Roumanian Society of Biological Sciences

microRNA a macro Revolution in Medical Biotechnologies

MIHAI BURLIBAŞA[1], LILIANA BURLIBAŞA[2], LORELAI BIANCA GAVRILĂ[3], VALERIA ROSALINDA GAVRILĂ[3], LUCIAN GAVRILĂ[2]
[1] *University of Medicine and Pharmacy „Carol Davila", Bucharest, Romania*
[2] *University of Bucharest, Institute of Genetics, Romania*
No 1-3 Aleea Portocalilor, Sector 6, Bucharest, Romania
[3] *Toronto University, Canada*
Corresponding author: e-mail: mburlibasa@gmail.com; liliana_burlibasa@yahoo.com.au

Abstract

Over the past decade RNA interference mechanism has emerged as a natural pathway for silencing gene expression. Starting with the field of plant biotechnology in early 90s, our knowledge about RNAi proved presently its enormous potential for engineering the control of gene expression, as well as for its use as a tool in functional genomics. The predominantly practical significance of RNAi is conferred by its application as a therapeutic mechanism. RNAi may yield RNA based drugs to treat human diseases and it proved to be efficient in control of some parasites interaction with their host genome.

This article is based on a review of the literature referring to RNA from the PubMed database.

Keywords: RNAi, medical biotechnologies, silencing genes

A brief history of nucleic acids discoveries awarded with Nobel Prize

The genetic material was identified as deoxyribonucleic acid in 1944 [1] and the double-helical nature of DNA was discovered in 1953 by Crick F. C., Watson J. D. and Wilkins M., recipients of the Nobel Prize in Physiology and Medicine, in 1962 [2]. After these landmark discoveries, the main problem for eukaryotes was to elucidate how nuclear DNA could govern protein synthesis in the cytoplasm. In 1961, Jacob F. and Monod J. L. presented a visionary gene control model, known as *operon hypothesis*, for which they received the Nobel Prize in Physiology and Medicine in 1965 [2]. In their proposed model a gene is transcribed into specific RNA species – messenger RNA (mRNA). Soon afterwards, in 1968, Nirenberg M and Khorana H.G. received the Nobel Prize in Physiology and Medicine for deciphering the genetic code that could assign specific codons (i.e. triplets of nucleotides) to one specific aminoacid from the known natural twenty amino acids. After all these findings RNA was believed to correspond to a continuous nucleotide sequence in the DNA, until Gilbert W. (Nobel Prize in Chemistry, 1980) discovered that in eukaryotes, the gene is a mosaic composed of sequences coding for aminoacids, called *exon,* which are separated by noncoding sequences that were called *introns*. In 1977, Sharp P. and Roberts R. (Nobel Prize in 1993) revealed that the mRNA sequence could be distributed discontinuously until it undergoes posttranscriptional processing (i.e. splicing) represented by cleavage and removal of *introns* followed by rejoining of *exons* into a mature mRNA. This mature RNA carries to the cytoplasm the genetic message for the polypeptide synthesis inside the ribosomes. The two researchers suggested that the mRNA sequences, the *exons,* are likely to be cut out from the primary transcript and spliced, while the *introns* are degraded. From this outstanding finding important evolutionary implications have emerged. In 1989 Altman S.

and Cech T. received the Nobel Prize in Chemistry for the finding that some RNA precursors are capable to catalyze their own replication and also the posttranscriptional processing of other RNA molecules. All these discoveries led to the *ribozyme concept*, one of the most unexpected findings after the double helix model of DNA [2].

From all these findings an "RNA world" has emerged, which is supposed to represent the first genetic material which lately evolved as the DNA world [2]. A large number of small RNA (snRNA) molecules are coupled and work together with proteins in ribonucleoprotein complexes (RNP). These complexes have several roles: they affect replication (telomerase), transcription (snRNA bound to elongation factors), translation (by signal recognition particle –SRP), chromatin architecture (X chromosome inactivation by XIST RNA), RNA processing (small nucleolar RNA-snoRNA) and RNA editing (by guide RNA).

The gene expression is one of the most important fundamental processes for all living organisms. In 1984 a link between RNA and the gene expression was shown in bacterium *Escherichia coli:* a special short molecule of RNA can inhibits translation through the binding to a complementary sequence in a specific mRNA. [3]

The gene expression is one of the most important fundamental processes for all living organisms. In recent years, new mechanisms of regulation of gene expression have been discovered. These mechanisms are based on complementary interaction of siRNA (small interfering RNA, 20-30 nucleotide sequences) with their mRNA target or nascent transcripts within chromatin. [4,5,6] This siRNA-mRNA recognition results in inhibition of translation and the interaction of siRNA with nascent transcript may be accompanied by heterochromatinization and chromatin silencing.

The first hints conferring the existence of the gene silencing mechanism called *RNA interference* emerged from work on genetic modification on plants in the late '80s and the beginning of '90s. Attempts to deepen the violet hue petunias by expressing higher level of an enzyme (chalconesynthase) involved in the synthesis of the anthocyanin pigment unexpectedly resulted in the appereance of many white flowers. Researchers observed that the introduction of extracopies of the gene had caused a decrease in its expression rather than the anticipated increase [7, 8]. The process which has been referred to for such a strange behavior of a transgene in a plant host genome was described as an epigenetic modification of DNA which left the DNA sequence unchanged, but instead had a negative effect upon the expression of the same DNA sequence. Later, similar relationship between the transcriptional status and epigenetic modifications of the genetic material has been identified in *Neurospora crassa* and *Caenorhabditis elegans* and was defined by the term Transcriptional Gene Silencing –TGS [9, 10, 11]. Another type of epigenetic control of gene expression has been later identified in plants and the other two model organisms: the so called Posttranscriptional Gene Silencing –PTGS [7, 8, 12, 13, 14] is a mechanism based on the initial described RNA turnover in cytoplasm, which may be triggered by the rapid increase in RNA concentration and consequently the activation of a RNA dependent RNA polimerase detected in transgenic plants obtained for viroses resistance. Such plants were transformed by cDNA for capsidial protein transfer in their genome and the corresponding RNA showed a rapid turnover that basically determined the PTG Silencing of that critical gene for virosis development [15]. Also, in the fungus *Neurospora crassa*, it was shown that an over expressed transgene can also induce gene silencing at the posttranscriptional level, a phenomenon referred to as *"quelling"* [16].

Analysis of viral infection in plants gave further insights into the mechanism of PTGS [2, 17, 18]. However, even if it was evident that RNA played a crucial role in gene silencing, the phenomenon remain unexplained until the discovery of RNA interference which provided

a most unexpected and exciting explanation that proved to offer many benefic medical and biotechnological consequences.

RNAi (RNA interference) refers to the introduction of homologous double stranded RNA (dsRNA) to target a gene's product in a specific way, resulting in null or hypomorphic phenotypes. The use of antisense RNA to interfere with a gene's activity in *C. elegans* was first utilized by Su Guo and Ken Kemphues to study *par-1* gene expression; it was reported that control sense RNA also produced a *par-1* mutant phenotype [19]. Subsequently, Fire and Mello (1998) [20] demonstrated that the presence of *dsRNA,* formed from the annealing of sense and antisense strands present in the in vitro preps, is responsible for producing the interfering activity. Introduction of *dsRNA* into an adult worm results in the loss of the targeted endogenous *mRNA* from both the adults and its progeny. Andrew Fire and Craig Mello published their break-through study on the mechanism of RNA *interference* in *Nature* in 1998. Due to the fact that both sense and antisense RNA could cause silencing, Mello argued that the mechanism could not just be a pairing of antisense RNA to mRNA, and he proposed the term *RNA interference* for the unknown mechanism [21]. Hailed the "**Breakthrough of the year**" in 2002 by *Science magazine* and representing a staple research tool in industry and academia, *RNAi technology* has gone from proof of principle in animals models to human clinical trials in less than 3 years. As a recognition of the importance of this breakthrough, the Nobel Prize was awarded to Fire and Mello, in 2006, 8 years after the publication of their paper in *Nature*.

RNAi pathway

The discovery of phenomenon known as *RNA interference* has demonstrated that exogenously administered or artificially expressed double stranded RNAs selectively inhibit expression of target genes with homologous nucleotide sequence [22]. This effect is associated with formation of small interfering RNA (siRNA) of 21-23 nucleotides in length. Small interfering RNA strands are crucial to the RNAi process and have complementary nucleotide sequences to the target RNA strand. A type of RNA transcribed from the genome itself, *microRNA (miRNA)*, works in the same way.

RNAi is an RNA-dependent gene silencing process that is controlled by the *RNA-induced silencing complex (RISC)* and is initiated by short *dsRNA* molecules in cytoplasm were they interact with the catalytic *RISC* component *argonaute*. [23]

Exogenous *dsRNA* initiates *RNAi* by activating the ribonuclease protein *Dicer* (a member of *RNase III family*) [23] which binds and cleaves double-stranded RNAs in an ATP-dependent processive manner, in fragments of 21-23 nucleotides with overhang bases on each end. These short *dsRNAs* are called *siRNA*. Such *siRNA* are then separated into single-strand molecules and integrated in active RISC complex. The active component of the *RNA-induced silencing complex* is an endonuclease called *argonaute protein* which cleaves the target *mRNA* strand complementary to their bound *siRNA*. Only one of the two strands which is known as the guide strand binds the argonaute protein and directs gene silencing. The other strand is degraded during *RISC* activation [24]. It is not yet understood how *RISC* complex locates complementary *mRNAs* in cell, but it is known that Argonaute protein is localized in specific regions in the cytoplasm called *P-bodies*, regions with high rates of *mRNA decay* [25]. The *miRNA* activity is also clustered in *P-bodies* [26] and disruption of these cytoplasmic particles decreases the efficiency of RNA interference, suggesting that they are involved in critical steps of the *RNAi* process [27].

Because of remarkable potency or *RNAi* in some organisms, an amplification step within the RNAi pathway has also been proposed. Amplification could occur by a copying of

the input *dsRNAs*, which would generate more *siRNA* or by specific mechanism of replication of the *siRNAs* themselves.

The phenomenon of RNA interference includes the endogenously induced gene silencing effects of *miRNA* as well as silencing triggered by foreign *dsRNA*. The *siRNA* derived from long *dsRNA* precursors differ from *miRNAs*. miRNAs especially those in animals have incomplete base pairing to a target and inhibit the translation of many different mRNAs with similar sequences. In contrast, *siRNAs* typically base-pair perfectly and induce mRNA cleavage only in a single specific target [28]. In *Drosophila* and *C. elegans miRNA* and *siRNA* are processed by distinct argonaute proteins and Dicer enzymes [29, 30].

In vivo Significance of RNAi and its possible applications
1. RNAi protects against viral infection;
2. RNAi secures genome stability by keeping mobile elements silent;
3. RNAi-like mechanisms keep chromatin condensed and suppress transcription
4. RNAi-like mechanisms repress protein synthesis and regulate the development of organisms;
5. RNAi might be a useful approach in future gene therapy.
6. RNAi offers a new experimental tool to repress genes in a specific way; [2]

Application of RNAi in medicine
Long before RNA interference had been established as an operating mechanism in mammalian cells, researchers working on worms had recognized the great power it promised as a research tool. The sequencing of the human genome has led to a situation in which the identities of very large numbers of genes are known but little is understood about their function. *RNA interference* allows rapid analysis of the effect of loss of gene function at the cellular level that would have taken much time by homologous recombination.

The recent success in triggering the *RNAi* pathway in vertebrate systems opens the door to direct use of *dsRNA* molecules as therapeutic agents with exquisitely controllable specificity to alleviate human disease.

There are presently several examples of RNAi uses in medical approaches. The presence of extremely low levels of viral *dsRNA* triggers an interferon response called *acute phase response*, and the activation of a *dsRNA responsive protein kinase (PKR)*. PKR phosphorylates and inactivates translation factor *EIF-2a* leading to activation of 2',5' oligo adenylate synthetase, finally resulting in *RNAase L* activation. This cascade induces a global non-specific suppression of translation, which in turn triggers apoptosis [31]. In contrast, small *dsRNA* called *siRNA* specifically switched off genes in human cells without initiating the acute phase response. Thus, these *siRNAs* are suitable for gene target validation and therapeutic applications in many species including humans.

Another application of *RNAi* includes multiple targets to neutralize HIV. These could be targets that block entry into the cell and disrupts the virus reproduction cycle inside the cells. This technology will help researchers dissect the biology of HIV infection and design drugs based on this molecular information [32]. Researchers from Hope Cancer Center in Duarte have developed a DNA-based delivery system in which human cells are generated that produce siRNA against REV protein, which is important in causing AIDS [33].

RNAi might also allow future treatments of human disease such as *Lou Gehrig's disease (amyotrophic lateral sclerosis, ALS)*. This pathological condition is inherited in a genetically dominant way within some families. This pattern of inheritance allowed identification of a specific gene that is linked to the death of *Betz* cells in some families, the gene for an enzyme called superoxide dismutase. *Superoxide dismutase* can protect cells from

molecular damage caused by free radicals of oxygen. Normally, oxygen despite de fact that is a corrosive chemical, is generally thought of as being an absolute necessity for human life, but the human body must also constantly protect itself from deleterious effects of highly chemically reactive oxygen molecules. Mutant forms of superoxide dismutase can lead to cell death. Existing treatment options for *Lou Gehrig's* disease are very limited. For example, Riluzole is a drug that inhibits the ability of some brain cells to generate the electrical signals that are transmitted directly to their axons [34]. The Nobel Prize-winning research on RNA inhibition might lead to new treatments for patients with *Lou Gehrig's disease* due to dominant mutations in the superoxide dismutase gene. Reduction in the level of the superoxide dismutase enzyme coded by the mutated gene has been studied in animal models of *Lou Gehrig's disease. Superoxide dismutase* is a major protein in the brain and spinal cord, so it is a challenge to find ways to significantly reduce production of this protein in the movement control neurons [35]. Working with laboratory mice as an experimental model system for the human disease *ALS,* Miller and coworkers showed that loss of muscle function could be slowed using *RNA interference* [36]. This result was obtained by using a virus to induce RNA interference in neurons. Recent results indicate that disease-causing superoxide dismutase that is present in non-neuronal cells also contributes to the death of movement control cells and progression of the disease [37]. These results from laboratory experiments suggest that if RNA-induced inhibition of mutant superoxide dismutase can be induced in the correct cells of the brain and spinal cord, it might be possible to slow progression of *Lou Gehrig's disease* in humans.

Researchers from *National Cancer Institute (NCI),* part of the *National Institute of Health* of USA, have developed a new method to identify genes that keep cancer cells active and that could be potential targets of anticancer therapies. The method uses RNAi technology for screening those genes in cancer cells that, when silenced, cause cancer cells to die or stop dividing. The screen is similar to those used to mutate and to study genes in laboratory. In this case, *RNAi* is pointed out to reduce the activity of a specific gene in a living cancer cell, and then to see whether the cell can survive. *RNAi* alters the levels of RNA in a cell, thereby reducing the amount of protein produced by the targeted gene [38].

DNA microarray technology has now enabled to evaluate under any condition the level of expression of every gene in the genome. This has led to a vast accumulation of information about genes whose expression is significantly altered in various disease states. The ability of *RNAi* to provide a relatively easy way to gene silencing has opened up the possibility of using collections of *siRNA*s to analyze the significance of thousands of different genes whose expression is known to be up-regulated in a disease.

Other approach is to use large pools of *RNAi viral vectors* and apply a selective pressure that only cells with the desired change in behaviour can survive. The identity of the genes *knocked down* in the surviving cells can then be identified by sequencing the *RNAi* vectors. This method is being used to investigate genes involved in neurodegenerative diseases, diabetes and cancer.

Over 30 pharmaceutical and biotechnology companies have elaborated an active RNAi based drug development program which is envisaged for the implementation of new therapeutics that acts to silence disease associated genes. This web focus (www.rnaiweb.com) [39] collects together the latest research covering the development of *RNAi* based tools for drug target and gene function analysis. These include *Sirna Therapeutics* (Colorado) for macular degeneration; *Avocel* (Sunnyvale, California) for hepatits C; *Alnylam Pharmaceuticals* (Cambridge) for Parkinson's disease; *CytRx* (Los Angeles, California) for obesity, type II diabetes and *ALS* etc. But the major challenge in turning *RNAi* into an

effective therapeutic strategy is the delivery of the *RNAi* agents, whether they are synthetic short double stranded RNAs or viral vectors directing production of *double stranded RNA*.

Given sufficient research into delivery methods, some of these diseases will probably be very soon treated effectively by *RNAi based therapeutics*.

Conclusions

The finding that cells have a special endogenous mechanism for suppressing the expression of homologous genes by recognizing and processing double-stranded RNA was totally unexpected and has dramatically expanded our knowledge of gene control.

The discovery of RNAi has provided us with a powerful new experimental tool to study the function of genes and also generates expectations about its future in medicine.

References

1. AVERY, OT, MACLEOD, CM and McCARTY, M – Studies on the chemical nature of the substance inducing transformation of *Pneumococcal types*. J. Exp. Med, **79**, 137-158 (1944)
2. http://nobelprize.org
3. MIZZUNO, T., CHOU, MY. and INOUYE, M – A unique mechanism regulating gene expression: Translational inhibition by a complementary RNA transcript (micRNA), Proc. Natl. Acad. Sci., **81** 1966-1970 (1984)
4. BERNSTEIN, E., and ALLIS, CD. – RNA meets chromatin, Genes Dev., **19**, 1635-1655 (2005)
5. CERUTTI, H., and CASAS-MOLLANO, JA. - On the Origin and Functions of RNA- Mediated Silencing: From Protists to Man, Curr. Genet., **50**, 81-99 (2006)
6. SONTHEIMER, EJ. - Assembly and function of RNA silencing com-. plexes. Nat**.** Rev**.** Mol. Cell Biol**. 6**, 127–138 (2005)
7. VAN DER KROL, AR., MUR, LA., BELD, M. MOL, JN., STUITJE, AR. – Flavonoid genes in petunia: addition of a limited number of gene copies may lead to a supression of gene expression. Plant Cell, **2 ,** 291-299 (1990)
8. NAPOLI, C., LERNIEUX, C., JORGENSEN, R – Introduction to a chimeric chalocone synthase gene into petunia results in reversible co-suppresion of homologous genes in trans, Plant Cell, **2,** 279-289 (1990)
9. MATZKE, M., PRIMING, M., TRNOVSKY, J., and MATZKE, A. – Reversible methylation and inactivation of marker genes in sequentially transformed plants. EMBO J. **8**, 643-649 (1989)
10. WASSENEGGER, M., HEIMES, S., RIEDEL, L., and SANGER, H. – RNA-directed de novo methylation of genomic sequences in plants. Cell **76**, 567-576 (1994)
11. PARK, YD., MOSCONE, EA., IGLESIS, VA., VAUCHERET, H., MATZKE, AJM., and MATZKE, MA. – Gene silencig mediated by promoter homology occurs at the level of transcription and results in meiotically heritable alterations in methylation and gene activity. Plant J., **9**, 183-194. (1996)
12. SMITH, CSJ., WATSON, CF., BIRD, CR., RAY, J., SCHUCH, W., and GRIERSON, D. – Expression of a truncated tomato polygalacturonase gene inhibits expression of the endogenous gene in transgenic plants. Mol. Gen. Genet. **224**, 447-481 (1990)
13. DE CARVALHO, F., GHEYSON, G., KUSHNIR, S., VAN MONTAGU, M., INZE, D – Suppression of β-1,3 –glucanase transgene expression in homozygous plants EMBO J., **11**, 2595-2602 (1992)
14. VAN BOKLAND, K, VAN DER GEEST, N., MOL, J., KOOTER, J. – Transgene-mediated suppression of chalcone synthase expression in *Petunia hybrida* results from an increase in RNA turnover. Plant J., **6**, 861-877 (1994)
15. CUCU,N, GAVRILA, L, GHETEA L. - Epigenetic information and variation of transgene expression in plants, Roumanian Biotechnological Letters, **2**, , 183-191 (1997)
16. RUVKUN**,** G. **-** Glimpses of a tiny RNA world. Science **294**: 797-799, (2001**)**
17. LINDBO, J, SILVA-ROSALES, L., PROEBSTING, W., and DOUGHERTY, W. – Introduction of a highly specific antiviral state in transgenic plants: Implications for regulation of gene expression and virus resistance. Plant Cell, **5**, 1749-1759 (1993)
18. DOUGHERTY, WG., and PARKS, TD. – Trangenes and suppression: telling us something new? Curr. Opin. Cell Biol. **7**, 399-405 (1995)

19. GUO, S., and KEMPHUES, K. - *par-1*, a gene required for establishing polarity in *C. elegans* embryos, encodes a putative ser/thr kinase that is asymmetrically distributed. Cell **81**: 611-620 (1995)
20. FIRE, A., XU, S., MONTGOMERY, MK., KOSTAS, SA., DRIVER, SE., and MELLO, CC. – Potent and specific genetic interference by double-stranded RNA in *Caenorabditis elegans*. Nature, **391**, 806-811 (1998)
21. ROCHELEAU, CE, DOWNS WD, LIN, R, WITTMAN, C, BEI, Y, CHA, YH, ALI, M, PRIESS JR and MELLO, CC - Wnt signaling and an APC-related gene specify endoderm in early *C. elegans* embryos. Cell 90, 707-716. (1997)
22. MELLO, CC and CONTE JR, D - Revealing the world of RNA interference. Nature 431, 338-342, (2004).
23. BERNSTEIN, E., CAUDY, A., HAMMOND, S., HANNON, G. - Role for a bidentate ribonuclease in the initiation step of RNA interference. *Nature*, **409**, 363–366, (2001)
24. RICHARD I. GREGORY, THIMMAIAH P. CHENDRIMADA, NEIL COOCH, and RAMIN SHIEKHATTAR - Human RISC Couples MicroRNA Biogenesis and Posttranscriptional Gene Silencing, Cell, **123**, 631-640 (2005)
25. SEN, G., WEHRMAN, T., BLAU, H. – mRNA translation is not prerequisite for small interfering RNA-mediated mRNAs cleavage, Differentiation, **73**, 287-293 (2005)
26. LIAN, S., JAKYMIW, A., EYSTATHIOY, T., HAMEL, FRITZLER, M., CHAN, E. – GW bodies, microRNAs and the cell cycle, Cell Cycle, **5**, 242-245 (2006)
27. JAKYMIW, A., LIAN, S., EYSTATHIOY, T., LI, S., SATOH, M., HAMEL, J., FRITZLER, M., CHAN, E. – Disruption of P bodies impairs mammalian RNA interference , Nat Cell Biol **7**, 1267-1274 (2005)
28. http://en.wikipedia.org/RNAinterference
29. OKAMURA. K., ISHIZUKA, A., SIOMI, H. AND SIOMI, M. C. - Distinct roles for Argonaute proteins in small RNA-directed RNA cleavage pathways. Genes Dev. 18(14): 1655-1666 (2004).
30. LEE SS. – Come one, come all, Sci Aging Knowl Environ. **18**, 18 (2004)
31. GILL J, ESTEBAN M. - Induction of apoptosis by the dsRNA-dependent protein kinase (PKR): mechanism of action. Apoptosi*s*;**5**:107–114.(2000)
32. ANANTHALAKSHMI, P and SUTTON, R. – Titers of HIV-based vectors encoding shRNAs are reduced by a Dicer-dependent mechanism, Mol Therapy, **16**, 378-386 (2008)
33. YU, JY., DERUITER, SL., and TURNER, DL., - RNA interference by expression of short interfering RNAs and hairpin RNAs in mammalian cells , PNAS , **99**, 6047-6052 (2002)
34. KONONENKO, N.I., SHAO L-R., DUDEK, FE. – Riluzole-sensitive slowly inactivating sodium current in rat suprachiasmatic nucleus neurons, J. Neurophys., **91**, 710-718 (2004)
35. SMITH, RA., MILLER, TM., YAMANAKA, K., MONIA, BP., CONDON, TP., HUNG, G., LOBSIGER, CS., WARD, CM., WEI, H., WANCEWICZ, BENNETT CF., and CLEVELAND, DW. – Antisense oligonucleotide therapy for neurodegenerative disease, J. Cli Inv., **116**, 2290-2296 (2006)
36. MILLER, M., KASPAR, KP., KOPS, GJ., YAMANAKA, K., CHRISTIAN, LJ., GAGE, FH., CLEVELAND, DW. – Virus –delivered small RNA silencing sustain strength in amyotrophic lateral sclerosis, Ann Neurology, **57**, 773-776 (2006)
37. BOILLÉE, S., YAMANAKA, K., LOBSIGER, CS., COPELAND, NG., JENKINS, NA., KASSIOTIS, G., KOLLIAS, G., CLEVELAND, DW – Onset and progression in inherited ALS determined by motor neurons and microglia, Science, **312**, 1389-1392 (2006)
38. http://www.cancer.gov
39. http://www.rnaiweb.com