell proliferation and developmental stages.

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