

We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

4,800

Open access books available

122,000

International authors and editors

135M

Downloads

Our authors are among the

154

Countries delivered to

TOP 1%

most cited scientists

12.2%

Contributors from top 500 universities



WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index
in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Numbers displayed above are based on latest data collected.

For more information visit www.intechopen.com



RNA Interference – A Hallmark of Cellular Function and Gene Manipulation

Ibrokhim Y. Abdurakhmonov

Additional information is available at the end of the chapter

<http://dx.doi.org/10.5772/62038>

Abstract

The discovery of RNA interference (RNAi) and its utilization in downregulation of specific target transcripts have revolutionized gene function analysis and elucidation of many key biochemical/genetic pathways. The insights into gene function, combined with a technology that made silencing of gene function possible using the potent, highly specific and selective RNAi approaches, provided the solution to longstanding complex obstacles in targeted crop improvements for agriculture, and disease therapies for medicine. In this introductory chapter, I aim briefly to cover the basics and peculiarities of RNAi and the advances made in understanding the mechanisms, components, function, evolution, application, safety and risk assessment of RNAi, while at the same time highlighting the related chapters of this book.

Keywords: Gene silencing, RNA interference, RNAi inducers and delivery, RNAi-based disease therapy, biosafety

1. Introduction

The “central dogma” of genetics as first presented by Francis Crick is that genes, packed inside the cell as the deoxyribonucleic acid (DNA) molecule, are transcribed into messenger ribonucleic acids (mRNA), which are subsequently translated into proteins (or enzymes). These final protein products provide all life functions, and together with DNA and RNA, constitute the molecules of life. Therefore, if there is a disruption (interference) of a gene function, messenger RNA synthesis, or protein translation, normal life processes get altered or even stopped. “No gene-no messenger”, or “no messenger-no protein”, has been the basis of understanding biological processes. One of the easy-to-access points in cellular processes is messenger RNA due to its cytoplasmic location, “naked” structure, comparatively short half-life, and temporal existence between transcription and translation. Further, mRNA is in between the chain of

events from DNA to protein; it has the universal chemical structure, consisting of only four nucleotides, regardless of the encoded message. In contrast, proteins are chemically much more variable, consisting of combinations of 21 different amino acids, with side chains that vary from very hydrophilic to highly hydrophobic. If mRNA is altered or eliminated before translation, there is no functional gene product, which results in changing the cellular process from the native state. This is the entire rationale of RNA interference (RNAi).

RNA interference is a process in eukaryotic cells in which double stranded endogenous or exogenous RNA molecules trigger a cytoplasmic response, which involves sequence specific target identification and destruction. This may include native messenger RNAs (mRNAs) that code vitally important proteins [1]. Any type of double-stranded RNA (dsRNA) molecules can activate RNAi where long dsRNAs, microRNAs (miRNAs) and small interfering RNAs (siRNAs) and their various forms and modifications are considered the main players/inducers [1]. Let us take a look at RNAi discovery history.

Plant scientists in the 1990s first used targeted gene silencing by introducing an antisense gene into plants. The first example was silencing of a nopaline synthase (NOS) gene, for which the silencing was only visible by loss of a band on a Northern blot and loss of NOS activity [2]. The second antisense gene used in plants targeted the petunia chalcone synthase (CHS) gene, encoding the first step in floral pigment production, and the result was visible in the loss of petal pigmentation [3]. Curiously, attempts to create dark pigmented petunia flowers by overexpression of the same CHS gene resulted in similar colorless petunia petals [4, 5]. It was thought that such a phenotype was “*due to post-transcriptional inhibition of gene expression via an increased rate of mRNA degradation*” [6]. The observed phenomenon was named as “*co-suppression*” of gene expression and the molecular mechanism behind “*co-suppression*” remained unknown for many years [7]. Later, a transient gene inactivation of the carotenogenic *albino-3* (*AL-3*) and *albino-1* (*AL-1*) genes was reported after transformation with homologous sequences in *Neurospora crassa* [8]. This phenomenon, named as gene “*quelling*”, was observed to be severely destructive but spontaneously and progressively reversible and unidirectional, resulting in mutant, intermediate, and wild-type phenotypes [8]. In the years to follow, the co-suppression phenomenon were attributed to inverted repeat T-DNA insertions, which result in RNA transcripts with internal complementary sequences that can fold back on themselves, generating double-stranded RNA and can seed the now well-known Argonaute/dicer silencing system.

Following these seminal discoveries, similar phenomena were discovered in other organisms including the nematode (*Caenorhabditis elegans*) and insects (*Drosophila melanogaster*) from studying the function of a *PAR-1* gene (required for establishing embryo polarity) in the former and alcohol dehydrogenase in the latter (*ADH*) [9, 10]. These studies not only demonstrated a wide range of functionality of “*co-suppression*” phenomenon but also prompted an intense effort to understand the exact mechanism causing this process. In one experiment, injection of dsRNAs associated with muscle protein production into nematodes successfully silenced the targeted gene. The effect on muscle production was not observed using either mRNA or antisense RNA [11]. With this work, for the first time, the agent directly responsible for “*co-suppression*” was identified and formally named as “*RNA interference*” or RNAi. This work was later recognized with the 2006 Nobel Prize.

In plants, the suppression of targeted genes during viral infections was discovered [12] and subsequently developed into a system by which plant gene function may be studied through inhibition by infection with viruses bearing a short sequence targeted against plant mRNAs [13]. This phenomenon was termed as "*virus-induced gene silencing*" (VIGS) and is often used to study gene function in plant species that are recalcitrant to transformation or just take a very long time to regenerate.

Over the past decade, RNAi has been demonstrated in many eukaryotes including humans as well as some prokaryotic life forms [14] and has been recognized to form an integral part of many gene regulatory networks during development. This revolutionary breakthrough in biological science has become a valuable *in vitro*, *in vivo*, and *ex vivo* manipulation of gene expression, allowing for large-scale studies of gene function. It is now a routine laboratory practice to introduce the desired gene-specific dsRNA inducers into cells and selectively, robustly, and systematically silence the targeted sequence signature revealing its cellular function. In addition, RNAi has become an efficient tool for agricultural biotechnology to improve production [15] and combat disease pests as well as for medicine and molecular pharmacology to cure complex infectious, inflammatory, and hereditary diseases [16].

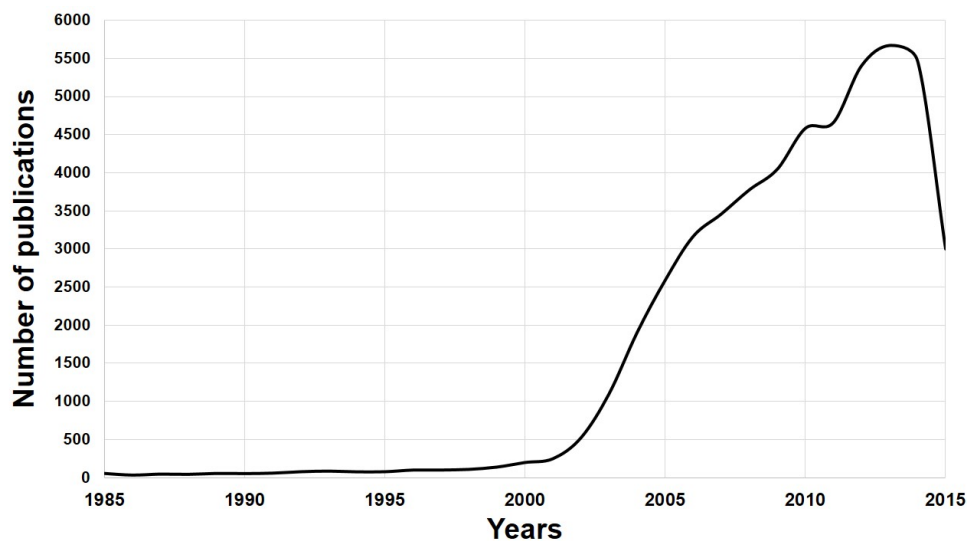


Figure 1. Dynamics of scientific publications devoted to the RNA interference for the past three decades. Source: PubMed [18] data sorted by the year of publications, which were retrieved by the search with the unquoted keyword "*RNA interference*"

RNAi research has rapidly advanced and expanded over the past decade, evidenced by increasing numbers of publications, research projects, and practical applications in both agriculture and medicine. For example, searching *Google Scholar* [17] with the unquoted keyword "*RNA interference*" retrieved over 1 million (1,110,000) documents. Repeating the same search with "*organism-specified RNA interference*" in *PubMed* database [18] on the same date returned a total of 50,824 indexed scientific documents with a major pick after 2002 reaching to over 1,000 scientific publications per year (Figure 1). The distribution of specified search results revealed a number of PubMed-indexed, RNAi-related publications for human (32,007), plant (3,701), animal (27,751), insect (4,145), fungal (690), and prokaryotic (119)

organisms. Moreover, the therapeutic application of RNAi is also expanding rapidly with 9,953 articles related to this topic and found in *PubMed* searching with “RNA interference therapy” keyword. In this brief introductory chapter, I aim to cover the basic understanding behind RNAi and an update knowledge on its applications, limitations, safety, and risks, highlighting and discussing some of the key points presented in this book.

2. Components, mechanism, and function

The principle mechanism of RNAi is complex, but very straightforward and easy to understand. RNAi is induced by the introduction of specific exogenous dsRNA either by virus genome RNAs, injection of synthetic dsRNAs or, in plants, is mediated by *Agrobacterium*. RNAi is also part of the normal development and dsRNAs are produced by endogenous genes encoding miRNA precursors or other long dsRNA molecules. In either case, the dsRNAs are recognized by the enzyme dicer and cleaved into short, double-stranded fragments of ~19-25 base pair long siRNAs [1]. These siRNAs are separated into two single-stranded RNAs (ssRNAs), which are referred to as the “*passenger*” and the “*guide*” strands. The passenger strand is degraded, while the guide strand is picked up by the RNA-induced silencing complex (RISC) that has enzymatic digestion activity and contains the key components of Argonaute (AGO) and P-element induced wimpy testis (PIWI) proteins [1]. The RISC proteins perform the unwinding of the *guide* and *passenger* strands in ATP-independent manner [19, 20]; however, ATP is required to unwind and remove the cleaved mRNA strand from the RISC complex after catalysis [21]. There are effector proteins such as RDE-4 (nematodes) and R2D2 (insects) that recognize exogenous dsRNAs and stimulate dicer activity. R2D2 also has a differentiating function for siRNA strands by stably binding to 5' end of the *passenger* strand, thus directing the *guide* strand to the RISC [22]. Here, it should be noted that the 5' end of the *guide* strand is involved in matching and binding the target mRNA while the 3' end physically arranges target mRNAs into the cleavage-favorable site of the RISC complex [21]. AGO/PIWI proteins localize within the specific P-body regions in the cytoplasm, considered to be a critical site for RNAi [23–25].

It is not clear as yet how the *guide* strand-bound active RISC complex finds mRNA targets within the cell, but it is known that this process is sequence-specific. Once the target mRNA is identified and captured through RNAi machinery, RISC cleaves the target mRNA rendering it untranslatable [1]. In most cases, the entire process is triggered by amplification of the cleavage process through synthesis of additional dsRNAs from primarily digested fragments of mRNA. Upon annealing to the mRNA target, the guide RNA may also be extended by RNA-dependent RNA polymerase (RdRP), resulting in extended “secondary” dsRNAs which in turn may lead to the formation of new siRNAs that enhance and further systematically spread the degradation of the target mRNA in cytoplasm [26, 27].

Although the pathways toward RNAi from exogenous and endogenous dsRNA converge at the RISC and use the same downstream RNAi machinery, there are also some clear differences in their processing and handling [1]. Endogenous dsRNAs cleaved by dicer (1) produce 20–25 bp fragments with a two-nucleotide overhang at the 3' end of siRNA duplex [1], while the length of exogenous dsRNAs-derived siRNAs, required for specificity, is unknown. Exoge-

nous dsRNAs are distinctly (2) handled by the above-mentioned effector proteins, RDE-4 or R2D2 [26, 27], whereas siRNA derived from endogenous dsRNAs (i.e., miRNA precursors) are handled by double-stranded miRNA precursor-binding DGCR8 and Drosha proteins with RNase III enzyme activity. Plants do not have Drosha homologs, instead, processing of miRNA to siRNAs is carried out by one of four dicer-like proteins. Endogenous miRNAs (3), except some plant miRNAs, typically have several mismatches to the target sequence, while siRNAs derived from exogenous dsRNAs usually are designed to have a perfect match to the target. Most importantly, (4) endogenous dsRNA-derived miRNAs are capable of mildly inhibiting the translation of hundreds of mRNAs [28–30], while exogenous dsRNA-derived ones usually silence only single specific target [31]. Depending on organisms, for instance in *C. elegans* and *D. melanogaster*, (5) distinct Argonaute proteins and dicer enzymes [32, 33] process miRNAs and exogenous siRNAs. Furthermore, endogenously processed miRNAs prevalently (6) interact with miRNA response elements (MREs) located within the 3'-UTRs region of target mRNAs. Upon binding to MREs, miRNAs can decrease the gene expression of various mRNAs by either inhibiting translation (in animals) or directly causing degradation of the transcript (in plants). In contrast, exogenous dsRNA-derived siRNAs may interact with any complementary sequence region of the target mRNA, causing direct cleavage of the transcript [1]. miRNAs may actually regulate translation of target mRNAs in dual ways, as translation regulation by miRNAs oscillates between repression and activation during the cell cycle through a yet unknown mechanism [34].

The main biological function of RNAi is regulation of gene activity of cells at the post-transcriptional level (PTGS) either by the inhibition of translation of mRNA or by direct degradation of the mRNA. In addition to PTGS, RNAi pathway components may contribute to maintenance of genome organization and structure, mediated by RNA-induced histone modification. Histone modification in turn affects heterochromatin formation and may silence gene activity at the pre-transcriptional level [35]. This process is referred to as “RNA-induced gene silencing (RITS) and requires dicer, siRNA and RISC component proteins such as AGO and R2D2 [36]. In addition, RNAi components and inducers (siRNA/dicer/AGO) may also possibly upregulate expression of genes in binding into a promoter region and through histone demethylation, a process dubbed RNA activation [37, 38].

Because of sequence-specific recognition, regulatory properties, and the possibility of systemic spreading of dsRNAs, RNAi is the key “sterilizing agent” of cells and tissues, and it functions as potent immune response against foreign nucleic acids from viruses, transposons, or transformation events which can invade and harm the genome and its stability [39]. The chapters presented in Section 2 of this book have a more detailed coverage of the history of the RNAi discovery, mechanism, and functional components and on the biological role of RNAi including natural small RNAs/microRNAs as well as long noncoding RNAs in gene regulations.

3. Differences among organisms

Although the RNAi pathway is a universal process in eukaryotic cells, and it consists of similar component(s), mechanisms, and functions as described above, there are some variations

among organisms in both up-take of exogenous dsRNAs and induction of RNAi. First, RNAi is systemic and heritable in plants and *C. elegans*. The systemic spreading of RNAi in plants occurs because of transfer of siRNAs between cells through plasmodesmata and the phloem [40]. Second, in plants, RNAi induces epigenetic silencing of genes through methylation of promoters of targeted genes which may be passed to the next generation [41], while in *Drosophila* and mammals this is not the case. Third, plant miRNAs have perfect or nearly perfect complementary to their target genes and directly cleave and degrade targeted mRNA. In contrast, animal miRNAs have one or more mismatches to target sequence and halt the translation process [42].

RNAi is not found in some eukaryotic protozoa (e.g., *Leishmania major* and *Trypanosoma cruzi*) [43, 44]. Some fungi (e.g., *Saccharomyces cerevisiae*) lack specific RNAi component(s) and the reintroduction of these missing components can recover RNAi [45, 46]. Further, prokaryotic organisms have distinctive RNA-dependent gene regulation system controlled by RNA products of translation-inhibiting genes. These regulatory RNAs are not processed by dicer enzymes, differentiating them from eukaryotic RNAi [47]. However, recently, the clustered regularly interspaced short palindromic repeats (CRISPR/Cas9) interference system has been characterized in prokaryotes, which is a gene silencing pathway analogous to eukaryotic RNAi systems [14]. The CRISPR interference system has its specific components, advantages, and limitations that are well described in the literature [48, 49], but will not be presented here. The chapter by Dr. Devi Singh and his colleagues in Section 2 of this book has a detailed coverage of RNAi in various organisms. RNAi in various organisms is also discussed in the chapter by Galay et al. presented in Section 6 of this book, highlighting the specifics of RNAi in ticks while Dr. Tayota and his colleagues present an interesting methodological paper on RNAi in the water flea in Section 3.

4. Evolution

Studies on components, mechanisms, and functions of RNAi have demonstrated variations among organisms, differences in eukaryotes and prokaryotes, and indicate that RNAi is derived from an ancestral immune defense function against transcripts of transposons and viruses [50, 51]. Although some eukaryotes might have lost RNAi components or, even, the entire pathway following the emergence of the Eukaryota, parsimony-based phylogenetic analyses suggest that an ancestral lineage of all eukaryotes possibly had a primitive RNAi capability including relevant components for some key functions such as histone modification [50]. Phylogenetic studies also indicate that miRNAs of plants and animals may have evolved independently, but the conservation of some key proteins involved in RNAi also indicate that the last common ancestor of modern eukaryotes already possessed an siRNA-based gene silencing system. The RNAi-like defense system of prokaryotes is functionally similar, but structurally distinct from the eukaryotic RNAi system [52]. It seems likely that a proto-RNAi system possessed at least some form of dicer-like, AGO, PIWI, and RdRP proteins. These basic components were shared by major eukaryotic lineages and functioned within an RNA degradation exosome complex [53].

Being an important component of an antiviral innate immune defense system in eukaryotes, RNAi components and various interaction/regulatory mechanisms, including the miRNA pathway, evolved later but at faster rates under strong directional selection [54]. This could have been a means of generating an improved response to the evolutionary arms race with viral genes. Correspondingly, some plant viruses have evolved the means to suppress the RNAi response in their host cells [55]. Extensive studies reported that an ancient duplication of RNAi components followed by species-specific gene duplications and losses provided evolutionary diversification, specificity and adaptation of the RNAi system in many organisms [56]. Chapter(s) presented in Section 2 has covered some evolutionary aspects of RNAi.

5. Applications

Since its first discovery as anti-sense gene suppression, *co-suppression* or *quelling* phenomenon, the sequence specificity, efficiency, and systemic spreading (in some organisms) characteristics of RNAi to suppress target gene expression have caught researchers' attention and soon became an attractive and powerful tool for gene function discovery in life sciences [1]. By full or partial suppression of target gene expression using RNAi, the change in cell physiology and/or developmental phenotype helps to reveal the function of the target gene. Therefore, a utilization of RNAi has revolutionized the annotation of cellular functions of many unknown and unique genes, adding to our understanding the complex genetic/biochemical pathways and their interactions. Thanks to its partial silencing effect, RNAi also helped to discover the function of genes when complete knockout would cause lethality [57]. Moreover, by targeting homologous sequences within a gene family, a single RNAi construct can suppress the expression of multiple members of a gene family, and thus reveal phenotypes that would have been missed in a single mutant due to redundancy in gene function.

The results of the functional genomics studies, advances in the understanding of the RNAi mechanism, improved design of trait-specific RNAi inducers (such as miRNAs), selection of target gene sequences combined with the development of proper delivery systems, as well as screens for "off-target" and cross reactivity have brought the practical applications of RNAi far beyond its initial experimental reach.

Agricultural application of RNAi through tissue culture-derived genetic modifications and transgenic research in a wide range of technical, food, and horticulture crops have been particularly successful and have solved many problems. Examples include, but are not limited to, crop yield and quality improvements [15, 58], food/nutrient quality improvements and fortification [59–62], decreasing the harmful precursors and carcinogens [63, 64], and improvement of plant pest and disease resistance [65–66]. Many of these applications are now evaluated for commercialization or are already in commercial production [67]. In this context, targeting far red (FR) photoreceptor gene (*PHYA1*) using RNAi approach [15], our team succeed to develop the world's first RNAi cotton cultivars with improved fiber quality and other key agronomic traits without adversely affecting the yield, which successfully passed multi-environmental large field trials and have been approved for cotton farming in Uzbekistan.

Therapeutic application of RNAi has also been successful in medicine and molecular pharmacology with examples in inflammatory and infectious disease [68-71], cancer [72-75], as well as hereditary and neurodegenerative diseases [76]. Indeed, for many other disorders RNAi may have great potential. To highlight advances made on this field, in Section 4 of this book, we present several relevant chapters on advances of RNAi application in key human diseases of blood, ocular, nervous, kidney, and oncogenic origin. In addition, Section 5 chapters discuss RNAi utilization in various immune and infectious diseases. Section 6 chapters present the latest advances of RNAi application in studies of insects and parasitic pests such as ticks. All of these chapters highlight various aspects of RNAi and add interesting insights to the present RNAi discussions.

6. Safety and risk assessment

Manipulation of the organisms' own genetic sequence signature(s) (cis-genesis) is usually considered safer compared to "trans-genesis" that utilizes "foreign" genetic material to create genetically modified (GM) crops and its products [77]. However, for RNAi, when broken down to ~21 nucleotides this quickly may lose its meaning, as a trans-RNAi will only work if it has sufficient homology to an endogenous target transcript. Chemically, RNA is "*generally recognized as safe (GRAS)*" or it is "*rarely formally considered in risk assessment*" [67]. Despite this and many other examples of successful application of RNAi technology in agriculture and medicine, there may be risks associated with high or repeated dosages of dsRNA, which inadvertently may interfere with unintended target sequences. A growing body of evidences suggests that testing for the safety and assessing possible risks associated with the use of RNAi-derived products sound practical, in particular, evidence of the remarkable stability of dsRNAs in the environment, their survival and resistance in the acidic conditions of the digestive tracts of higher organisms, and consequent transmissibility of dsRNA from foods to humans/animals. Further, production of possibly harmful "secondary" dsRNAs [67] by primary RNAi inducers raised an early warning signal regarding the GRAS signature of any RNA molecule and the possibility of risks for human health and environment.

Safety concerns about RNAi-based drugs are exemplified by the lethality of 23 out of 49 distinct RNAi therapy experiments in mice because of potential "off-target" effects that could shut down non-targeted gene(s) with sequence similarity to therapeutic RNAi inducer [78]. This observed lethality, however, could be due to "oversaturation" of the dsRNA pathway and delivery issues of short hairpin RNAs [79] that needs to be optimized for harmless therapeutic applications. There are several suggested approaches to minimize or eliminate such "off-target", "oversaturation" or delivery issues, in particular through the use of (1) comprehensive *in silico* target and off-target analyses [80], (2) modified designing of RNAi inducers with improved target selectivity, and (3) efficient delivery systems.

There may also be concerns about the uptake of intact plant miRNA by consumers through plant diet. Plant microRNAs and some long dsRNA molecules, with sequence complimentary and perfect matches to endogenous human genes, were demonstrated to survive the digestive

tract of humans and can freely and routinely enter the blood system [67, 81]. *In vitro* human cell culture experiments further showed that such plant siRNA entered into human blood system could silence endogenous human genes due to sequence complementarity. While this may require attention of regulatory systems on one hand, on the other hand, human consumption of food crops with natural occurring siRNAs is considered safe and so far has not caused any dramatic biohazards or risk [81]. The chapters in Section 3 of this book also present updated information on RNAi delivery methods (e.g. Tayota et al.); synthesis, chemical and structural modifications, and designing for high specificity and selectivity of RNAi inducers (see Gvozdeva and Chernolovskaya), and limitations of RNAi and possible alternative technology such as ER-targeted intrabodies for gene silencing (see chapter by Backhaus and Böldicke).

Risk assessment and available protocols/guidelines are in the early stages of development. Some suggest that dsRNA-derived products must be subject to risk assessment studies [67]. Other findings indirectly support the safety of RNAi [81, 82], provided its use is within specific dosage ranges, the correct delivery system is in place and RNAi inducers without possible off-target effects, unintended gene silencing and secondary dsRNA production can be designed. However, it is always advisable to admit to possible risks of any novel genomic technology, including RNAi, and consider potential biohazards and evaluate risks for environmental health, before release of a new product [58, 81–85]. To accomplish this, Heinemann et al. [67] proposed the following five-step guidance: (1) to perform detailed *in silico* comparative bioinformatics analyses for targets of designed dsRNA and identify possible “off-targets” in key consumers; (2) to experimentally quantify designed dsRNAs, and the processing of any other unknown sequence signatures or secondary dsRNA as a result of introducing intended RNAi inducer into recipient or its product; (3) to test possible biohazards and risks due to exposure of RNAi product in animal and human cell/tissue culture; (4) to conduct animal feeding experiments for the long-term physiological and toxicological patterns and possible chronic effects; and (5) to perform clinical trials of RNAi-derived products in humans.

7. Conclusions and future perspectives

Thus, being a revolutionizing discovery in genome biology to characterize functions of any desired unknown genetic sequences, the discovery of RNAi has significantly widened our knowledge on core cellular processes. This knowledge has created opportunities and solutions to longstanding obstacles in conventional agriculture and medicine, offering a bright future to curing complex human and animal diseases, improve crop production and protection, and a sustained global food security through proper manipulation of key genes with agricultural or medicinal importance. Although key issues on specificity, selectivity, and delivery of RNAi inducing structures still exist, and some safety risks associated with the use of RNAi products have been recognized, the general believe is that RNAi is a safer technology than transgenomics utilizing “foreign” genetic information. Safe applications, however, require proper designing, dosage and delivery of RNAi inducers, and before its delivery for wide consumer market, the safety risks should be assessed. Addressing the advances made over the past three decades in RNAi research and commercialization, in this book, we have compiled and

presented a diverse collection of chapters contributed by the science research communities. We all believe that RNAi, in combination with the rapidly expanding genomic information in key organisms and novel genome editing tools, will become even more powerful and efficient, and that we will all enjoy its benefits far into the future.

Acknowledgements

I thank the Academy of Sciences of Uzbekistan and Committee for Coordination Science and Technology Development of Uzbekistan for basic science FA-F5-T030; and several applied (FA-A6-T081 and FA-A6-T085) and innovation (I-2015-6-15/2 and I5-FQ-0-89-870) research grants. I am particularly grateful and thank the Office of International Research Programs (OIRP) of the United States Department of Agriculture (USDA) – Agricultural Research Service (ARS) and U.S. Civilian Research & Development Foundation (CRDF) for international cooperative grants P121, P121B, and UZB-TA-2292, which are devoted to study, development, application, risk assessment, and commercialization of RNAi cotton cultivars and their products. I sincerely thank Dr. Alexander R. van der Krol, Wageningen University, Netherlands, and Dr. Eric J. Devor, Iowa State University, the USA, for their critical reading and suggestions to this chapter manuscript.

Author details

Ibrokhim Y. Abdurakhmonov*

Address all correspondence to: genomics@uzsci.net

Center of Genomics and Bioinformatics, Academy of Science, Ministry of Agriculture and Water Resources, and “UzCottonIndustry” Association of the Republic of Uzbekistan, Tashkent, Uzbekistan

References

- [1] Hannon GJ. RNA interference. *Nature*. 2002;418:244–51. DOI: 10.1038/418244a
- [2] Rothstein SJ, Dimaio J, Strand M, Rice D. Stable and heritable inhibition of the expression of nopaline synthase in tobacco expressing antisense RNA. *Proc Natl Acad Sci U S A*. 1987;84:8439–8443. DOI:10.1073/pnas.84.23.8439
- [3] van der Krol AR, Lenting PE, Veenstra J, van der Meer IM, Koes RE, Gerats AGM, Joseph N. M. Mol JNM, Stuitje AR. An anti-sense chalcone synthase gene in transgenic plants inhibits flower pigmentation. *Nature* 1988;333:866–869. DOI: 10.1038/333866a0

- [4] 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 suppression of gene expression. *Plant Cell*. 1990;2:291–99. DOI:10.1105/tpc.2.4.291
- [5] Napoli C, Lemieux C, Jorgensen R. Introduction of a chimeric chalcone synthase gene into petunia results in reversible co-suppression of homologous genes in trans". *Plant Cell*. 1990; 2:279–289. DOI:10.1105/tpc.2.4.279
- [6] van Blokland R, van der Geest N, Mol JNM, Kooter JM. Transgene-mediated suppression of chalcone synthase expression in *Petunia hybrida* results from an increase in RNA turnover. *Plant J*. 1994;6:861–877. DOI:10.1046/j.1365-313X.1994.6060861.x.
- [7] Mol JNM, van der Krol AR. Antisense nucleic acids and proteins: fundamentals and applications. New York: Marcel Dekker; 1991. 231 p. ISBN 0-8247-8516-9.
- [8] Romano N, Macino G (1992). Quelling: transient inactivation of gene expression in *Neurospora crassa* by transformation with homologous sequences. *Mol Microbiol*. 1992;6:3343–3353. DOI:10.1111/j.1365-2958.1992.tb02202.x
- [9] Guo S, Kempthues K. par-1, a gene required for establishing polarity in *C. elegans* embryos, encodes a putative Ser/Thr kinase that is asymmetrically distributed. *Cell*. 1995;81:611–620. DOI:10.1016/0092-8674(95)90082-9
- [10] Pal-Bhadra M, Bhadra U, Birchler J. Cosuppression in *Drosophila*: gene silencing of alcohol dehydrogenase by white-Adh transgenes is polycomb dependent. *Cell*. 1997;90: 479–490. DOI:10.1016/S0092-8674(00)80508-5
- [11] Fire A, Xu S, Montgomery M, Kostas S, Driver S, Mello C. Potent and specific genetic interference by double-stranded RNA in *Caenorhabditis elegans*. *Nature*. 1998;391: 806–811. DOI:10.1038/35888. PMID 9486653
- [12] Ratcliff F, Harrison B, Baulcombe D. A similarity between viral defense and gene silencing in plants. *Science*. 1997;276:1558. DOI:10.1126/science.276.5318.1558.
- [13] Godge MR, Purkayastha A, Dasgupta I, Kumar PP. Virus-induced gene silencing for functional analysis of selected genes. *Plant Cell Rep*. 2008;27: 209-219. DOI:
- [14] Hale C, Kleppe K, Terns RM, Terns MP. Prokaryotic silencing (psi)RNAs in *Pyrococcus furiosus*. *RNA*. 2008;14:2572–2579. DOI: 10.1261/rna.1246808
- [15] Abdurakhmonov IY, Buriev ZT, Saha S, Jenkins JN, Abdugarimov A, Pepper AE. Cotton *PHYA1* RNAi enhances major fiber quality and agronomic traits of cotton (*Gossypium hirsutum* L). *Nature Communications*. 2014;4:3062; DOI:10. 1038/ncomms4062
- [16] Kupferschmidt K. A Lethal Dose of RNA. *Science*. 2013;341:732–733. DOI:10.1126/science.341.6147.732
- [17] Google Scholar. 2015. Available from: <http://scholar.google.com> [Accessed 2015-09-06]

- [18] PubMed database. 2015. Available from: <http://www.ncbi.nlm.nih.gov/pubmed> [Accessed from 2015-09-06]
- [19] Matranga C, Tomari Y, Shin C, Bartel D, Zamore P. Passenger-strand cleavage facilitates assembly of siRNA into Ago2-containing RNAi enzyme complexes. *Cell*. 2005;123: 607–620. DOI:10.1016/j.cell.2005.08.044
- [20] Leuschner P, Ameres S, Kueng S, Martinez J. Cleavage of the siRNA passenger strand during RISC assembly in human cells. *EMBO Rep*. 2006;7:314–320. DOI: 10.1038/sj.embor.7400637
- [21] Haley B, Zamore B. Kinetic analysis of the RNAi enzyme complex. *Nature Structural & Molecular Biology*. 2004;11:599–606. DOI:10.1038/nsmb780
- [22] Tomari Y, Matranga C, Haley B, Martinez N, Zamore P; Matranga; Haley; Martinez; Zamore. A protein sensor for siRNA asymmetry. *Science*. 2004;306:1377–1380. DOI: 10.1126/science.1102755
- [23] Sen G, Blau H. Argonaute 2/RISC resides in sites of mammalian mRNA decay known as cytoplasmic bodies. *Nat Cell Biol*. 2005;7: 633–636. DOI:10.1038/ncb1265. PMID 15908945
- [24] Lian S, Jakymiw A, Eystathioy T, Hamel J, Fritzler M, Chan E. GW bodies, microRNAs and the cell cycle. *Cell Cycle*. 2006;5:242–245. DOI:10.4161/cc.5.3.2410
- [25] 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*. 2005;7:1267–1274. DOI:10.1038/ncb1334
- [26] Parker G, Eckert D, Bass B. RDE-4 preferentially binds long dsRNA and its dimerization is necessary for cleavage of dsRNA to siRNA. *RNA*. 2006;12:807–818. DOI: 10.1261/rna.2338706
- [27] Liu Q, Rand T, Kalidas S, Du F, Kim H, Smith D, Wang X. R2D2, a bridge between the initiation and effector steps of the *Drosophila* RNAi pathway. *Science*. 2003;301: 1921–1925. DOI:10.1126/science.1088710
- [28] Lim LP, Lau NC, Garrett-Engle P, Grimson A, Schelter JM, Castle J, Bartel DP, Linsley PS, Johnson JM. Microarray analysis shows that some microRNAs downregulate large numbers of target mRNAs. *Nature*. 2005;433:769–773. DOI:10.1038/nature03315. PMID 15685193
- [29] Selbach M, Schwanhäusser B, Thierfelder N, Fang Z, Khanin R, Rajewsky N. Widespread changes in protein synthesis induced by microRNAs. *Nature*. 2008;455: 58–63. DOI:10.1038/nature07228
- [30] Baek D, Villén J, Shin C, Camargo FD, Gygi SP, Bartel DP. The impact of microRNAs on protein output. *Nature*. 2008;455: 64–71. DOI:10.1038/nature07242

- [31] Pillai RS, Bhattacharyya SN, Filipowicz W. Repression of protein synthesis by miRNAs: how many mechanisms? *Trends Cell Biol* 2007;17:118–126. DOI:10.1016/j.tcb.2006.12.007
- [32] Okamura K, Ishizuka A, Siomi H, Siomi M (2004). "Distinct roles for Argonaute proteins in small RNA-directed RNA cleavage pathways". *Genes Dev.* 2004;18:1655–1666. DOI:10.1101/gad.1210204
- [33] Lee Y, Nakahara K, Pham J, Kim K, He Z, Sontheimer E, Carthew R. Distinct roles for *Drosophila* Dicer-1 and Dicer-2 in the siRNA/miRNA silencing pathways. *Cell.* 2004;117:69–81. DOI:10.1016/S0092-8674(04)00261-2
- [34] Vasudevan S, Tong Y, Steitz JA. Switching from Repression to Activation: MicroRNAs Can Up-Regulate Translation. *Science.* 2007;318:1931–1934. DOI:10.1126/science
- [35] Holmquist G, Ashley T. Chromosome organization and chromatin modification: influence on genome function and evolution. *Cytogenet Genome Res.*2006;114:96–125. DOI:10.1159/000093326
- [36] Verdel A, Jia S, Gerber S, Sugiyama T, Gygi S, Grewal S, Moazed D. RNAi-mediated targeting of heterochromatin by the RITS complex. *Science.* 2004;303:672–676. DOI:10.1126/science.1093686
- [37] Li LC, Okino ST, Zhao H, Pookot D, Place RF, Urakami S, Enokida H, Dahiya R. Small dsRNAs induce transcriptional activation in human cells. *Proc Natl Acad Sci USA.*2006;103:17337–17342. DOI:10.1073/pnas.0607015103
- [38] Check E. RNA interference: hitting the on switch. *Nature.* 2007;448: 855–858. DOI:10.1038/448855a
- [39] Stram Y, Kuzntzova L. Inhibition of viruses by RNA interference. *Virus Genes.* 2006;32:299–306. DOI:10.1007/s11262-005-6914-0
- [40] Lodish H, Berk A, Matsudaira P, Kaiser CA, Krieger M, Scott MP, Zipurksy SL, Darnell J. *Molecular Cell Biology.* 5th ed. New York; W.H; 2004.963 p. ISBN 978-0-7167-4366-8.
- [41] Jones L, Ratcliff F, Baulcombe DC. RNA-directed transcriptional gene silencing in plants can be inherited independently of the RNA trigger and requires Met1 for maintenance. *Curr Biol.* 2001;11:747–757. DOI:10.1016/S0960-9822(01)00226-3
- [42] Saumet A, Lecellier CH. Anti-viral RNA silencing: do we look like plants ?. *Retrovirology.* 2006;3:3. DOI:10.1186/1742-4690-3-3
- [43] DaRocha W, Otsu K, Teixeira S, Donelson J. Tests of cytoplasmic RNA interference (RNAi) and construction of a tetracycline-inducible T7 promoter system in *Trypanosoma cruzi*. *Mol Biochem Parasitol.* 2004;133:175–86. DOI:10.1016/j.molbiopara.2003.10.005

- [44] Robinson K, Beverley S. Improvements in transfection efficiency and tests of RNA interference (RNAi) approaches in the protozoan parasite *Leishmania*. *Mol Biochem Parasitol.* 2003;28:217–228. DOI:10.1016/S0166-6851(03)00079-3
- [45] Aravind L, Watanabe H, Lipman DJ, Koonin EJ. Lineage-specific loss and divergence of functionally linked genes in eukaryotes. *Proc Natl Acad Sci USA.* 2000;97:11319–11324. DOI:10.1073/pnas.200346997
- [46] Drinnenberg IA, Weinberg DE, Xie KT, Nower JP, Wolfe KH, Fink GR, Bartel DP. RNAi in budding yeast. *Science* 2009;326:544–550. DOI:10.1126/science.
- [47] Morita T, Mochizuki Y, Aiba H. Translational repression is sufficient for gene silencing by bacterial small noncoding RNAs in the absence of mRNA destruction". *Proc Natl Acad Sci USA.* 2006;103:4858–486. DOI:10.1073/pnas.0509638103
- [48] Larson MH, Gilbert LA, Wang X, Lim WA, Weissman JS, Qi LS. CRISPR interference (CRISPRi) for sequence-specific control of gene expression. *Nature Protocols* 2013;8:2180–2196. DOI:10.1038/nprot.2013.132
- [49] Gilbert LA, Larson MH, Morsut L, Liu Z, Brar GA, Torres SE, Stern-Ginossar N, Brandman O, Whitehead EH, Doudna JA, Lim WA, Weissman JS, Qi LS. CRISPR-mediated modular RNA-guided regulation of transcription in eukaryotes. *Cell.* 2013;154:442–451. DOI:10.1016/j.cell.2013.06.044
- [50] Cerutti H, Casas-Mollano J. On the origin and functions of RNA-mediated silencing: from protists to man". *Curr Genet.* 2006;50:81–99. DOI:10.1007/s00294-006-0078-x
- [51] Buchon N, Vaury C. RNAi: a defensive RNA-silencing against viruses and transposable elements. *Heredity.* 2006;96:195–202. DOI:10.1038/sj.hdy.6800789.
- [52] Shabalina S, Koonin EV. Origins and evolution of eukaryotic RNA interference *Ecol Evol.* 2008;23:578–587. DOI: 10.1016/j.tree.2008.06.005
- [53] Anantharaman V, Koonin E, Aravind L. Comparative genomics and evolution of proteins involved in RNA metabolism. *Nucleic Acids Res.* 2002;30:1427–64. DOI: 10.1093/nar/30.7.1427
- [54] Obbard DJ; Jiggins FM; Halligan DL; Little TJ. Natural selection drives extremely rapid evolution in antiviral RNAi genes. *Curr Biol.* 2006;16:580–585. DOI:10.1016/j.cub.2006.01.065
- [55] Lucy A, Guo H, Li W, Ding S. Suppression of post-transcriptional gene silencing by a plant viral protein localized in the nucleus. *EMBO J.* 2000;19:1672–1680. DOI:10.1093/emboj/19.7.1672
- [56] Hu Y, Stenlid J, Elfstrand M, Olson A. Evolution of RNA interference proteins dicer and argonaute in Basidiomycota. *Mycologia.* 2013;105:1489–1498. DOI: 10.3852/13-171
- [57] Voorhoeve PM, Agami R. Knockdown stands up. *Trends Biotechnol.* 2003;21:2–4. DOI:10.1016/S0167-7799(02)00002-1

- [58] Saurabh Satyajit, Vidyarthi AS, Prasad D. RNA interference: concept to reality in crop improvement. *Planta*. 2014;239:543–564. DOI:10.1007/s00425-013-2019-5
- [59] Sunilkumar G, Campbell L, Puckhaber L; Stipanovic R, Rathore K. Engineering cottonseed for use in human nutrition by tissue-specific reduction of toxic gossypol. *Proc Natl Acad Sci USA*. 2006;103:18054–18059. DOI:10.1073/pnas.0605389103
- [60] Siritunga D, Sayre R. Generation of cyanogen-free transgenic cassava. *Planta*. 2003;217:367–373. DOI:10.1007/s00425-003-1005-8
- [61] Le L, Lorenz Y, Scheurer S, Fötisch K, Enrique E, Bartra J, Biemelt S, Vieths S, Sonnewald U. Design of tomato fruits with reduced allergenicity by dsRNAi-mediated inhibition of ns-LTP (Lyc e 3) expression. *Plant Biotechnol J*. 2006;4:231–242. DOI: 10.1111/j.1467-7652.2005.00175.x
- [62] Niggeweg R, Michael A, Martin C. Engineering plants with increased levels of the antioxidant chlorogenic acid. *Nat Biotechnol*. 2004;22:746–54. DOI:10.1038/nbt966
- [63] Gavilano L, Coleman N, Burnley L, Bowman M, Kalengamaliro, Hayes A, Bush L, Siminszky B. Genetic engineering of *Nicotiana tabacum* for reduced nicotine content". *J Agric Food Chem*. 2006;54:9071–9078. DOI:10.1021/jf0610458
- [64] Allen R, Millgate A, Chitty J, Thisleton J, Miller J, Fist A, Gerlach W, Larkin P. RNAi-mediated replacement of morphine with the nonnarcotic alkaloid reticuline in opium poppy". *Nat Biotechnol*. 2004;22:1559–1566. DOI:10.1038/nbt1033
- [65] Zadeh A, Foster G. Transgenic resistance to tobacco ringspot virus. *Acta Virol*. 2004;48:145–152
- [66] Mao YB, Cai WJ, Wang JW, Hong GJ, Tao XY, Wang LJ, Huang YP, Chen XY. Silencing a cotton bollworm P450 monooxygenase gene by plant-mediated RNAi impairs larval tolerance of gossypol. *Nat Biotechnol*. 2007;25:1307–1313. DOI: 10.1038/nbt1352
- [67] Heinemann JA, Agapito-Tenfen SZ, Carman JA. A comparative evaluation of the regulation of GM crops or products containing dsRNA and suggested improvements to risk assessments. *Environ Int*. 2013;55:43–55. DOI: 10.1016/j.envint.2013.02.010
- [68] Crowe S. Suppression of chemokine receptor expression by RNA interference allows for inhibition of HIV-1 replication. *AIDS*. 2003;17 Suppl 4:S103–S105. DOI: 10.1097/00002030-200317004-00014
- [69] Kusov Y, Kanda T, Palmenberg A, Sgro J, Gauss-Müller V. Silencing of Hepatitis A Virus Infection by Small Interfering RNAs. *J Virol*. 2006;80:5599–610. DOI:10.1128/JVI.01773-05
- [70] Jia F, Zhang Y, Liu C. A retrovirus-based system to stably silence hepatitis B virus genes by RNA interference. *Biotechnol Lett*. 2006;8:1679–1685. DOI:10.1007/s10529-006-9138-z

- [71] Hu L, Wang Z, Hu C, Liu X, Yao L, Li W, Qi Y. Inhibition of Measles virus multiplication in cell culture by RNA interference. *Acta Virol.* 2005;49:227–234. DOI: Not available
- [72] Putral L, Gu W, McMillan N. RNA interference for the treatment of cancer". *Drug News Perspect.* 2006;19:317–324. DOI:10.1358/dnp.2006.19.6.985937
- [73] Izquierdo M. Short interfering RNAs as a tool for cancer gene therapy". *Cancer Gene Ther.* 2005;12:217–227. DOI:10.1038/sj.cgt.7700791
- [74] Li C, Parker A, Menocal E, Xiang S, Borodyansky L, Fruehauf J. Delivery of RNA interference. *Cell Cycle.* 2006;5:2103–2109. DOI:10.4161/cc.5.18.3192
- [75] Takeshita F, Ochiya T. Therapeutic potential of RNA interference against cancer. *Cancer Sci.* 2006;97:689–696. DOI:10.1111/j.1349-7006.2006.00234.x
- [76] Raoul C, Barker S, Aebischer P. Viral-based modelling and correction of neurodegenerative diseases by RNA interference. *Gene Ther.* 2006;3:487–495. DOI:10.1038/sj.gt.3302690
- [77] Hou H, Atlihan N, Lu ZX. New biotechnology enhances the application of cisgenesis in plant breeding. *Front Plant Sci.* 2014;5:389. DOI: 10.3389/fpls.2014.00389.
- [78] Check E. RNA treatment kills mice. *Nature.* 2006; DOI:10.1038/news060522-10
- [79] Grimm D, Streetz K, Jopling C, Storm T; Pandey K, Davis C, Marion P, Salazar Kay F, Kay M. Fatality in mice due to oversaturation of cellular microRNA/short hairpin RNA pathways. *Nature.* 2006;441:537–541. DOI:10.1038/nature04791
- [80] Qiu S, Adema C, Lane T. A computational study of off-target effects of RNA interference. *Nucleic Acids Res.* 2005;33:1834–1847. DOI:10.1093/nar/gki324
- [81] Petrick JS, Brower-Toland B, Jackson AL, Kier LD. Safety assessment of food and feed from biotechnology-derived crops employing RNA-mediated gene regulation to achieve desired traits: a scientific review. *Regul Toxicol Pharmacol.* 2013;66:167–176. DOI: 10.1016/j.yrtph.2013.03.008
- [82] Kamthan A, Chaudhuri A, Kamthan M, Datta A. Small RNAs in plants: recent development and application for crop improvement. *Front Plant Sci.* 2015;6:208. DOI: 10.3389/fpls.2015.00208
- [83] Lemgo GN, Sabbadini S, Pandolfini T, Mezzetti B. Biosafety considerations of RNAi-mediated virus resistance in fruit-tree cultivars and in rootstock. *Transgenic Res.* 2013;22:1073–1088. DOI: 10.1007/s11248-013-9728-1.
- [84] Ramesh SV. Non-coding RNAs in crop genetic modification: considerations and predictable environmental risk assessments (ERA). *Mol Biotechnol.* 2013;55:87–100. DOI: 10.1007/s12033-013-9648-6
- [85] Auer C, Frederick R. Crop improvement using small RNAs: applications and predictive ecological risk assessments. *Trends Biotechnol.* 2009;27:644–651. DOI: 10.1016/j.tibtech.2009.08.005