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RNA INTERFERENCE: TRENDS IN SMALL REGULATORY RNAS AND GENE SILENCING

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ABSTRACT

RNAi pathway is a biologically conserved phenomenon which regulates the expression of genes through transcriptional or post-transcriptional gene silencing mechanism. The pathway involves the network of different proteins to degrade the target mRNA and triggered by small regulatory RNAs. These small regulatory RNAs are generated via action of dicer enzyme on long double-stranded RNA or hairpin RNA. Herein, we have explained the significant classes of small RNAs, their functions, and mechanism of RNAi as well. Along with, we have highlighted the recent findings in RNAi, glimpse of available small RNAs repositories, and softwares/tools for their efficient designing.

KEYWORDS: ***RNAi, Small RNAs, Small RNA databases, Designing tools/softwares.***

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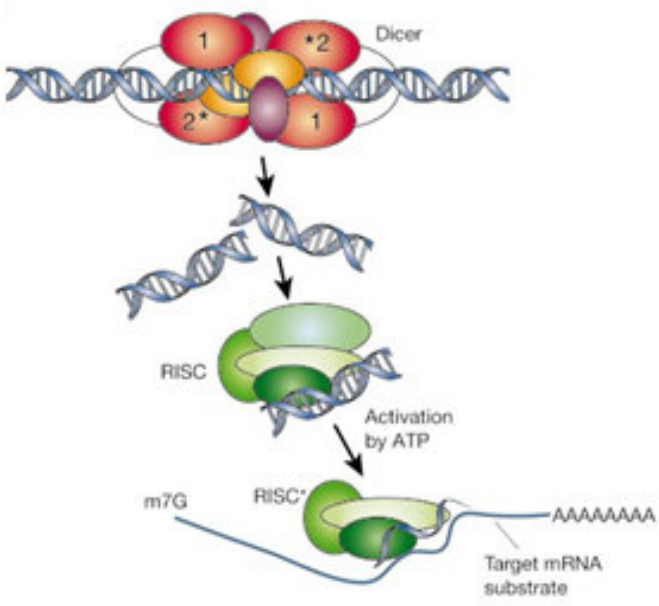
INTRODUCTION

RNA interference (RNAi) is a biologically conserved gene silencing pathway and has founded a new paradigm for getting insight of eukaryotic gene regulation[1]. It has unveiled the novel host defenses against transposons and viruses. It has become a powerful knockdown tool for the functional annotation of genome and countenance highly specific and efficient gene silencing in mammalian systems, and other animals as well[2,3]. In addition to determining the gene functions, it is holding a promise as a therapeutic strategy also. In 1990, the phenomenon was first reported by Jorgensen & Napoli and in 1992 by Macino & Romano in *Neurospora crassa*[4]. Guo and Kemphues reported that the process of gene silencing is heritable in *Caenorhabditis elegans*[5,6]. The immense potential of RNAi in research and therapeutics has been valued in 2006 by awarding the Nobel Prize in Medicine to Andrew Fire (Stanford University School of Medicine) and Craig Mello (University of Massachusetts Medical School) for uncovering RNAi[7]. It is a highly sequence-specific phenomenon and a single difference in nucleotides (nts) among the targets results in striking differences in gene silencing. Thus, this attribute of RNAi can be used to target the alleles with a single nucleotide mutation from their wild type[8]. This phenomenon has also been noticed in nematodes, protozoa, animals, viruses, insects, bacteria, plants and fungi[9-11]. In plants, post-transcriptional gene silencing (PTGS) and in fungi, quelling is fundamentally related phenomenon as they are also triggered by double-stranded (ds) RNA.

MECHANISM OF RNAi

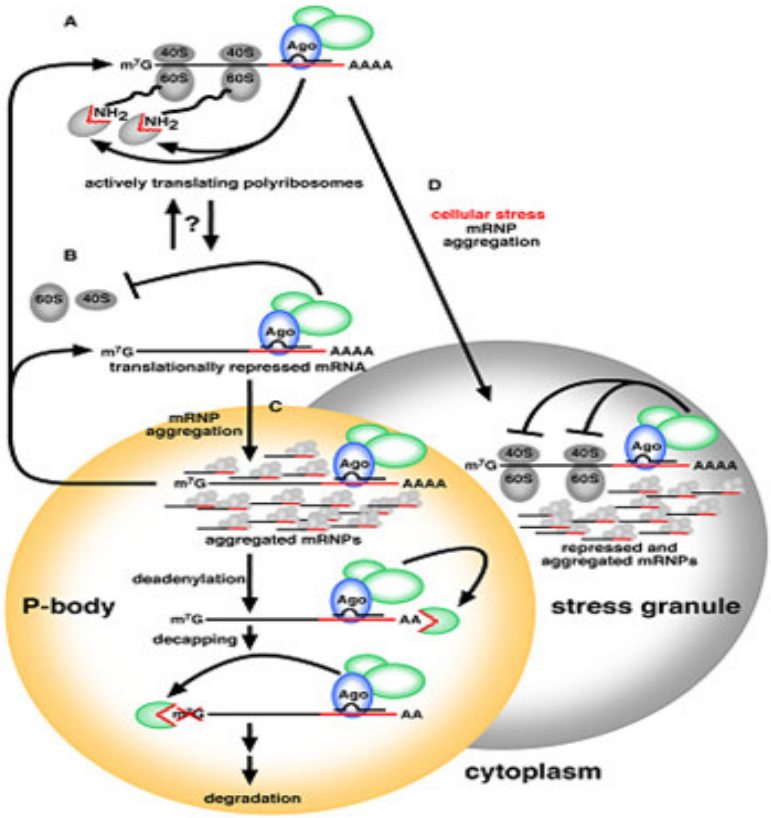
The RNAi machinery is consisting of three core components: Dicer enzyme, Argonaute protein family and RNA-dependent RNA polymerases (RDRP). To date, the proposed model to explain RNAi machinery is that the pathway is triggered by double-stranded (ds) small RNA (~21-26 nucleotide), which in turn binds to endogenous homologous messenger RNA (mRNA) and mediates its demolition[12,13], causing the silencing of gene of interest. RNAi pathway generation has briefly explained in figure 1.



Enzyme dicer initiate the RNAi and processes long double-stranded RNA or hairpin RNA into ~22-nucleotide small double-stranded RNAs[14]. These RNAs are then incorporated into RNA Induced Silencing Complex (RISC) (green) and RISC then uses the unwound small RNAs as a guide to substrate selection[15]. Reproduced with the permission from[8].

Figure 1
Diagram representing the Dicer binding and cleavage of dsRNA.

These small RNAs are generated by cleaving of dsRNA by endogenous enzyme Dicer. Dicer is having an RNase III-like activity. It acts in a complex with some other proteins such as Argonaute protein (Ago) family members, and supposed to work as a dimeric enzyme[14-15]. Subsequently, small RNAs associate to Ago proteins that together form a nuclease complex, termed as RNA-Induced Silencing Complex (RISC). Unwinding of small RNAs causes activation of RISC. As RISC becomes activated, small RNAs hybridize the target mRNA through complementary base pairing. The mRNA is then cleaved, causing silencing of corresponding gene. Translational repression of mRNA by Ago proteins has described in figure 2. RNAi has also been generated with synthetic siRNAs[16], hairpin RNAs[17], plasmid vector-based RNAi techniques[18,19], and virus-based RNAi systems[20].



These proteins stifle translation either during polysome translation (A) or during initiation of translation (B). Aggregation of stifled Ago containing mRNPs to P bodies degrade repressed mRNAs via deadenylation and decapping enzymes (C). During cellular stress polysome aggregate to form stress granules and Ago proteins stifle those mRNA translation which are inefficient for stress response (D). Reproduced with the permission from[12].

Figure 2
A model representing translational repression by Ago proteins.

The Argonaute protein family can be split into two subfamilies a) Piwi subfamily and b) Ago subfamily[21-23]. Ago subfamily is cosmically expressed, whereas Piwi subfamily's expression is restricted to germ cells[24,25]. Members of Piwi subfamily are characterized by amino-terminal: Piwi-Argonaute-Zwille (PAZ) domain and carboxy-terminal: PIWI domains[26] as their structural features (figure 3). The molecular weight of Argonaute protein has been estimated about 100 kDa[27].

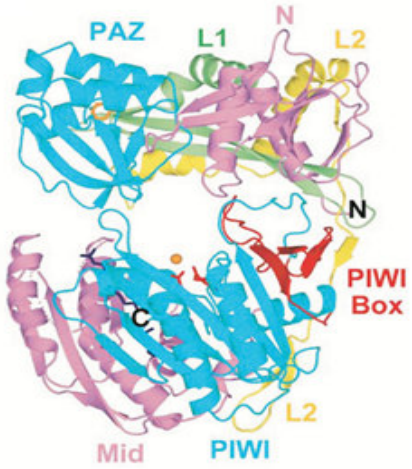


Figure 3
Structure of the Argonaute protein from Aquifex aeolicus representing that PAZ domain (blue) is linked to the amino-terminal domain (N, magenta) by linker 1 (L1, green), and connected with the MID domain (magenta) followed by the PIWI domain (blue) by linker 2 (L2, yellow) at the carboxy-terminal. Reproduced with the permission from[28].

SMALL RNAS

Small RNAs are known to be non-coding molecules. Their size varies from 20 to 40 nts. They play potential roles in repression of translation or RNA demolition, transcriptionally, by affecting chromatin structure or post-transcriptionally, by affecting translation or mRNA stability [28-30]. Thus they are a key player in RNA silencing. They are mobile molecules and guide the RISC to the target mRNA, resulting in gene degradation. They come in many pretexts based on their RNA precursor and biogenesis, such as: stRNAs, siRNAs, tncRNAs, miRNAs, qiRNAs, tRFs, milRNAs, piRNAs[31-34], scnRNAs and heterochromatic siRNAs[35]. Besides exogenous siRNAs, some endogenous siRNAs: rasiRNAs, tasiRNAs, casiRNAs, natsiRNAs have also been reported in plants and animals[36-40]. Among these some small RNAs are known to be used as RNAi reagent for cell-based High Throughput Screenings (HTSs). A comparative study of various small RNAs found in eukaryotes have been done in table I. Available useful databases of various classes of small RNAs and various tools & softwares to design them have been mentioned in table II and table III, respectively. Table IV is containing the web links of various companies and labs for designing of effective small RNAs.

Table 1
A comparative study of various small RNAs

Small RNAs	Full Name	length (Nt)	Organism	Precursor	Biogenesis	References
tasiRNAs	Trans-acting Acting siRNAs	~21	Plants	miRNA-cleaved *TAS* RNAs	DCL4	Rana 2007[12], Fire 1998[13]
piRNAs	Piwi-interacting RNAs	~23-31	In animal's germ cells	Transposons and repetitive DNA elements	AGOfamily proteins:PIWIL1, PIWIL2,PIWIL3, PIWIL4,AGO3,AGO4, AGO6	Carmell 2002[14],Hammond 2000[15], Elbashir2001[16] Paul 2002[18]
tncRNA	Tiny non-coding RNA	~20	_		Dicer	Ambros 2007[31]
casiRNA	*cis*-acting siRNAs	24	Plants	Transposons, repeats	DCL3	Hamilton 2002[38], Xie 2004[39]
rasiRNA	Repeat-associated siRNA	~24-26 (in plants) ~24-27 (in fruit flies)	Plants, fruit fly	longdsRNAs derived from repetitive sequences	Dicer	Hamilton 2002[38], Xie 2004[39]
natsiRNA	Natural antisense transcript-derived siRNAs	~21-24	Plants	Bidirectional transcripts induced by stress	DCL1,DCL2	Katiyar 2006[40]
qiRNAs	QDE-2-interacting Small RNAs	~21	Described only in *N. crassa*	Aberrant RNAs (aRNAs)	QDE-1, QDE-3, Dicer	Lee 2009[41], Li 2010[42]
tRFs	tRNA-derived RNA fragments	~20-22	*S. cerevisiae, M, oryzae, A. fumigatus*, Human	tRNA	Dicer, RNaseZ	Kawaji 2008[43], Nunes 2011[44]
siRNAs	Small-interfering RNAs	~21-24	Eukaryotes	dsRNA	Dicer, AGO	Nunes 2011[44]
esRNAs	Endogenous short RNAs	~21	*M. circinelloides*	Exonic-siRNAs	RdRp1,Dicer-like2 (DCL2)	Nicolas 2010[45]
miRNAs	MicroRNA	~21-25	Plants and Animals	Endogenous miRNA genes	DCL1, AGO	Winter 2009[46]
scnRNAs	Small-scan RNAs	~28	*Tetrahymena thermophila*	long dsRNAs by	Dicer	Sui 2002[19], Liu 2004[47]
21U-RNA piRNAs	_	~21	*Caenorhabditis elegans*	Individual transcription of each piRNA	Dicer-independent	Ruby 2006[48]
26G RNA	_	~26	*Caenorhabditis elegans*	Enriched in sperm	RdRP	Ruby 2006[48], Ambros 2007[31]
smRNA	Small modulatory RNA	~20	_	_	_	Kuwabara 2004[49]

Table 2
Known small RNAs repositories

Repositories	Attributes	Source links
HIVsirDB a) siRNAmaps b) HIVsirblast c) siRNAalign	A manually curated and freely accessible database of 750 siRNAs which can inhibit HIV.	http://crdd.osdd.net/raghava/hivsir/
HuSida	An open access human siRNA database.	http://www.human-siRNA-database.net.
siRNAdb	The siRNA sequence database gives a view of siRNA experimental data.	http://siRNA.cgb.ki.se
GenomeRNAi	Cell-based RNAi phenotypes database.	http://rnai.dkfz.de
COLT	A database of pooled shRNA with a web interface for shRNA querying and visualization.	http://colt.ccbr.utoronto.ca/cancer
RNAiAtlas	A database of siRNA libraries of human genome.	http://rnaiatlas.ethz.ch/
ASRP	Arabidopsis Small RNA Project Database.	http://asrp.cgrb.oregonstate.edu
Rfam	An open access database of miRNA and RNA family.	http://www.sanger.ac.uk/Software/Rfam/mirna/ or http://rfam.sanger.ac.uk/

Table 3
Useful web servers/ softwares for designing of effective shRNA/siRNA

Softwares/Web servers	Attributes	Source links
VIRsiRNAPred	A siRNA prediction web server against essential human virus genes, based on Support Vector Machine (SVM)	http://crdd.osdd.net/servers/virsirnapred/ submit.php
siDirect	A web tool for designing efficient and target-specific siRNA for mammalian RNAi.	http://sidirect2.rnai.jp/.
desiRM	A web application to design highly effective complementary and mismatched siRNAs.	http://www.human-siRNA-database.net.
AsiDesigner	Software for designing siRNAs for silencing of multiple genes simultaneously along with the capacity to search for off-targets with FASTA and BLAST algorithms.	http://sysbio.kribb.re.kr/AsiDesigner/
DEQOR	A web tool to design siRNA based on state-of-the-art parameters scoring system.	http://cluster-1.mpi-cbg.de/Deqor/deqor.html
OligoWalk	A web application to design siRNA which utilizes hybridization thermodynamics to predict the free energy changes during the binding of oligonucleotides to a target RNA.	http://rna.urmc.rochester.edu/servers/oligowalk
dsCheck	A web-based highly sensitive software for reckoning off-target effects for RNAi studies.	http://dsCheck.RNAi.jp/
E-RNAi	The E-RNAi, a web-based tool to design and evaluate RNAi reagents, siRNAs and esiRNAs as well.	http://e-rnai.dkfz.de/
SiDESIGN Center	An advanced web tool to design siRNA based on the likelihood of identifying functional siRNA.	http://www.dharmacon.com/designcenter/designcenterpage.aspx
Sfold (siRNA)	For rational siRNA designing.	http://sfold.wadsworth.org/cgi-bin/sirna.pl
siVirus	A web-based software for designing of highly effective, off-target minimized antiviral siRNAs and finding of highly conserved target sites for antiviral RNAi.	http://sivirus.rnai.jp/
TROD	T7 RNAi Oligo Designer (TROD) is a web tool to design oligodeoxynucleotide sequences for *in vitro* synthesis of siRNA duplexes.	http://www.cellbio.unige.ch/RNAi.html
passRNAMiner	A web-based server to predict small RNA phase clusters in plants.	http://bioinfo3.noble.org/pssRNAMiner/
psRNATarget	A server for analyzing small RNA targets in plants.	http://plantgrn.noble.org/psRNATarget/
siDRM	Disjunctive Rule Merging (DRM) algorithm based software for effective selection of siRNAs.	http://siRecords.umn.edu/siDRM/
siPRED	A support vector regression and neural networks based prediction server.	http://predictor.nchu.edu.tw/siPRED
DSIR	Web tool for siRNA (19 or 21 nt) and shRNA prediction against selected target gen.	http://biodev.extra.cea.fr/DSIR/ DSIR.html

Table 4
***Web links of various companies and labs for effective designing of small RNAs*.**

Labs/Company	Web source
Ambion	www.ambion.com/techlib/misc/siRNA_finder.html
Hannon Lab	katahdin.cshl.org:9331/portal/scripts/main2.pl
Integrated DNA Technologies	http://scitools.idtdna.com/site/order/designtool/index/DSIRNA_CUSTOM
Invitrogen	http://rnaidesigner.thermofisher.com/rnaiexpress/
Qiagen	www1.qiagen.com/Products/GeneSilencing/CustomSiRna/SiRnaDesigner.aspx
Whitehead Institute	jura.wi.mit.edu/siRNAext

RNAi IN FUNGI

The fungi kingdom is estimated to have more than 1 million species. In fungi, RNAi pathway performs three major functions: heterochromatin formation, gene regulation and genomic defense. Studies of different fungal system have revealed the occurrence of diverse classes of small RNAs and their biogenesis pathways. Major RNAi studies have been done on *Schizosaccharomyces pombe*, *Neurospora crassa*, *Cryptococcus neoformans, Saccharomyces cerevisiae, Saccharomyces castellii, Ustilago maydis, Candida albicans, Aspergillus fumigates, Aspergillus nidulans, Aspergillus oryzae, Mucor circinelloides* and *Mucor grisea*[50]. In filamentous fungi, posttranscriptional gene silencing exists, whereas heterochromatin formation at transcriptional level results in gene silencing in fission yeast[51]. As discussed above RNAi have three components (Dicer, Argonaute, RDRP) in its core, but gene silencing in *Aspergillus nidulans* is RDRP independent, and requires RsdA, a PAZ PIWI domain (PPD) protein[52].

RNAi SCREENING

Recently RNAi screening is being used to get insight on a gene function in the organisms which were not accessible for gene manipulation earlier such as planarians[53]. The screening is helping in getting the insight on health-relevant filed such as: fertility, biocide resistance, viability and innate immunity[54,55]. Cell-based RNAi screening, RNAi screening *in-vivo,* and *ex-vivo* screening are having a strong impact in understanding cancer biology, RNA biology, general cell biology, signal transduction, interactions between bacterial or viral pathogens and host cells[56]. The accessibility of transcriptomic data for cell-lines, tumors and tissues has made possible the interpretation of cell-based RNAi screening[57,58]. The RNAi reagents: dsRNAs, shRNAs, endoribonuclease-prepared siRNAs and siRNAs, are used for cell-based HTSs. But, in comparison to cell-based RNAi screening, RNAi screening *in-vivo* provides a straightforward and fast route for phenotype screening. RNAi libraries, so far, have been developed for ticks, Hydra, planarians, Lepidoptera, many plants and trypanosomes[54,55]. In this regard Retroviral Short Hairpin RNA (shRNA) and synthetic siRNA libraries are publicly available for high-throughput genetic screening and have been mentioned under table II. In addition, still there is a need for improved gene models, development of algorithms for designing more efficient RNAi reagents, generation of better genome-scale RNAi libraries, and improved methods for delivering RNAi reagents to support HTSs.

CONCLUDING REMARKS

In the past few years, RNAi has revolutionized studies of determining gene functions over other genetic approaches and manipulations. Perhaps, it has become a predominant means of appraising loss of gene function in most organisms and addressing the most fundamental queries in biological processes. Gene silencing via RNAi has led to the generation and screening of genome-wide siRNA libraries. It has opened a new era for designing specific methods to silence the gene of interest by mining the basic cellular mechanism in most of the eukaryotic cells. Treatment of genetic diseases and gene function analysis are now the most important applications of RNAi. This can be achieved by *in-vivo* delivery of shRNAs or synthetic siRNAs via viral particles, transfection of plasmid DNAs, liposome-peptide complex, lipoplexes, dendriplexes, polyplexes, microspheres and microsponges. Advancement in the understanding of mammalian RNAi has made it feasible to use this mechanism as therapeutics against a variety of human diseases. It has shown a demonstrable therapeutic potential against neurodegenerative diseases such as: Alzheimer, Amyotrophic Lateral Sclerosis (ALS), Spinocerebellar Ataxia (SCA), Huntington and Prion in animal models. Thus, RNAi has the admiring potential to cure the undruggable diseases, and its diverse applications in medicines, plants, agriculture and biology is apparently limitless.

CONFLICT OF INTEREST

Conflict of interest declared none.

REFERENCES

1. Finnegan EJ and Matzke MA. The small RNA world. J. Cell Sci. 2003; 116: 4689-4693.
2. Sandy P, Ventura A and Jacks T. Mammalian RNAi: a practical guide. Biotechniques. 2005; 39: 215-24.
3. Napoli C, Lemieux C and Jorgensen R. Introduction of a Chimeric Chalcone Synthase gene into Petunia Results in Reversible Co-Supression of Hmologous Genes in trans. Plant Cell. 1990; 2: 279-289.
4. Romano N and Macino G. Quelling: transient inactivation of gene expression in Neurospora crassa by transformation with homologous sequences. Mol. Microbiol. 1992; 6: 3343-53.
5. Grishok A, Tabara H and Mello CC. Genetic requirements for inheritance of RNAi in C. elegans. Science. 2000; 287: 2494-2497.
6. Voinnet O and Baulcombe DC. Systemic signalling in gene silencing. Nature. 1997; 389: 553.
7. Kong Q. RNAi, a novel strategy for the treatment of prion diseases, J. Clin. Invest. 2006; 116: 3101-3103.
8. Hannon GJ. RNA interference. Nature. 2002; 418: 244-251.
9. Dykxhoorn DM, Novina CD and Sharp PA. Killing the messenger, short RNAs that silence gene expression. Nat. Rev. Mol. Cell Biol. 2003; 4: 457-467.
10. Meister G and Tuschl T. Mechanisms of gene silencing by double-stranded RNA. Nature. 2004; 431: 343-349.
11. Wang J, Lu Z and Wientjes MG. Delivery of siRNA Therapeutics, Barriers and Carriers. The AAPS Journal. 2010: 12: 4.

12. Rana TM. Illuminating the silence: understanding the structure and function of small RNAs. Nat. Rev. Mol. Cell Biol. 2007; 8: 23-36.
13. Fire A, Xu S and Montgomery MK. Potent and specific genetic interference by doublestranded RNA in Caenorhabditis elegans. Nature. 1998; 391: 806–811.
14. Carmell MA, Xuan Z and Zhang MQ. The Argonaute family, tentacles that reach into RNAi, developmental control, stem cell maintenance, and tumorigenesis. Genes Dev. 2002; 16: 2733-2742.
15. Hammond SM, Bernstein E and Beach D. An RNA-directed nuclease mediates posttranscriptional gene silencing in *Drosophila* cells. Nature. 2000; 404: 293-296.
16. Elbashir SM, Harborth J and Lendeckel W. Duplexes of 21-nucleotide RNAs mediate RNA interference in cultured mammalian cells. Nature. 2001; 411: 494-498.
17. Paddison PJ, Harborth J and Lendeckel W. Duplexes of 21-nucleotide RNAs mediate RNA interference in cultured mammalian cells. Nature. 2001; 411: 494-498.
18. Paul CP, Good PD and Winer I. Effective expression of small interfering RNA in human cell. Nat. Biotechnol. 2002; 20: 505-508.
19. Sui G, Soohoo C and Affar B. A DNA vector-based RNAi technology to suppress gene expression in mammalian cells. Proc. Natl. Acad. Sci. 2002; 99: 5515-5520.
20. Brummelkamp TR, Bernards R and Agami R. Stable suppression of tumorigenicity by virus-mediated RNA interference. Cancer Cell. 2002; 2: 243-247.
21. Girard A, Sachidanandam R and Hannon GJ. A germline-specific class of small RNAs binds mammalian Piwi proteins. Nature. 2006; 442: 199-202.
22. Hutvagner G and Simard MJ. Argonaute proteins, key players in RNA silencing. Nat. Rev. Mol. Cell Biol. 2007; 9: 22-32.
23. Peters L and Meister G. Argonaute proteins, mediators of RNA silencing. Mol. Cell. 2007; 26: 611-623.
24. Sasaki T, Shiohama A and Minoshima S. Identification of eight members of the Argonaute family in the human genome. Genomics. 2003; 82: 323-330.
25. Meister G, Landthaler M and Patkaniowska A. Human Argonaute2 mediates RNA cleavage targeted by miRNAs and siRNAs. Mol. Cell. 2004; 15: 185-197.
26. Bohmert K, Camus I and Bellini C. AGO1 define a novel locus of Arabidopsis controlling leaf development. EMBO J. 1998; 17:170-180.
27. Cerutti L, Mian N and Bateman A. Domains in gene silencing and cell differentiation proteins, the novel PAZ domain and redefinition of the piwi domain. Trends Biochem. Sci. 2000; 25: 481-482.
28. Yuan YR, Pei Y and Ma JB. Crystal structure of A. aeolicus Argonaute, a site-specific DNA guided endoribonuclease, provides insights into RISC-mediated mRNA cleavage. Mol. Cell. 2005; 19: 405-419.
29. Park W, Li J and Song R. CARPEL FACTORY, a Dicer homolog, and HEN1, a novel protein, act in microRNA metabolism. Curr. Biol. 2002; 12: 1484-1495.
30. Ambros V and Chen X. The regulation of genes and genomes by small RNAs. Development. 2007; 134:1635-41.
31. Ambros V, Lee R., Lavanway A, Williams PT and Jewell D. MicroRNAs and other tiny endogenous RNAs in *C. elegans*. Curr. Biol. 2003; 13: 807-818.
32. Lee R and Ambros V. An extensive class of small RNAs in *Caenorhabditis elegans.* Science. 2001; 294: 862-864.
33. Lau N, Lim L and Weinstein E. An abundant class of tiny RNAs with probable regulatory roles in *Caenorhabditiselegans*. Science. 2001; 294: 858-862.
34. Lee R, Feinbaum R and Lagos-Quintana M. Identification of novel genes coding for small expressed RNAs. Science. 2001; 294: 853-858.
35. Reinhart B and Bartel D. Small RNAs correspond to centromere heterochromatic repeats. Science. 2002; 297: 1831.
36. Vazquez F. Endogenous *trans*-acting siRNAs regulate the accumulation of *Arabidopsis* mRNAs. Mol. Cell. 2004; 16: 69-79.
37. Peragine A, Yoshikawa M and Wu G. Poethig RS. *SGS3* and *SGS2*/*SDE1*/*RDR6* are required for juvenile development and the production of *trans*-acting siRNAs in *Arabidopsis*. Genes Dev. 2004; 18: 2368-2379.
38. Hamilton A, Voinnet O, Chappell L and Baulcombe D. Two classes of short interfering RNA in RNA silencing. EMBO J. 2002; 21**:** 4671-9.
39. Xie Z, Johanson LK, Gustafson AM, Kasschau KD, Lellis AD, Zilberman D, Jacobsen SE and Carrington Jc. Genetic and functional diversification of small RNA pathways in plants. PLoS Biol 2004; 2: E104.
40. Katiyar-Agarwal S, Morgan R, Dahlbeck D, Borsani O, Villegas A Jr, Zhu Jk, Staskawicz BJ and Jin H. A pathogen-induced endogenous siRNA in plant immunity. Proc. Natl. Acad. Sci. 2006; 103: 18002-7.
41. Lee HC, Chang SS, Choudhary S, Aalto AP, Maiti M and Bamford DH. qiRNA is a new type of small interfering RNA induced by DNA damage. Nature. 2009; 459: 274-7.
42. Li L, Chang SS and Liu Y. RNA interference pathways in filamentous fungi. Cell Mol. Life Sci. 2010; 67: 3849-63.
43. Kawaji H, Nakamura M, Takahashi Y, Sandelin A, Katayama S, Fukuda S, Daub CO, Kai C, Kawai J, Yasuda J, Carninci P and Hayashizaki Y. Hidden layers of human small RNAs. BMC Gemonics. 2008; 9: 157.
44. Nunes CC, Gowda M, Sailbery J, Xue M, Chen F, Brown DE, Oh Y, Mitchell TK and Dean RA. BMC Genomics. 2011; 12: 288.
45. Nicolas FE, Moxon S, de Haro JP, Calo S, Grigoriev IV, Torres-Martinez S, Moulton V, Ruiz-Vazquez RM and Dalmay T. Endogenous short RNAs generated by Dicer 2 and RNA-dependent

RNA polymerase 1 regulate mRNAs in the basal fungus Mucor circinelloides. Nucleic Acids Res. 2010; 38: 5535-41.
46. Winter J, Jung S, Keller S, Gregory RI and Diederichs S. Many roads to maturity: microRNA biogenesis pathways and their regulation. Nat. Cell Biol. 2009; 11: 228-34.
47. Liu Y, Mochizuki K and Gorovsky MA. Histone H3 lysine 9 methylation is required for DNA elimination in developing macronuclei in Tetrahymena. Proc. Natl. Acad. Sci. 2004; 101: 1679-84.
48. Ruby JG, Jan C, Player C, Axtell MJ, Lee W, Nusbaum C, Ge H and Bartel DP. Large-scale sequencing reveals 21U-RNAs and additional microRNAs and endogenous siRNAs in C. elegans. Cell. 2006; 127**:**1193-207.
49. Kuwabara T, Hsieh J, Nakashima K, Taira K and Gage FH. A small modulatory dsRNA specifies the fate of adult neural stem cells. Cell. 2004; 116: 779-93.
50. Dang Y, Yang Q, Xue Z and Liu Y. RNA interference in fungi: pathways, functions, and applications. Eukaryot Cell. 2011; 10: 1148-55.
51. Chicas A, Forrest EC, Sepich S, Cogoni C and Macino G. Small interfering RNAs that trigger posttranscriptional gene silencing are not required for the histone H3 Lys9 methylation necessary for transgenic tandem repeat stabilization in Neurospora crassa. Mol Cell Biol. 2005; 25:3793-801.
52. Hammond TM and Keller NP. RNA silencing in Aspergillus nidulans is independent of RNA-dependent RNA poplymerases. Genetics. 2005; 169: 607-17.
53. Perrimon N, Ni JQ and Perkins L. In vivo RNAi: today and tomorrow. Cold Spring Harb Perspect Biol. 2010; 2: a003640.
54. Schumann Burkard D, Jutzi P and Roditi I. Genome-wide RNAi screens in bloodstream form trypanosomes identity drug transporters. Mol Biochem Parasitol. 2011; 175: 91-4.
55. Baker N, Alsford S and Horn D. Genome-wide RNAi screens in African trypanosomes identify the nifurtimox activator NTR and the eflornithine transporter AAT6. Mol Biochem Parasitol. 2011 176**:** 55-7.
56. Mohr S, Bakal C and Perrimon N. Genomic screening with RNAi: results and challenges. Annu. Rev. Biochem. 2010; 79**:** 37-64.
57. Neumuller RA and Perrimon N. Where gene discovery turns into systems biology: genome-scale RNAi screens in Drosophilla. Wiley Interdicip Rev. Syst Biol Med. 2010; 3: 471-8.
58. Nijwening JH & Beijersbergen RL. Using large-scale RNAi screens to identify novel drug targets for cancer. Investig Drugs J. 2010; 13: 772-7.

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