RNA Interference

Introduction

- RNA interference (RNAi) is a genetic regulatory system that functions to silence the activity of specific genes.
- RNAi occurs naturally, through the production of nuclear-encoded pre-microRNA (pre-miRNA), and can be induced experimentally, using short segments of synthetic double-stranded RNA (dsRNA).
- The synthetic dsRNA employed is typically either a small hairpin RNA (shRNA) or a short interfering RNA (siRNA).
- In both the natural and the experimental pathways, an enzyme known as DICER is necessary for the formation of miRNA from pre-miRNA or of siRNA from shRNA.
- The miRNA or siRNA then binds to an enzyme-containing molecule known as RNA-induced silencing complex (RISC).
- The miRNA-RISC or siRNA-RISC complex binds to target, or complementary, messengerRNA (mRNA) sequences, resulting in the enzymatic cleavage of the target mRNA.
- The cleaved mRNA is rendered nonfunctional and hence is “silenced.”

Introduction

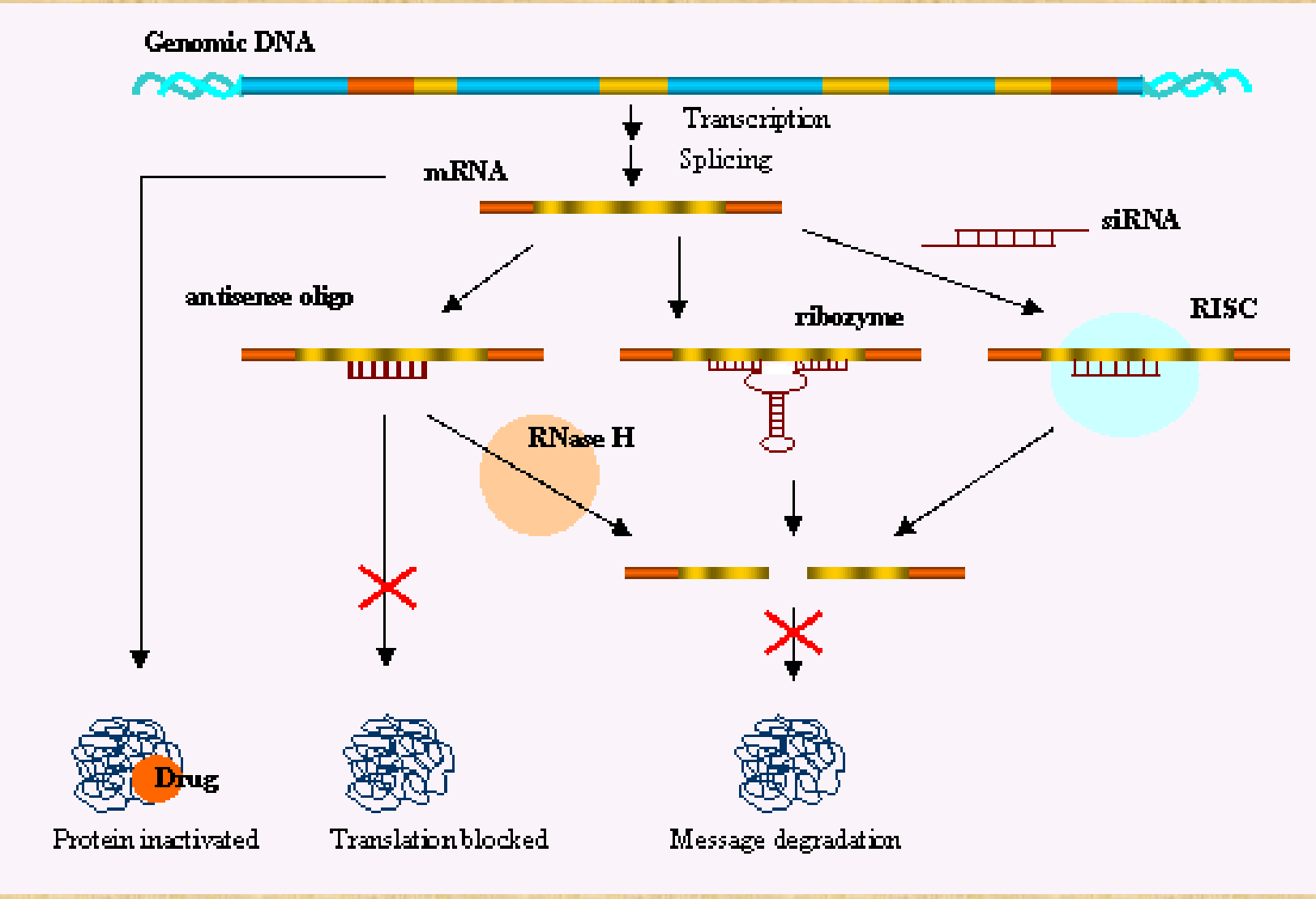
The ability of interfering RNA to silence genes was discovered in the 1998s by American scientists Andrew Z. Fire and Craig C. Mello, who shared the 2006 Nobel Prize for Physiology or Medicine for their work. Fire and Mello successfully inhibited the expression of specific genes by introducing short double-stranded RNA (dsRNA) segments into the cells of nematodes (*Caenorhabditis elegans*). The dsRNA segments underwent enzymatic processing that enabled them to attach to molecules of messenger RNA (mRNA) possessing complementary nucleotide sequences. The attachment of the two RNAs inhibited the translation of the mRNA molecules into proteins.

Two types of small ribonucleic acid (RNA) molecules – microRNA (miRNA) and small interfering RNA (siRNA) – are central to RNA interference. RNAs are the direct products of genes, and these small RNAs can direct enzyme complexes to degrade messenger RNA (mRNA) molecules and thus decrease their activity by preventing translation, via post-transcriptional gene silencing.

Introduction

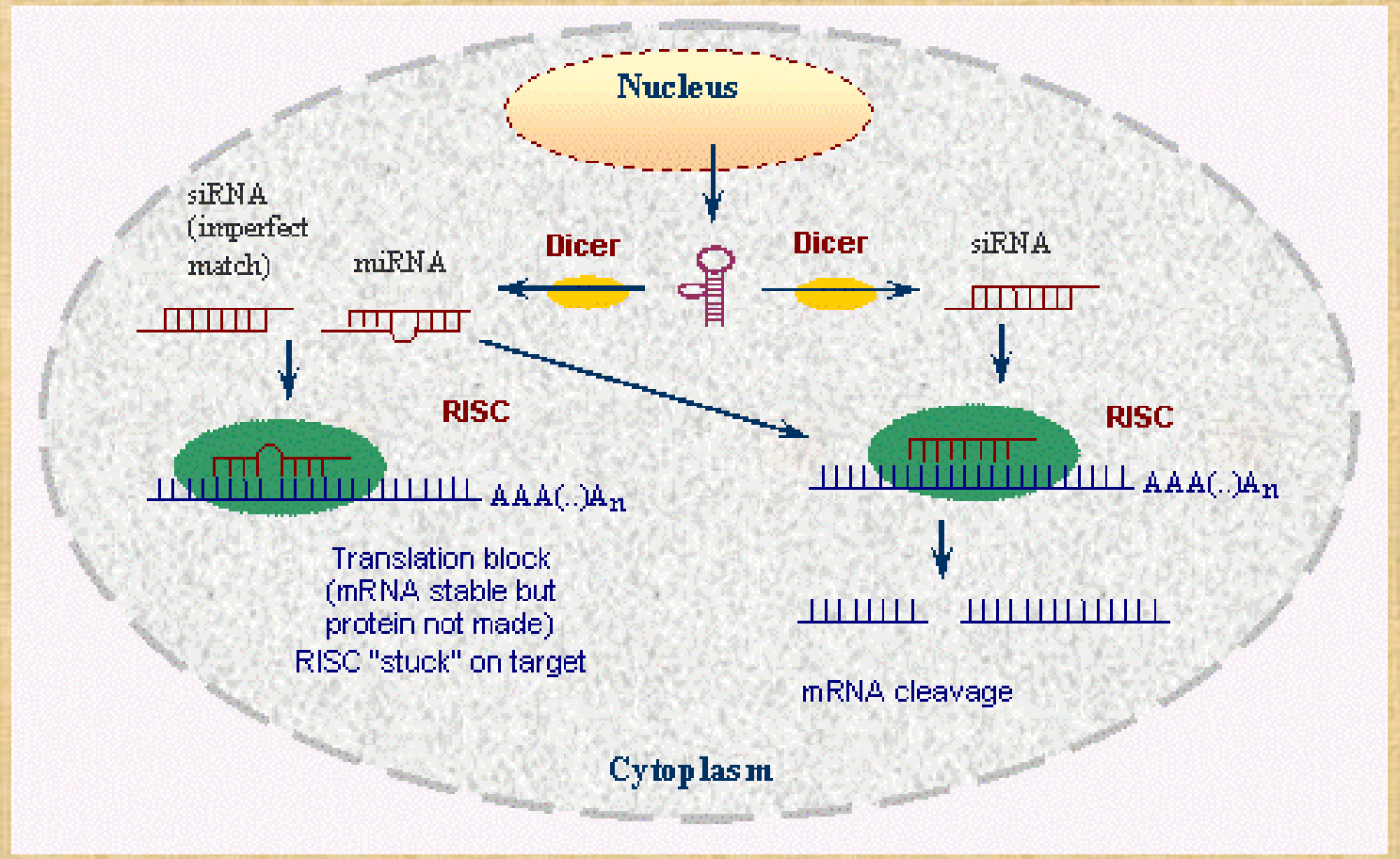
Gene silencing by RNAi is a natural genetic mechanism in eukaryotes that takes place following transcription (the synthesis of mRNA from DNA). Special microRNA (miRNA) segments, each of which is approximately 20 nucleotides in length, are encoded by the genomes of eukaryotic organisms. Each miRNA is produced from a precursor transcript (pre-miRNA). After the pre-miRNA migrates from the nucleus into the cytoplasm, it is cleaved into a mature miRNA by an enzyme known as DICER. The mature miRNA molecule then binds to an RNA-induced silencing complex (RISC), which contains multiple proteins, including a ribonuclease enzyme. The miRNA nucleotide sequence directs the protein complex to bind to a complementary sequence of mRNA. Once bound to the mRNA, the miRNA-RISC complex then enzymatically cleaves targeted sites on the mRNA molecule, thereby inhibiting the translation of the gene into a protein, which effectively silences the gene (Figure).

RNAi plays an important role not only in regulating genes but also in mediating cellular defense against infection by RNA viruses, including influenza viruses and rhabdoviruses, a group that contains the causative agent of rabies. In fact, a number of plants and animals have evolved antiviral RNAi genes that encode short segments of RNA molecules with sequences that are complementary to viral sequences. This complementarity enables interfering RNA produced by the cell to bind to and inactivate specific RNA viruses. RNAi also is an innate mechanism by which cells can suppress the activity of transposons, or “jumping genes.” Certain types of transposable elements are able to produce mobile copies of themselves, which subsequently are inserted into various regions of the genome, giving rise to repetitive sequences of DNA. These insertions generally are of little concern. However, some insertions lead to increased or decreased gene activity and can give rise to disease in humans. For example, certain types of cancer and Duchenne muscular dystrophy, a hereditary muscle-wasting disorder, are associated with insertions of transposons.

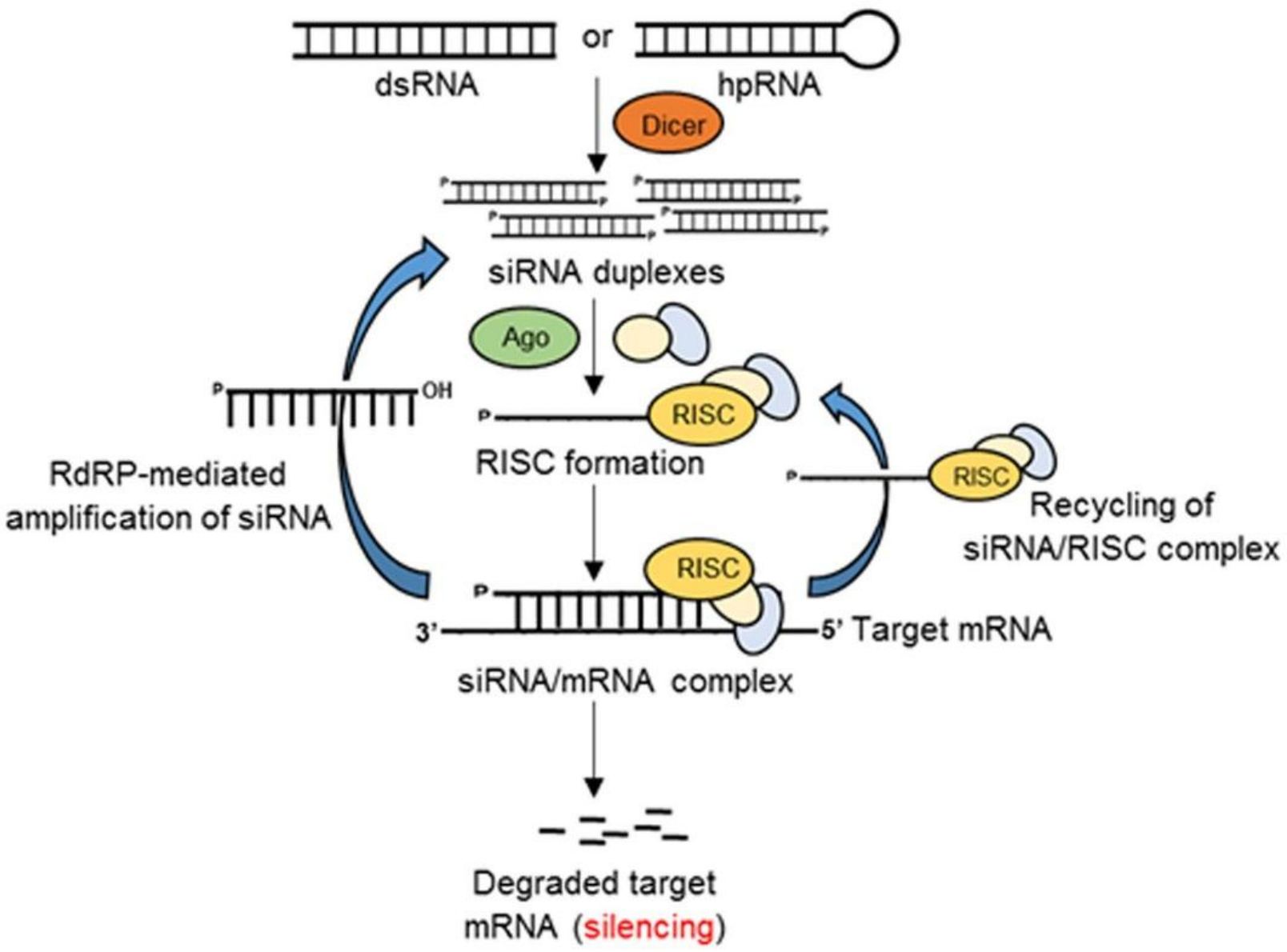


Antisense and RNAi are referred as gene knockdown technologies: the transcription of the gene is unaffected; however, gene expression, i.e. protein synthesis, is lost because mRNA molecules become unstable or inaccessible. Furthermore, RNAi is based on naturally occurring phenomenon known as Post-Transcriptional Gene Silencing (PTGS).

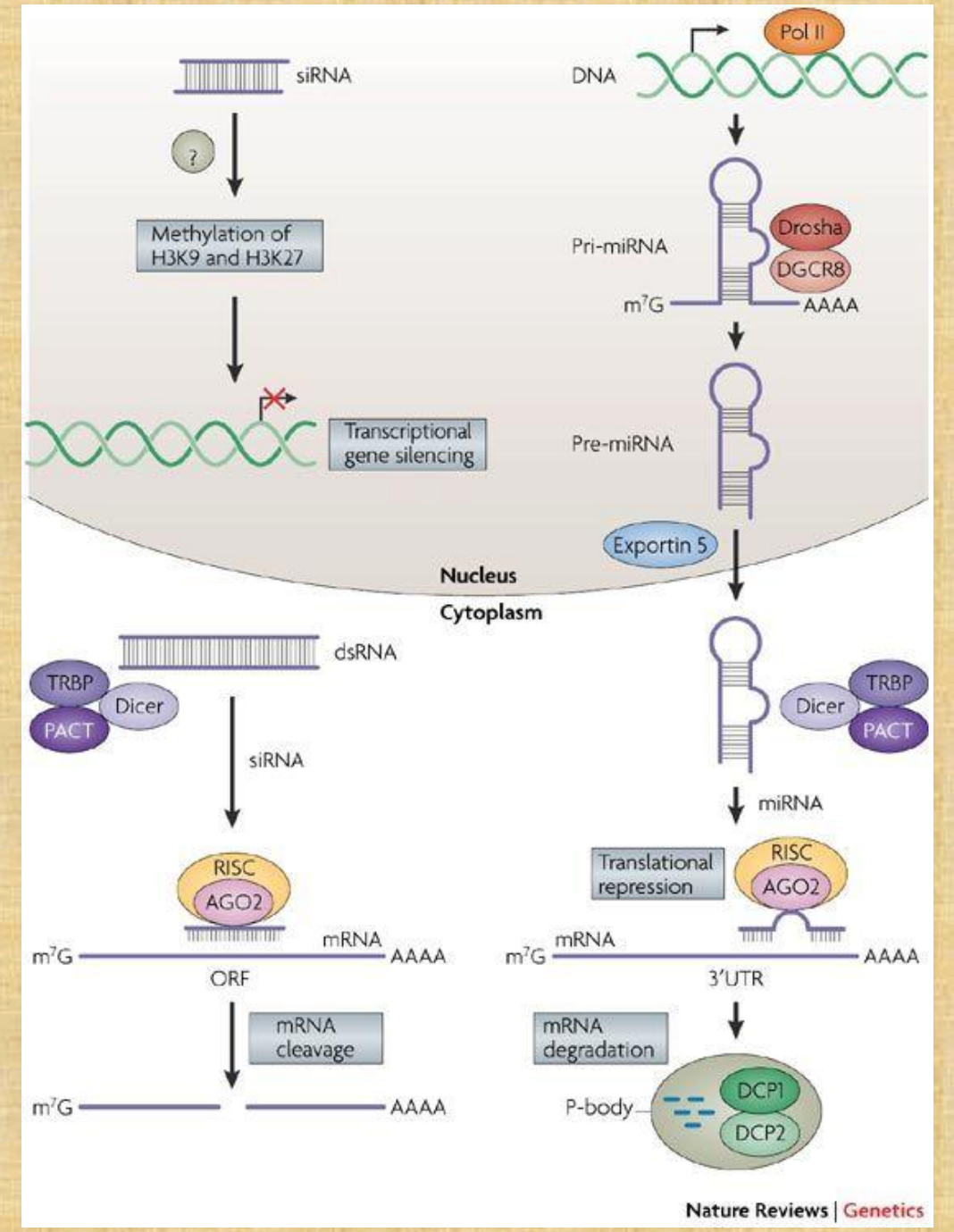
RNAi Pathway



A simplified model for the RNAi pathway is based on two steps, each involving ribonuclease enzyme. In the first step, the trigger RNA (either dsRNA or miRNA primary transcript) is processed into an short, interfering RNA (siRNA) by the RNase II enzymes Dicer and Drosha. In the second step, siRNAs are loaded into the effector complex RNA-induced silencing complex (RISC). The siRNA is unwound during RISC assembly and the single-stranded RNA hybridizes with mRNA target. Gene silencing is a result of nucleolytic degradation of the targeted mRNA by the RNase H enzyme Argonaute (Slicer). If the siRNA/mRNA duplex contains mismatches the mRNA is not cleaved. Rather, gene silencing is a result of translational inhibition



Schematic of RNAi-mediated gene silencing in eukaryotes. Double-stranded RNAs or hairpin RNAs (hpRNAs) generate small siRNA duplexes by the action of Dicer. The guide RNA strand binds with Argonaute (Ago) and other proteins to form an RNA-induced silencing complex (RISC). The siRNA/RISC complex then binds the complementary sequence of the target mRNA resulting in the degradation of the target transcript or inhibition of translation. The components of siRNA/mRNA complex can be recycled to the RISC complex or generate siRNA duplexes by the action of RNA-dependent RNA-polymerase (RdRP).



Mechanism of siRNA and miRNA

Both siRNA and miRNA can play a role in epigenetics through a process called RNA induced transcriptional silencing (RITS).

Table adapted from Mack, 2007

	siRNA	miRNA
Occurrence	Occurs naturally in plants and lower animals. Whether or not they occur naturally in mammals is an unsettled question.	Occurs naturally in plants and animals.
Configuration	Double stranded	Single stranded
Length	21-22 nt	19-25 nt
Complementarity to target mRNA	100% perfect match; therefore, siRNAs knock down specific genes, with minor off-target exceptions.	Not exact; therefore, a single miRNA may target up to hundreds of mRNAs.
Biogenesis	Regulate the same genes that express them.	Expressed by genes whose purpose is to make miRNAs, but they regulate genes (mRNAs) other than the ones that expressed them.
Action	Cleave mRNA	Inhibit translation of mRNA
Function	Act as gene silencing guardians in plants and animals that do not have antibody-or cell-mediated immunity.	Regulators (inhibitors) of genes (mRNAs)

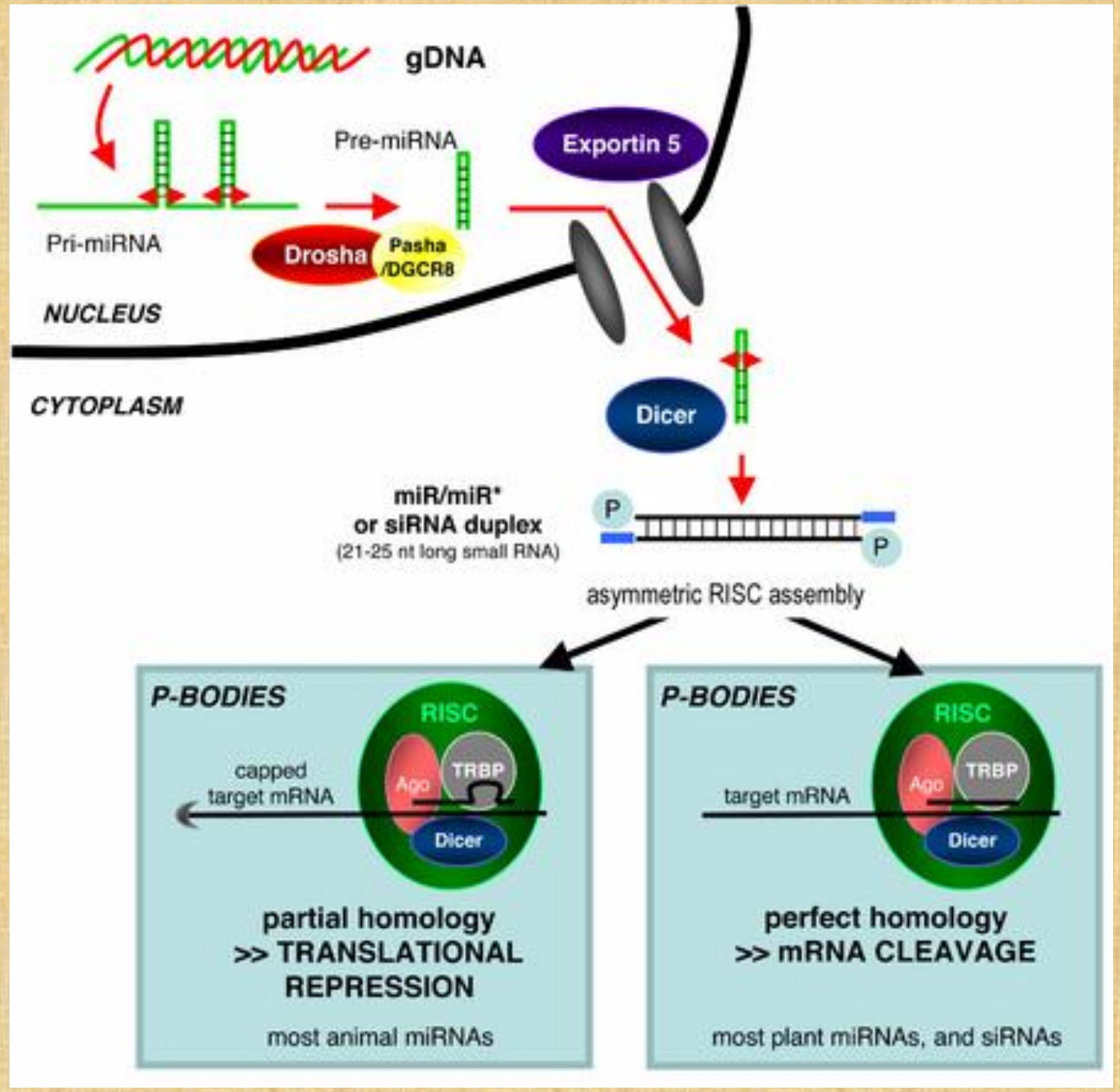


Illustration of the major differences between plant and animal gene silencing. Natively expressed microRNA or exogenous small interfering RNA is processed by dicer and integrated into the RISC complex, which mediates gene silencing.

Mechanism of RNA Interference

The components of the RNAi machinery were identified. Double-stranded RNA binds to a protein complex, Dicer (RNA nucleases), which cleaves it into fragments (21- to 25-nucleotide RNA fragments (siRNA). Another protein complex, RISC, binds these fragments. One of the RNA strands is eliminated but the other remains bound to the RISC complex and serves as a probe to detect mRNA molecules. When an mRNA molecule can pair with the RNA fragment on RISC, it is bound to the RISC complex, cleaved and degraded. The gene served by this particular mRNA has been silenced.

Applications

RNAi technology is proving to be useful to analyze quickly the functions of a number of genes in a wide variety of organisms.

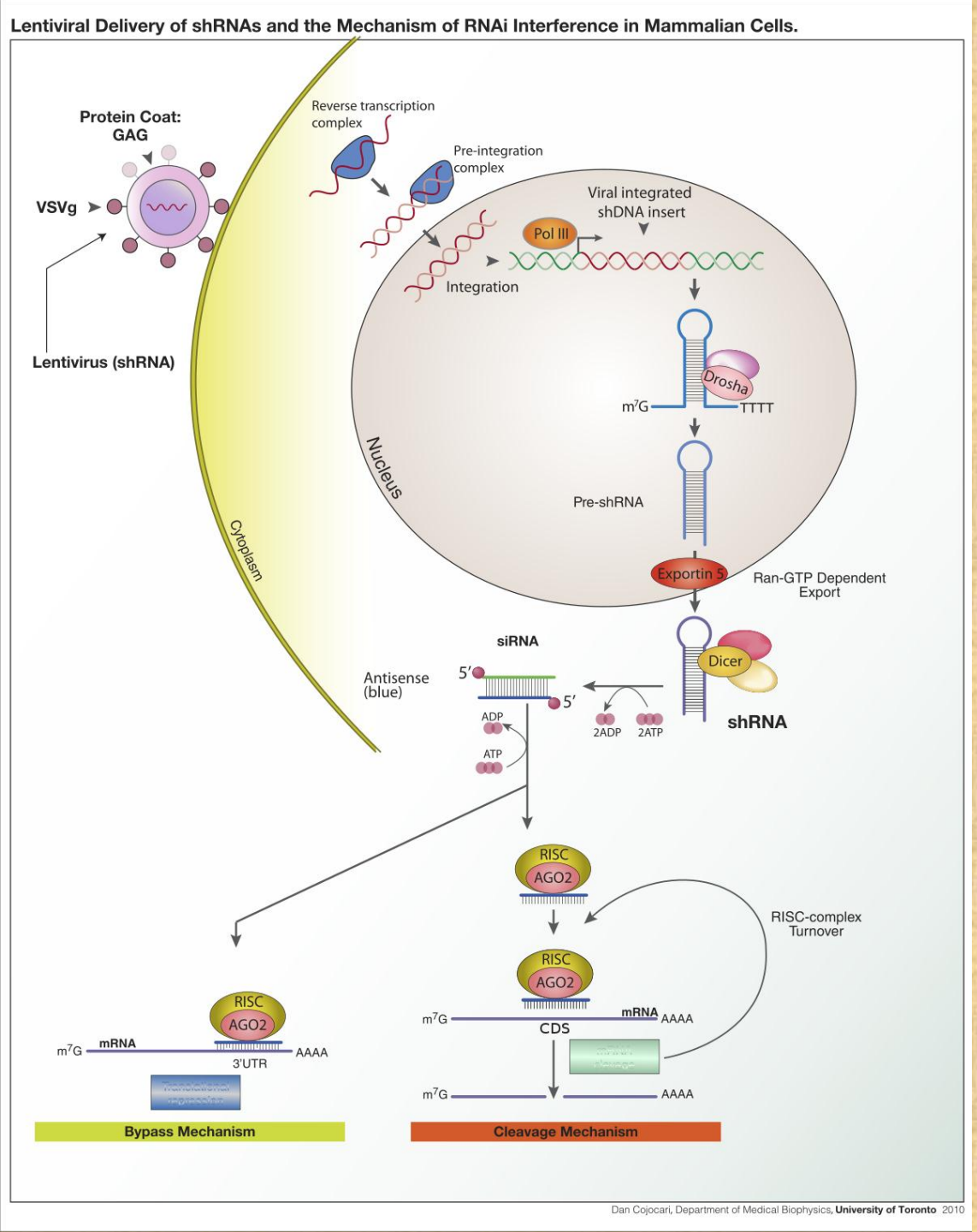
In plants, gene knockdown-related functional studies are being carried out efficiently when transgenes are present in the form of hairpin (or RNAi) constructs. Plant endotoxins could also be removed if the toxin biosynthesis genes are targeted with the RNAi constructs. Recently, the theobromine synthase of the coffee plant was knocked down with the hairpin construct of the transgene, leading to the production of decaffeinated coffee plants. Virus-induced gene silencing has also been proven to be a successful approach for plant genetics.

RNAi may facilitate drug screening and development by identifying genes that can confer drug resistance or genes whose mutant phenotypes are ameliorated by drug treatment, providing information about the modes of action of novel compounds. siRNAs are potential therapeutic reagents because of their power to down regulate the expression pattern of mutant genes in diseased cells.

siRNAs have been shown to inhibit infection by human immunodeficiency virus, poliovirus, and hepatitis C virus in cultured cell lines. siRNAbased therapy seems to have a great potential to combat carcinomas, myeloma, and cancer caused by overexpression of an oncoprotein or generation of an oncoprotein by chromosomal translocation and point mutations. However, independent of its biomedical applications, RNAi appears to be a forthcoming method for functional genomics.

References

- RNA Interference: Biology, Mechanism, and Applications, Neema Agrawal, P. V. N. Dasaradhi, Asif Mohmmed, Pawan Malhotra, Raj K. Bhatnagar, and Sunil K. Mukherjee, MICROBIOLOGY AND MOLECULAR BIOLOGY REVIEWS, Dec. 2003, p. 657–685.
- Andrew Fire, SiQun Xu, Mary K. Montgomery, Potent and specific genetic interference by double stranded RNAin *Caenorhabditis elegans* Steven A. Kostas*†, Samuel E. Driver‡ & Craig C. Mello *Nature* volume 391, pages806–811(1998)
- https://www.britannica.com/science/RNA-interference
- https://www.frontiersin.org/articles/10.3389/fpls.2017.00200/full
- https://www.thermofisher.com/in/en/home/references/gibco-cell-culture-basics/transfection-basics/rnai-non-coding-rna-research.html



Lentiviral delivery of designed shRNAs and the mechanism of RNA interference in mammalian cells.

Nobel price 2006

"for their discovery of RNA interference - gene silencing by double-stranded RNA"

Andrew Z. Fire

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Stanford University
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Craig C. Mello

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University of Massachusetts
Medical School
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Andrew Fire and Craig C. Mello shared the 2006 Nobel Prize in Physiology or Medicine for their work on RNA interference in the nematode worm *Caenorhabditis elegans*, which they published in 1998.

Potent and specific genetic interference by double-stranded RNA in *Caenorhabditis elegans*

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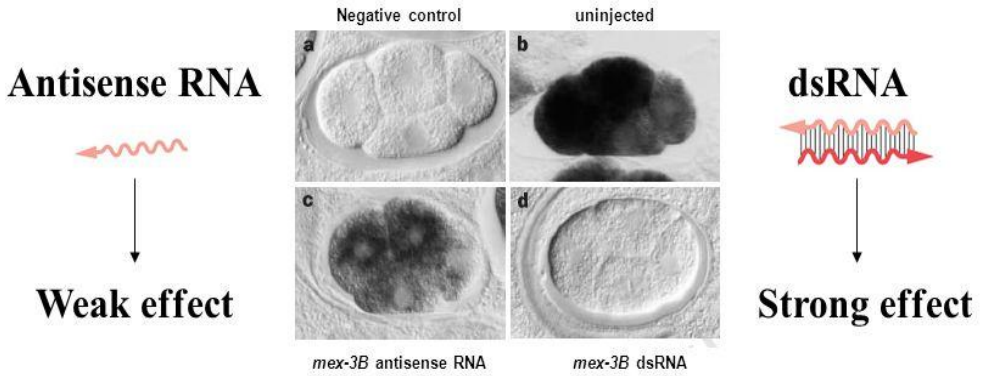
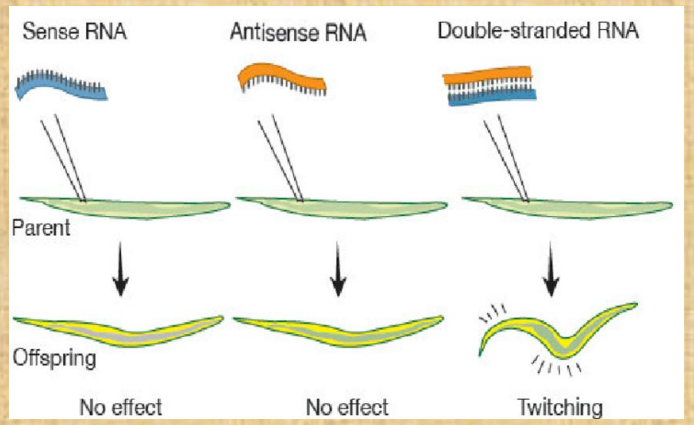
Nature **volume 391**, pages806–811(1998)

Double-stranded RNA causes silencing: RNA Interference!



Double-stranded RNA was a contaminant in antisense experiments

1998	Fire et al.	First described RNAi phenomenon in C. elegans by injecting dsRNA into *C. elegans*, which led to an efficient sequence-specific silencing and coined the term "RNA Interference".



Andrew Fire and Craig Mello were investigating how gene expression is regulated in the nematode worm *Caenorhabditis elegans* . Injecting mRNA molecules encoding a muscle protein led to no changes in the behavior of the worms. The genetic code in mRNA is described as being the 'sense' sequence, and injecting 'antisense' RNA, which can pair with the mRNA, also had no effect. But when Fire and Mello injected sense and antisense RNA together, they observed that the worms displayed peculiar, twitching movements. Similar movements were seen in worms that completely lacked a functioning gene for the muscle protein. When sense and antisense RNA molecules meet, they bind to each other and form double-stranded RNA. Could it be that such a double-stranded RNA molecule silences the gene carrying the same code as this particular RNA? Fire and Mello tested this hypothesis by injecting double-stranded RNA molecules containing the genetic codes for several other worm proteins. In every experiment, injection of double-stranded RNA carrying a genetic code led to silencing of the gene containing that particular code. The protein encoded by that gene was no longer formed.

The individual steps of miRNA biogenesis.

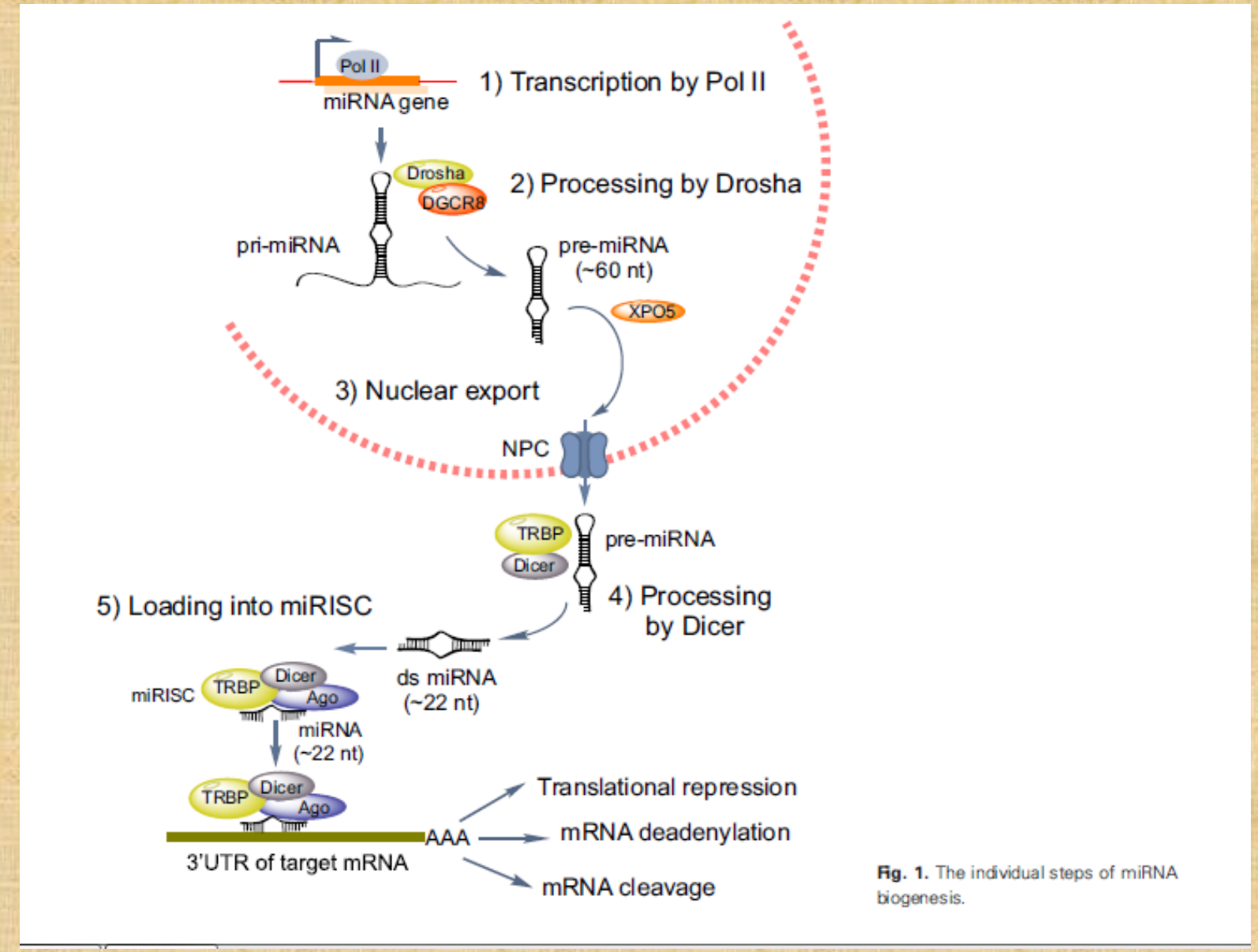


Fig. 1. The individual steps of miRNA biogenesis.

Table 1. Enzymes and sophisticated molecules participating in antisense technology

Enzyme	Function
DICER (DCL)	Cleave the dsRNA for biogenesis of siRNAs and miRNAs
Argonautes (AGO)	Specialized proteins that function binding modules of small RNAs component and coordinate gene silencing together with RISC
RNA-induced silencing complex (RISC)	Unwind the double-stranded siRNA produced by dicer and guide it to cleave the target mRNA in RNA interference methods
Ribonuclease H (RNase H)	Cleave the target mRNA in the case of antisense RNA methods

RNAi in crop improvement

Fruit improvement				
Beta-caroteins	RNAi	BCH	Potato	Van Eck et al. (2007)
Carotenoids and flavonoids	RNAi	DEF1	Tomatoes	Davuluri et al. (2005)
Seedless fruit improvement	RNAi	CHS	Tomato	Schijlen et al. (2007)
Enhanced shelf life	RNAi	*MaMADS1/S2*	Banana	Elitzur et al. (2016)
Reduce ethylene	RNAi	ACC synthase	Tomato	Aarti Gupta et al. (2013)
Biotic stress resistance				
Bacteria resistance				
Leaf blight	RNAi	OsSSI2	Rice	Younis et al. (2014)
Fungal resistance				
Sheath blight pathogen	RNAi	RPMK1-1/-2	Rice	Ila Mukul Tiwari et al. (2017)
Apple scab fungus	RNAi	GFP & THN	Apple	Fitzgerald et al. (2004)

Virus resistance				
Tobacco mosaic virus	asRNA	CP	Tobacco	Powell et al. (1989)
PMMoV	RNAi	PMMoV replicase	Pepper	Dalakouras et al. (2020)
Insect resistance				
Helicoverpa armigera	RNAi	CYP6AE14	Cotton	Younis et al. (2014)
Nematode	RNAi	Mi-msp2	Arabidopsis	Joshi et al. (2019)
Whitefly	RNAi	v-ATPase	Lettuce	Ibrahim et al. (2017)
Abiotic stress tolerance				
Salt tolerance	RNAi	TaPUB1	Wheat	Wenlong Wang et al. (2020)

(*Continued*)

Improved traits	RNA tools	Targeted gene	Crops	References
Phytate accumulation	RNAi	GmMIPS1	Soybean	Kumar et al. (2019)
Drought tolerance	RNAi	GhSnRK2	Cotton	Bello et al. (2014)
Drought tolerance	RNAi	GbMYB5	Cotton	Chen et al. (2015)
Male sterility				
Parthenocarpy	RNAi	TA29	Tobacco	Nawaz-ul-Rehman et al. (2007)
Male sterility	RNAi	SmTAF10/13	Tomato	Toppino et al. (2011)