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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, 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).

Key words:

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 it was referred to as co-suppression, the similar mechanism also observed in Neurospora crassa but the term is quelling. An introduced chalcone synthase gene in petunia has suppressed by the expression of both the transgene and the homologous gene [1,2]. This phenomenon suggest that an increased copy of expressed gene leads to silenced by dsRNA in the way of mRNA degradation (PTGS) or DNA methylation (TGS) [3]. Some transgenes has to silence the expression of homologous loci was first observed in plants after that subsequently identified in other eukaryotes like nematodes, fungal, insect and protozoan [4].

In 1998 Andrew fire and Craig mello was published in *nature* that the dsRNA mediated gene silencing in *c.elegans* after their experiment, Mello was coined the term RNA interference. They prefer to inject sense, anti-sense and dsRNA into the worm *C.elegans* which results has there is no effects on *C.elegans* by injection of sense and anti-sense RNA, but phenotypic effect was observed in *C.elegans* with dsRNA. Fire and Mello concluded in their experiments, such as gene silencing was triggered efficiently by injected dsRNA, but weakly or not at all by sense or anti-sense ssRNA, silencing only on homologous of dsRNA, other mRNA were not affected, dsRNA had to directly complimentary to the mature mRNA. 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 19-28nt in length exist in diverse organisms, on the basis of origin and biogenesis it has broadly classified into two types such as siRNA and miRNA. The siRNA are 22nt in length derived from a long double standard RNA, whereas miRNAs are single stranded (ssRNA) of 19-25nt in length which is derived from dsRNA of hairpin shaped precursor catalyzed by Dicer enzyme. TasiRNAs are 21nt, generated from an intronic region of the gene in *Arabidopsis* recently it has been identified in nematode worms. RasiRNAs are founds in plants, Trypanosoma brucei, Drosophila melanogater and fission yeast. The function of rasiRNA is to form heterochromatin in repetitive elements of the sense anti-sense orientation of genome. Small scan RNA (scnRNA) is approximately 30nt in length, it has been identified from Ciliated protozoans (*Tetrahymena thermophila*) during conjugation an abolition of internal eliminated segment sequence (ranging from 0.5kb to >20kb) by methylation of H3K9 [6,7].

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S. No	Classes	sub-classes	Organism	Function
1.	miRNA (19-25nt)	yet not identified	*C.elegans, Droshophila Melanogaster*	Diverse Function, degradation of mRNA repression of translation.
2.	siRNA	Tasi RNA (21nt)	*Arabidopsis*,*Nematode*.	Regulatory endogenous cellular function
		Rasi RNA (24-26nt)	*Trypanosoma brucei,*	Heterochromatin in repetitive element of the sense, anti-sense orientation of genome
		Scn RNA (28nt)	*Tetrahymena thermophila*	Methylation of H3K9.
3.	tnc RNA (~20nt)	yet not identified	*C.elegans*	unknown.
4.	smRNA (~20nt)	yet not identified	hyppo campal	neural differentiation and its function as a transcriptional modulator.
5.	piRNA (26-31nt)	yet not identified	Mouse testes	Spermatogenesis.

TasiRNA: Trans-acting siRNA, RasiRNA: Repeat assosciated siRNA, ScnRNA: Small scan RNA, tncRNA: Tiny non-coding RNA, smRNA: Small modulatory RNA, piRNA: Piwi-interacting RNA.

Recently tiny non-coding RNA (tncRNA) and small modulatory RNA (smRNA) also noted in small RNA classes that they are identified through cloning experiments. tncRNAs are shorter than the miRNA, similar to miRNA that has been identified from *C.elegans.* smRNA is identified from adult hyppo campal neutral stem cell. smRNA is expressed at the early stage of neural differentiation and its function as a transcriptional modulator. Till now unclear the biogenesis of smRNA in cells [6]. piRNA genes are identified in mouse testes and enriched in chromosomes 2, 4, 5 and 17 but it slightly enriched in intergenic regions. piRNA sequences are more relevance to retrotransposons and the majority of piRNAs are clustered in short genomic loci (<1kb to >100kb). Its function may be engage in spermatogenesis possibly by regulating meiosis and/or suppressing of tetrotransposons [7].

Mechanism of RNAi

Key Proteins Involved in RNAi: Dicer is an enzyme highly conserved that is identified in all eukaryotic organisms. For example human dicer homologues are multi domain protein of ~200kDa, 1,922 amino acids in length, its consists of two RNase III domains (RIIIDa and RIIIDb) and double standard RNA binding domain (dsRBD) apart from these, 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 is to hydrolyze ATP and unwind an RNA duplex. There is one Ago family protein in *S.pombe*, more than 20 in *C.elegans*, five in *Drosophila*, eight in human and ten in *Arabidopsis*. Ago protein is about ~100kDa that contain two domain namely PAZ and PIWI [6,8]. PAZ domain is ~130 amino acids which located at the center of the protein and interact with the 3' overhang of dsRNA. The PIWI domain containing ~300 amino acids resembles structural homology to RNase H.

Human drosha enzyme is classified under RNase III family protein, it is a large proteins of ~160kDa, 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 still unclear [8].

Transcriptional Gene Silencing: In mammals and plants, hypermethylation of cytosine in DNA and Histone methlylation occurs in histone H3 at lysine K9 (H3K9) is directed by small non-coding RNA that leads to the formation of heterochromatin complex (transcriptional inactive form). This methylation process is carried out by DNA methyltrasferase (DNMT) and Histone methyltrasferase enzyme (HMT) [9]. The RNA dependent DNA and/or histone methylation might be different but these functions are to control the gene expression and act as an epigenetic marker [10]. RNA direct DNA methylation process (RdDM) 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) which depends of HEN1 protein and domain rearrangement methyltransferase2 (DRD2) [10]. In nucleus, dsRNA triggered gene silencing has been initiated by an aberrant transgene, inverted repeat sequence of dsRNA, or secondary siRNA produced by RNA dependent RNA polymerase (RdRP), 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].

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 can be identified in some eukaryotes. For examples, humans and *Caenorhabditis elegans* have only one dicer enzyme, *Drosophila* have two Dicer enzymes (Dicer-1 and Dicer-2), short interference RNA production is associated with Dicer-2, but not Dicer-1. In Drosophila at embryos stage, maturation and the function of miRNAs and siRNAs are required Ago1 and Ago2 in respectively for the assembly of RICS complex [12]. *Arabidopsis thaliana* have four Dicer enzymes, Dicer-2, 3 and Dicer-4 produces different size of siRNAs (21, 22, 24 nucleotide in length) whereas Dicer-1 produce variable sizes of miRNA [11].

MicroRNAs are a large family of endogenous RNA, a short single standard 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, named as 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 its function is to export the priRNA into cytoplasm [14]. The Dicer (RNase III) cleaves the pri-miRNA to form short 22nt miRNA with 2nt 3' overhang. The mature miRNA has recognized by Argonaute protein and Dicer to form RNA induced silencing complex (RISC) which the results as cleaves a complementary mRNA called as PTGS [15]. In plants, miRNA target interaction are more complementary and within the coding regions but animal miRNA targets are interrupted by gaps, mismatches and occurs in the 3'UTR of mRNA. Some miRNAs are mostly responsible for translational repressor [16].

There are different sources of long dsRNA like it can be synthesis from bidirectional transcription from inverted repeats of transgenes, transposons, transcription from converge promoters [14,17]. Double standard RNA can be formed by pairing of the sense RNAs and anto-sense RNAs arising by aberrant transcription of the same genes [18]. RNA-dependent RNA polymerase (RdRP) could be involved in producing dsRNA that can trigger PTGS [4]. RdRP was present in wide organism like plants, worms, fungi and fission yeast. This enzyme has converted the primary and aberrant transcripts into dsRNA [14]. Short interfering RNAs are generated by Dicer from exogenous or endogenous long dsRNA, a short siRNA is incorporated into a ribonucleoprotein (argonaute protein) which forms a RICS complex. As a final RICS complex contain single-stranded RNA molecule, more often a 2nt 3' overhang RNA and the other strand is eliminated at last endonucleolytically cleavage takes place i.e. target mRNA has degraded [19,20] 3' overhangs are more efficiently processed than blunt ended RNA molecules [19]. The initial RICS containing siRNA is inactive until it is transformed into active form, which involves loss of one strand [15].

CONCLUSION

The small RNAs classes and sub classes are exist in many eukaryotes but the biogenesis, pathways and the functions are quite different from one another. For example in plants the major role of RNAi has an anti-viral mechanism, in mammals more numbers of small RNAs identified in germ cells. The mystery of RNAi is to control the gene expression at all stages i.e., transcriptional, post transcriptional and translational level. Its action function is more advance to ribozyme and anti-sense RNA technology. These results suggest that eukaryotes genome itself have gene down regulation mechanism while cell proliferation and developmental stages.

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