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Review:

Antisense RNA: the new favorite in genetic research*

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Abstract: Antisense RNA molecule represents a unique type of DNA transcript that comprises 19–23 nucleotides and is complementary to mRNA. Antisense RNAs play the crucial role in regulating gene expression at multiple levels, such as at replication, transcription, and translation. In addition, artificial antisense RNAs can effectively regulate the expression of related genes in host cells. With the development of antisense RNA, investigating the functions of antisense RNAs has emerged as a hot research field. This review summarizes our current understanding of antisense RNAs, particularly of the formation of antisense RNAs and their mechanism of regulating the expression of their target genes. In addition, we detail the effects and applications of antisense RNAs in antivirus and anticancer treatments and in regulating the expression of related genes in plants and microorganisms. This review is intended to highlight the key role of antisense RNA in genetic research and guide new investigators to the study of antisense RNAs.

Key words: Antisense RNA; Formation mode; Regulatory mechanism; Application field; Genetic research
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1 Introduction

The conventional central dogma of genetics is that DNA stores genetic information and proteins execute the biological functions, while RNA serves as a bridge in the transmission of genetic information. However, only approximately 20000 genes (≤2% of the total DNA) are translated into proteins, and >90% of genes are transcribed into noncoding RNAs (ncRNAs) in the human genome (Qi and Du, 2013). Notably, >98% of RNAs formed in human cells are ncRNAs (Rusk, 2015). These findings raise the following question: why do ncRNAs in higher life forms account for >90% of the RNA produced in cells? In addition, ncRNAs constitute a highly diverse and complex group and are also referred to as "junk RNA" (Boland, 2017). Moreover, the specific physiological functions of ncRNAs are mainly performed by proteins (Rusk, 2015).

With the discovery over the past decade or two of small interfering RNA (siRNA), microRNA (miRNA), long noncoding RNA (lncRNA), and PIWI-interacting RNA (piRNA), ncRNAs have become a highly popular topic of research. Antisense RNAs represent a specific type of ncRNA used for regulating genetic activity at multiple levels in cells, such as at DNA, RNA and chromosome structures, transcription and translation, and RNA and protein stabilities (Rusk, 2015). Antisense RNAs are unique DNA transcripts. They are small, noncoding, and diffusible molecules, containing 19–23 nucleotides that complement mRNA. With the development of antisense RNAs, their application will gradually replace traditional technology for gene-specific silencing. Therefore, antisense RNA, once considered as a "yard waste" of RNA, has come to be recognized as a potential extraordinary treasure in intracellular gene regulation.

§ The two authors contributed equally to this work

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In this review, we summarize the current understanding of antisense RNAs, particularly of the formation and their mechanism regulating the expression of their target genes. In addition, we highlight the effects and applications of antisense RNAs in antivirus and anticancer treatments and in regulating gene expression in plants and microorganisms.

2 Antisense RNA: formation and regulatory mechanisms

Previous studies on the mechanism of the *lac* repressor suggested that the mechanism was similar to the antisense RNA mechanism (Fig. 1) (Jacob and Monod, 1961). The operator, which controls gene expression, attaches either with the genes (Model I) or with the cytoplasmic messengers of flanking genes (Model II). The mechanism described in Model II is more similar than that in Model I to the antisense RNA mechanism, because the *lac* repressor is a protein transcription factor (Appasani, 2004). Currently, studies on miRNAs, siRNAs, lncRNAs, and piRNAs represent a hotspot in the research on antisense RNAs. Thus, we illustrate below the formation and regulatory mechanism of antisense RNAs by describing these aspects according to the aforementioned four types of antisense RNA.

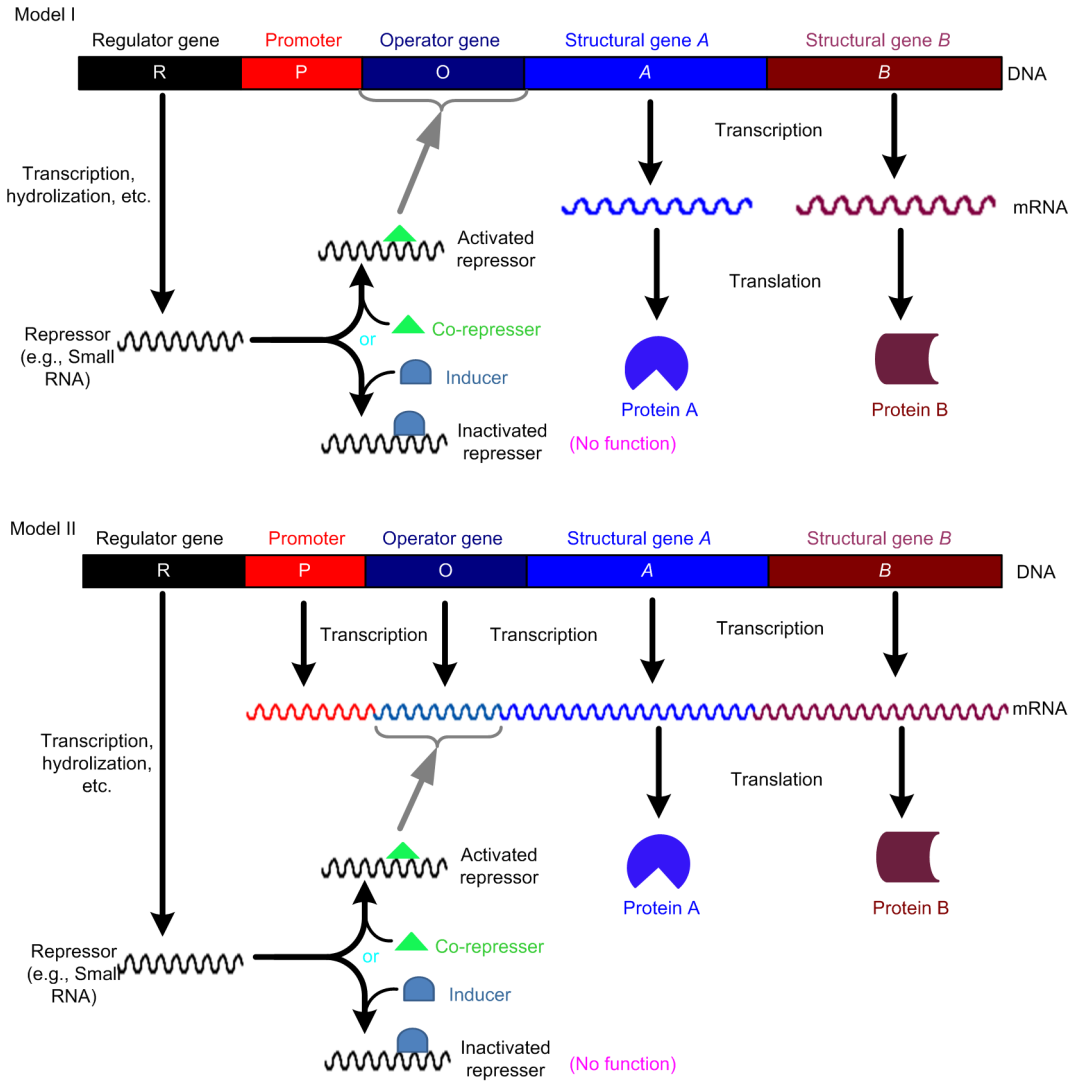

Fig. 1 Two models of the regulation of protein synthesis

Based on the description of Jacob and Monod (1961), two models were considered to have a similar mechanism for antisense RNA, especially Model II. For antisense RNA, small RNA can be used as repressor

2.1 Formation and regulatory mechanisms of miRNAs

miRNAs were first identified as RNA molecules from *Caenorhabditis elegans*, including *lin-4* (Lee et al., 1993) and *let-7* (Lau et al., 2001). miRNA is a single-stranded RNA (ssRNA) that contains 18–25 nucleotides and differs from the long RNA transcripts of noncoding DNA, which is referred to as the primary transcripts of miRNAs (pri-miRNAs) (Mohr and Mott, 2015). Although miRNA is considered as an RNA transcript generated from DNA, it cannot be translated into a protein. In contrast, it is used as an inhibitor of the expression of its target coding gene (Mohr and Mott, 2015). Pri-miRNA is a long RNA transcript that contains at least one hairpin-like miRNA precursor (Adams, 2017). Then the precursor is processed by enzyme ribonuclease (RNase) III (i.e. Drosha and DGCR8/Pasha) in the nucleus to generate precursor miRNA (pre-miRNA) (Mohr and Mott, 2015). Next, intranuclear pre-miRNA is transferred to the cytoplasm by Exportin-5 (Kim et al., 2016), and then forms a novel pre-miRNA featuring stem and loop structures (Ling et al., 2013). In the cytoplasm, the novel pre-miRNAs are cleaved at the hairpin stem region by RNase III (i.e. Dicer) to generate mature miRNAs (Kim et al., 2016). The mature miRNAs can be connected by the Argonaute protein family with RNA-induced silencing complex (RISC) to activate RISC (Riley et al., 2012), thus leading to the degradation of the target mRNA or the repression of translation (Nishimura and Fabian, 2016). At this point, miRNAs regulate gene expression by the base complementarity between mRNAs and miRNAs rather than by mRNA degradation (Schmiedel et al., 2015). In certain cases, however, the combination of double-stranded RNAs (dsRNAs) and miRNAs will trigger mRNA degradation. Therefore, miRNAs also play a major role in mediating mRNA degradation at the region of 20 base pairs (bp) (Mohr and Mott, 2015; Schmiedel et al., 2015). Since miRNAs regulate the gene expression via the partial complementarity of bases, one miRNA can regulate at least one mRNA, or one mRNA can be regulated by multiple miRNAs (Schmiedel et al., 2015). Thus, miRNAs perform diverse functions in regulating the expression of the coding genes. The mechanisms of miRNAs and their functions are described in Fig. 2.

2.2 Formation and regulatory mechanisms of siRNAs

siRNA is a small exogenous dsRNA (contains about 20 nucleotides), which is artificially synthesized in the process of RNA interference (RNAi) in vitro or transferred from the nucleus into the cytoplasm by transporters (Lam et al., 2015; Valiunas et al., 2015). In *C. elegans*, dsRNA is transferred to the cytoplasm by the transmembrane protein SID-1, and then processed by DCR-1 to form the double-stranded siRNA. These double-stranded siRNAs are connected to small interfering RISC (siRISC) by RISC, and then are unfolded by a helicase to activate the siRISC. Subsequently, the activated siRISC is associated with the target mRNA in a sequence-specific manner (Saurabh et al., 2014). RDE-2 and MUT-7 help the siRISC to associate with the target mRNA (Valiunas et al., 2015). RISC generated by a single siRNA can degrade the complementary target mRNA specifically and completely, thereby inhibiting the translation process (Saurabh et al., 2014). Antisense siRNAs can also be used as primers in the RNA-dependent RNA polymerase reaction, which uses an mRNA as the template to produce additional antisense siRNAs (Saurabh et al., 2014). Previous studies indicated that intracorporal dsRNAs can also be derived from virus activation, transposon activation, specific repetitive sequences, or other unknown mechanisms except for exogenous import (Creasey et al., 2014). In Fig. 2, siRNA formation and regulatory mechanisms are detailed.

Deciphering the relationship between miRNAs and siRNAs is a big challenge because both are small molecules in the cell and are generated from dsRNAs. Moreover, the biosynthetic pathways and regulatory mechanisms of these molecules are similar (Fig. 2). However, miRNAs and siRNAs mediate RNAi via different mechanisms. miRNAs are highly evolutionarily conserved and small single-stranded ncRNA molecules that regulate gene expression by inhibiting translation rather than by affecting transcript stability. In contrast, siRNAs are small double-stranded coding RNA molecules that regulate gene expression through the degradation of the target mRNAs at the posttranscriptional level. miRNA is only partially complementary with a part of mRNA in the 3'-untranslated region (UTR), whereas siRNA is completely complementary

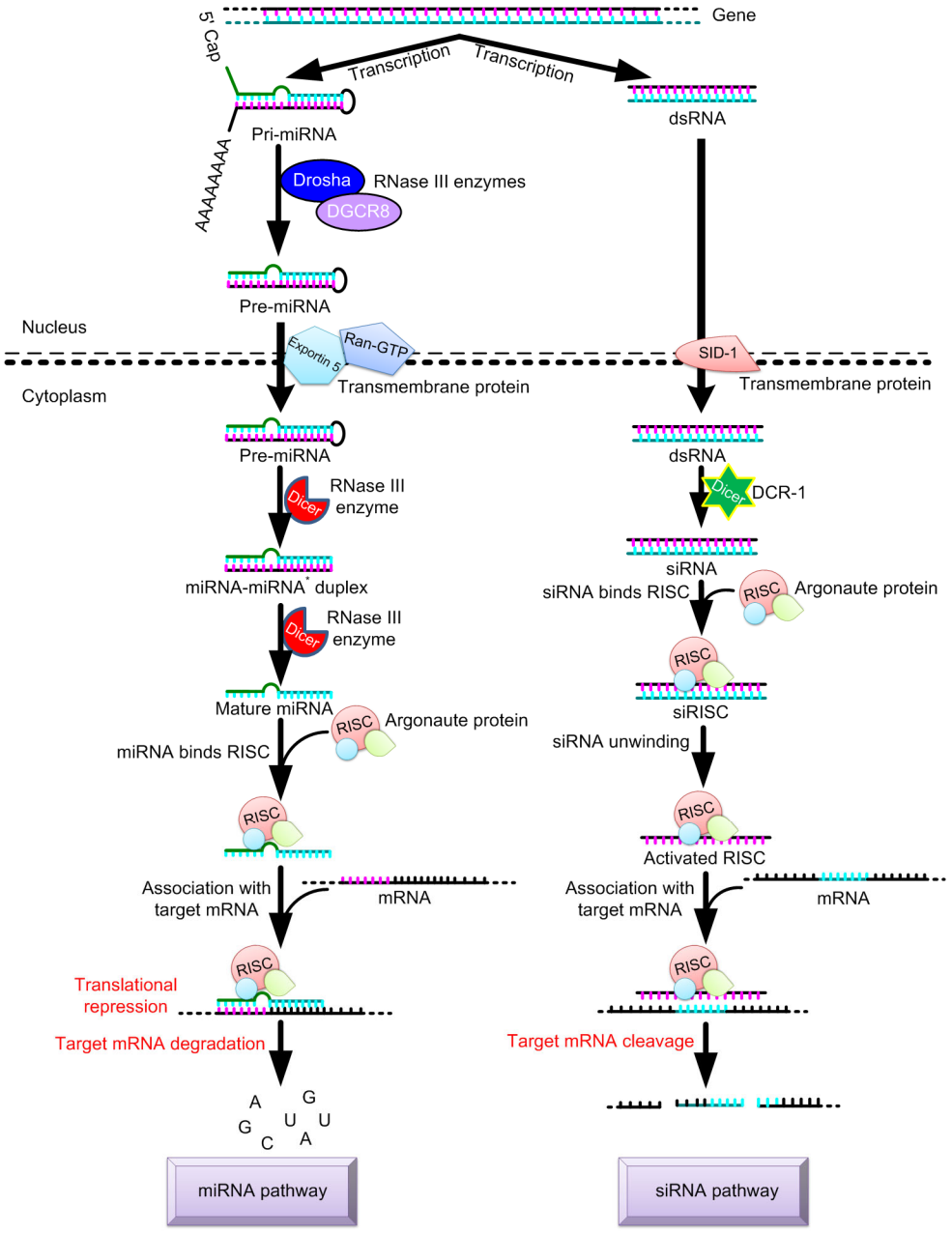

Fig. 2 Formation mode and regulatory mechanisms of miRNAs and siRNA

The aqua green line and pink line represent complementary base; RNA-induced silencing complex (RISC) represents RNA-induced silencing complex; A, G, C, and U represent the nucleotides (Note: for interpretation of the references to color in this figure legend, the reader is referred to the web version of this article)

with mRNA. Although the combination of dsRNAs and miRNAs will trigger the degradation of mRNAs and then prevent protein translation, the translational repression is mainly because of miRNA blocking the protein translation machinery. Conversely, siRNAs trigger the cleavage of the target mRNAs and thereby prevent protein translation. In Tables 1 and 2, the similarities and differences between miRNA and siRNA are summarized.

2.3 Formation and regulatory mechanisms of antisense lncRNAs

lncRNA is an mRNA-like transcript that ranges from 200 bp to 1000 kb in length. lncRNAs are the

Table 1 Similarities between miRNA and siRNA

Item	Similarity
Length and characteristic	About 22 nucleotides and 5'-end phosphorylated
Synthetic substrate	Both are generated from the processing dsRNA or RNA precursors
Dicer enzyme	Cleaving by Dicer enzyme and having the characteristics of Dicer products
Agonaunte family	Requiring the participation of Agonaunte protein family
RISC	A part of RISC, overlapping on the mediating mechanism of silence
Mode of action	Preventing the translation of target gene, leading to degradation of mRNA and acting on post-transcriptional level and translational level
Evolutionary relationship	siRNA is a complement of miRNA, whereas miRNA replaces siRNA in the course of evolution

RISC: RNA-induced silencing complex

Table 2 Differences between miRNA and siRNA

Item	Difference	
	miRNA	siRNA
Characteristic	Own normal regulatory mechanisms for organism; highly conserved; time-ordered and tissue-specific	Usually exogenous, induced after viral infections or artificial insertion; abnormal and highly specific
Molecular structure	ssRNA	dsRNA, 3' end with two unpaired nucleotides, usually UU
Biosynthesis, mature process, and place	Pri-miRNAs are processed in the nucleus by Drosha and transferred from the nucleus into cytoplasm by Exportin-5. The mature miRNA is formed from the pre-miRNA hairpin stem region by cleaving with the cytoplasmic RNase III enzyme Dicer in cytoplasm. miRNA is produced in the nucleus and cytoplasm	Dicer cleaves dsRNA to produce siRNA in cytoplasm
Machining process	miRNA processing is asymmetrical, but miRNA is just a side arm of the pre-miRNA, other parts of degradation	siRNA locates symmetrically in both arm sides of the precursor of the dsRNA
Argonaute protein	Different Argonaute protein	Different Argonaute protein
Biological function	Regulating the growth of plants and animals, organ development, cell apoptosis, and proliferation; regulating the differentiation of hematopoietic stem cells and embryonic stem cells; involving in regulating the genes expression of oncogenes and tumor suppressor	Resisting virus defense mechanism, silencing the overexpression of mRNA, and protecting the genome from the destruction of the transposons
Mechanism of action	Mature miRNAs with miRNP ribosome complex are used to identify target mRNAs, and to stop the translation of the target mRNA by partly complementing with mRNAs. Moreover, the formation of dsRNA through the binding of miRNA triggers the degradation of mRNA transcript	Single-stranded siRNA combined with RISC is used to guide the completely complementary combination between RISC and mRNA, then via its own helicase activity to unlock siRNAs. siRNA antisense strand identifies the target mRNA fragments and degrades the fragments, and then mRNA fragments further are degraded by extracellular enzyme
Influence on RNA	Degrading the target mRNA and regulating the translational levels, but not affecting the stability of the mRNA	Degrading the target mRNA and affecting the stability of the mRNA
Acting site	miRNA combines with the 3'-UTR region of the target gene	siRNA can act on any part of mRNA
Combined with target genes	Completely complementary, incompletely complementary, and mismatch phenomenon	Completely complementary
Specificity of the effect on the target mRNA	Low: one mutation does not affect the effect of miRNA	High: one mutation can easily change RNA interference silencing effect

RISC: RNA-induced silencing complex; miRNP: miRNA ribonucleoprotein complex

least conserved RNA molecules, and they do not function in protein synthesis (Legeai and Derrien, 2015). According to their position in the genome, lncRNAs can be divided into five types: (1) sense, (2) antisense, (3) bidirectional, (4) intronic, and (5) intergenic (Mattick, 2009). lncRNA is mainly transcribed by RNA polymerase II, and then undergoes co-transcriptional modifications (e.g. 5'-capping, polyadenylation, and pre-lncRNA splicing). Lastly, lncRNA is folded to form secondary and/or tertiary structures (Legeai and Derrien, 2015). These lncRNAs combine with gene transcripts to form a complementary double-stranded structure, which can be processed by the enzyme Dicer to produce endogenous siRNAs. Furthermore, the unpaired lncRNA sequences can also complement mature miRNA molecules. Here, our discussion is mainly focused on antisense lncRNAs, which regulate the gene expression at either transcriptional or posttranscriptional level via diverse biological mechanisms, including transcription interference, RNA–DNA interaction, or RNA–RNA interaction in the nucleus and cytoplasm (Villegas and Zaphiropoulos, 2015). Antisense lncRNAs are involved in X-chromosome inactivation, genomic imprinting, and the occurrence and development of certain diseases (Villegas and Zaphiropoulos, 2015; Khani et al., 2016).

2.4 Formation and regulatory mechanisms of piRNAs

piRNAs were first identified in mammalian testes, and most piRNAs contain 26–32 nucleotides (Zuo et al., 2016), which differ from miRNAs and siRNAs in size, biogenesis, expression pattern, and possibly function (Zhu et al., 2015). In combination with the members of PIWI protein family, piRNAs play a major role in regulating gene expression (Ross et al., 2014). Although the specific functions and biogenesis of piRNAs are still being elucidated, a previous study demonstrated that piRNAs are critical for regulating the growth and development of germ cells (Nishida et al., 2015). Currently, long ssRNA transcripts are presumed to be processed by an endonuclease to form 5'-end of mature piRNAs (Haase et al., 2010). A previous study indicated that human PIWI proteins can interact with the enzyme Dicer, but it remains unknown whether the piRNA biological process is associated with RNase III (i.e. Dicer and Drosha) (Ross et al., 2014). However, one speculation is that piRNA might be produced from a double-stranded RNA-DNA that is modified by the reverse transcriptase of retrotransposons (Rajan and Ramasamy, 2014). Moreover, piRNAs mainly exist in mammalian germ cells and stem cells (Mourier, 2011). piRNA combines with PIWI-family proteins to form PIWI-RNA complex, which regulates gene-silencing pathways (Ross et al., 2014). Given the functions of PIWI proteins, the role of piRNAs might have three aspects: (1) silencing the gene-transcription process, (2) maintaining the functions of stem cells and the reproductive system, and (3) regulating mRNA stability and gene translation (Ross et al., 2014).

3 Antisense RNAs: effects and applications

Antisense RNAs can be used as powerful tools for regulating genetic activity at multiple levels. Thus they have become the new favorite molecules in genetic research. Here, we highlight the effects and the applications of antisense RNAs in antivirus and anticancer treatments, and in regulating the expression of related genes in plants and microorganisms.

3.1 Effects and applications of antisense RNA in antivirus treatments

Viruses, including DNA and RNA viruses, are the most successful life forms on earth (Schmidt, 2009). Examples are the human immunodeficiency virus (HIV), hepatitis C virus (HCV), influenza virus (IV), etc. Viruses have posed serious dangers to humans and animals, whereas effective vaccines and antiviral drugs are not available for most. Therefore, antisense RNA has been welcomed by biomedical scientists as a potentially powerful new tool to target viruses (Cullen, 2014).

HIV is a lentivirus that causes persistent infection, which leads to the apoptosis of immune regulatory cells and hampers efforts to develop effective drugs and prophylactic vaccines (Wang et al., 2017). Lu et al. (2004) showed that HIV-1 replication was suppressed when a therapeutic antisense gene was expressed using a lentiviral vector constructed based on HIV-1. This recombinant lentiviral vector contains 937 bp of antisense sequence and can be used to repress HIV-1 envelope gene expression. Furthermore,

a recombinant antisense RNA with the "constitutive transport element" of "Rev response element" can be used to inhibit HIV-1 replication (Ward et al., 2009). For HCV treatment, antisense RNA can be efficiently employed to inhibit the translation of HCV RNAs in human hepatocellular carcinoma cells via introducing antisense RNAs with the highly conserved 5' region of the HCV genome at genetic loci 1–402 (Wakita et al., 1999). Additional examples of the use of antisense RNAs for inhibiting HCV replication are summarized in the previous review (Verstegen et al., 2015). In addition, antisense RNAs are also widely used in IV treatment, which causes considerable morbidity and mortality in humans and animals (Salomon and Webster, 2009). For example, Ge et al. (2003) reported that siRNAs with the conserved regions of viral genomes can strongly inhibit the replication of IV. In another study, Zhou et al. (2008) used the siRNA expression plasmid pBabe-Super to inhibit the accumulation of IV in embryonated chicken eggs, BALB/c mice, and cell lines, indicating that siRNAs targeting nucleocapsid proteins play a highly specific role in inhibiting the production of RNA in infected cells. These findings provide a theoretical basis for developing antisense RNA as a therapy for influenza infection in humans and animals.

3.2 Effects and applications of antisense RNA in anticancer treatments

Cancer is not only one of the biggest global killers in humans, but also one of the fastest-growing fatal diseases (Cavalli, 2013). Although cancer is widely researched, early discovery and therapy techniques are still lacking. The generation of cancer is widely recognized to be an extremely complex process, including the inactivation of cancer-suppressing genes and the activation of cancer-inducing genes (Hansen et al., 2015). Fortunately, considerable evidence has now demonstrated that antisense RNAs can play a critical role in cancer diagnosis and treatment.

In cancer, ncRNAs serve as potential biomarkers (Koch, 2014). For cancer treatment, effective and clinically relevant biomarkers are essential (Bustin and Murphy, 2013), and several studies indicated that the abnormal expression of antisense RNA can be used as an indicator in cancer diagnosis. For example, Iorio et al. (2005) found that the expression levels of 15 miRNAs are significantly different between normal and cancerous tissues. The discovery of novel anticancer treatments for cancer patients is an immediate requirement, and new drugs must be identified so that the expression of carcinogenic factors and related genes could be regulated. For example, Stat5 or survivin has been reported to be overexpressed in various human cancer cells and primary tumors. Therefore, many researchers use antisense RNAs to silence Stat5 or survivin expression, thus inhibiting the growth and apoptosis of tumor cells (Wang et al., 2005; Zhang et al., 2012).

Antisense RNA can also be used as cancer-promoting genes (e.g. miR-21, miR-155, miR-17-20) and cancer-suppressing genes (e.g. miR-15, miR-16, miR-143) (Tsang et al., 2015). Researchers can use antisense RNA to either promote the expression of cancer-suppressing genes or inhibit the expression of cancer-promoting genes, and then examine the relevant targets and the effective treatment of cancer.

3.3 Applications of antisense RNA in plants

In plants, antisense RNAs are mainly used in the inhibition of fruit maturation, virus resistance, flower coloration, starch synthesis, male sterility, and fertility (Tiwari et al., 2014). Furthermore, antisense RNAs also play a key role in the suppression or elimination of the expression of genes involved in the synthesis of harmful substances in food.

Ethylene can be used to regulate the expression of genes involved in metabolic processes, and thus control the time of maturation and extend the preservation of fruit (Kim et al., 2015). Previously, antisense RNA of 1-aminocyclopropane-1-carboxylate (ACC) oxidase from tomato was shown to be capable of inhibiting the expression of the rate-limiting enzymes in the biosynthetic pathways of ethylene, and thus postpone the maturation of fruit (Oeller et al., 1991). At present, the correlational studies are ongoing and have been extended to fruits such as pears, apples, bananas, and mangoes. Except for the maturation phase, plant viruses represent one of the critical factors, because they influence the crop yield and quality (Luckanagul et al., 2015). Antisense RNA can be used in plant virus resistance because antisense sequence inhibits RNA biosynthesis. Day et al. (1991) first reported on the use of antisense RNA to inhibit DNA viruses, and results indicated that antisense DNA of the AL1-coding gene from the geminivirus tomato golden mosaic virus (TGMV) was used to suppress TGMV replication.

Starches include amylose and amylopectin, but amylose is considered undesirable in the starch industry. However, we can use antisense RNA to improve the starch components in plants. For example, Salehuzzaman et al. (1993) successfully used the endogenous granule-bound starch synthase (*GBSS*) antisense gene to inhibit *GBSS* gene expression for the first time. This led to the absence of GBSS protein and the production of amylose-free potato starch. Antisense RNA can also be used to change flower colors. van der Krol et al. (1988) successfully changed the color of petunia by using antisense RNA for the first time. Since then, substantial progress has been made in the use of antisense RNA for altering flower color. For example, Aida et al. (2000) found that the amount of anthocyanin was decreased, whereas flavones and flavonols were markedly increased during transferring dihydroflavonol-4-reductase antisense gene into *Torenia* plants. Moreover, antisense RNA is frequently used for silencing or removing the genes involved in the biosynthesis of hazardous substances. For example, Dodo et al. (2008) used antisense RNA to silence the expression of Ara h 2 protein-coding gene in the peanut, which indicated that Ara h 2 concentration in transgenic peanut plants was decreased by approximately 25% compared with the parental plants.

Male sterility is one of the most critical traits in plants, and it can be used to ensure purity during constructing hybrid plants (Sandhu et al., 2007). Currently, antisense RNA is used for producing transgenic plants that exhibit male sterility or for restoring the reproductive capacity of male plants (Nizampatnam and Kumar, 2011). van der Meer et al. (1992) confirmed first that the antisense chalcone synthase (*CHS*) gene not only inhibits the biosynthesis of flavonoids, but also concomitantly causes male sterility in transgenic petunia plants. Sandhu et al. (2007) also used antisense RNA to suppress the expression of Mutator S (MutS) homolog (i.e. Msh1) in tomato and tobacco, and found that cytoplasmic male sterility occurred because of rearrangements in mitochondrial DNA.

3.4 Applications of antisense RNA in microorganisms

Metabolic pathways are modified via mutation breeding, gene overexpression, and gene knockout. However, the application of metabolic engineering technology is challenging because of the complexity of the microbial genetic system. However, antisense RNA plays a key role in the regulation of gene expression in microorganisms (Yang et al., 2015), because it involves a simple operation that avoids the complexity of knockout technology (Szafranski et al., 1997).

Moralejo et al. (2002) found that silencing the expression of *pepB* (encoding aspergillopepsin B, which degrades thaumatin in vitro) from *Aspergillus awamori* via inserting an antisense cassette in *pepB* led to an increase in the yield of thaumatin. In addition, xylitol dehydrogenase 1 (*xdh1*) antisense gene can be used to suppress the expression of xylitol dehydrogenase-coding gene in *Trichoderma reesei*, and thereby increases xylitol production (Wang et al., 2005). These results indicated that antisense RNA can be used in silencing the expression of genes involved in degrading desired products or intermediate metabolites. Moreover, antisense RNA can be used to investigate the regulation mechanisms of intracellular genes in microorganisms. For example, García-Rico et al. (2007) used antisense RNA to attenuate the expression of *pga1* (encoding heterotrimeric G protein α-subunit) in *Penicillium chrysogenum* NRRL 1951, and found that *pga1* plays a crucial role in controlling development and cell growth in this fungus.

The misuse and overuse of antibiotics have resulted in the emergence of multidrug-resistant organisms, and thus novel antibacterial targets and inhibitors must be discovered in order to meet clinical requirements (Ji and Lei, 2013). Antisense RNA has also been used to identify and evaluate novel antibacterial targets. Patil et al. (2013) introduced the antisense RNA of the bacterial protein YidC to down-regulate the expression of YidC in *Escherichia coli*, which indicated that this impaired the growth of the host cells, and YidC is a promising candidate target for screening antibacterial agents.

4 Conclusions and future prospects

When the first antisense RNA was discovered by Simons et al. (1983), few have imagined the impact that this new class of small ncRNAs would have on the study of development and disease biology, botany,

plasmid biology, and other areas of biology. The research on antisense RNA has steadily grown and has been marked by certain breakthroughs. Moreover, major achievements have also been made in the application of antisense RNA in biology. However, the underlying mechanisms of antisense RNA functions remain incompletely understood, and only a few in vivo functions of antisense RNAs have been demonstrated to date in organisms, especially in the case of piRNAs and lncRNAs. Increasing numbers of antisense RNA molecules will be subjected to scientific scrutiny in future studies. More importantly, the discovery of the small RNAs and their functions has markedly altered the manner in which we view gene regulation. Antisense RNAs will keep researchers extremely busy for years to come, because a substantial amount of information regarding these molecules is lacking and warrants investigation.

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Compliance with ethics guidelines

Jian-zhong XU, Jun-lan ZHANG, and Wei-guo ZHANG declare that they have no conflict of interest.

This article does not contain any studies with human or animal subjects performed by any of the authors.

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中文概要

题　目：反义 RNA：遗传研究领域的新宠儿

概　要：该综述较为全面地概述了当前反义 RNA 的研究现状，特别是它的形成模式以及参与调节目的基因的调节机制。同时，还概述了反义 RNA 在病毒和癌症治疗以及在遗传改造植物和微生物中的应用，为拟计划开展反义 RNA 研究的学者提供了指导。

关键词：反义RNA；形成模式；调节机制；应用领域；遗传研究