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# Unleashing miRNA Technology for Seed Quality Enhancement

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The rapid escalation of the global population necessitates a commensurate increase in food production to meet burgeoning demands. While conventional crop breeding methods have historically enhanced crop quality and yield, they are encumbered by their protracted timelines and labor-intensive nature. Consequently, there is a pressing need for novel strategies to swiftly integrate new genes and traits into elite crop varieties, thereby amplifying yield, fortifying nutritional content, and endowing resistance against both biotic and abiotic stresses. Gene silencing emerges as a promising avenue for modulating gene expression within cells, thereby playing a pivotal role in crop enhancement endeavors. This mechanism entails the degradation of specific gene RNA instructions, thereby thwarting protein synthesis. Antisense technology, a cornerstone technique in this domain, operates by impeding the translation of mRNA into proteins through the introduction of complementary nucleic acids into cells. Demonstrating remarkable efficacy, it serves as a potent tool for suppressing undesirable genes and augmenting desirable traits in crops. At its core, antisense technology operates on the principle of base-pairing between antisense nucleic acid sequences and their complementary sense RNA strands, thereby precluding protein synthesis. These complementary nucleic acid sequences may manifest as synthetic oligonucleotides, typically oligo deoxyribonucleotides (ODNs) comprising fewer than 30 nucleotides, or longer antisense RNA (asRNA) sequences. For instance, the sense and antisense RNA strands can be illustrated by the pairing of "5′ACGU3′ mRNA" with "3′UGCA5′ Antisense RNA."

**History**

The inception of antisense technology traces back to Dr. Hal Weintraub's pioneering work at the Basic Science Division. It was in the early 1980s when Weintraub and colleagues elucidated the inhibitory effect of antisense RNA (asRNA) on gene expression within mouse cells. The concept of asRNAs as viable drug targets took root in 1982, with the seminal discovery by Zamecnik and Stephenson. Their identification of an antisense oligonucleotide targeting the viral RNA of the Rous sarcoma virus marked a pivotal moment, demonstrating its efficacy in impeding viral replication and protein synthesis.

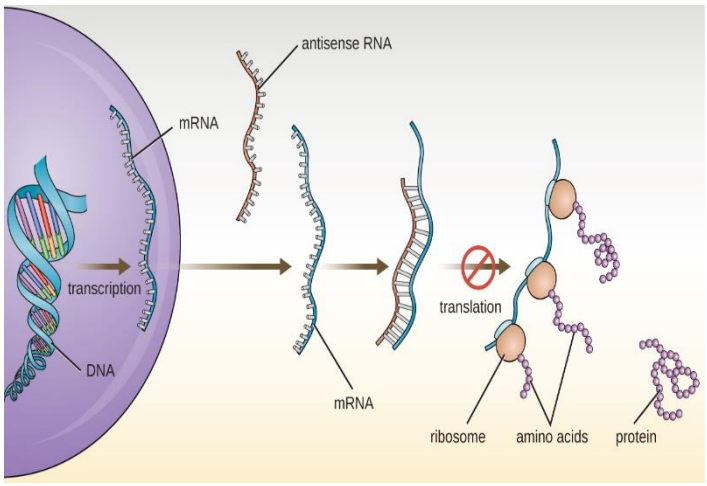


**Fig. 1 General Outline Antisense Mechanism**

Antisense oligonucleotides (AS-ONs) serve as artificially synthesized oligomers engineered to selectively hybridize with target RNA molecules, thereby exerting precise control over the expression of specific genes. This technology, operating at the level of messenger RNA (mRNA), effectively halts the synthesis of proteins encoded by these targeted genes. By binding or hybridizing with the mRNA of interest, antisense nucleic acid sequences disrupt the normal processing of genetic information within the cell through various mechanisms. Notably, the arsenal of anti-mRNA strategies deployed for knock-down or knock-out of translation includes single-stranded antisense oligonucleotides, ribozymes, and RNA interference (RNAi).

The RNAi pathway, a ubiquitous mechanism found across diverse eukaryotic organisms, including animals, initiates with the action of the enzyme Dicer. Dicer cleaves long double-stranded RNA (dsRNA) molecules into shorter fragments known as small interfering RNAs (siRNAs), typically comprising around 21 nucleotides. Following cleavage, each

siRNA undergoes unwinding, giving rise to two single-stranded RNAs: the passenger strand and the guide strand. While the passenger strand is rapidly degraded, the guide strand integrates into the RNA-induced silencing complex (RISC). One of the most extensively studied outcomes of this process is post-transcriptional gene silencing, wherein the guide strand pairs with complementary sequences within messenger RNA molecules, thereby triggering cleavage by Argonaute 2 (Ago2), the catalytic component of RISC. Intriguingly, in certain organisms, this silencing mechanism demonstrates systemic spread, despite the initially limited concentrations of siRNA molecules.

MicroRNAs (miRNAs) represent a class of genomically encoded non-coding RNAs intricately involved in the regulation of gene expression, particularly throughout various stages of development. The overarching phenomenon of RNA interference encompasses both the endogenous gene silencing effects induced by miRNAs and the silencing triggered by exogenous double-stranded RNA (dsRNA). While mature miRNAs bear structural resemblance to small interfering RNAs (siRNAs) derived from exogenous dsRNA, the maturation process of miRNAs entails extensive post-transcriptional modifications. Originating from significantly longer RNA-coding genes, miRNAs initially manifest as primary transcripts, termed pri-miRNAs. Within the nucleus of the cell, these pri-miRNAs undergo processing by the microprocessor complex, comprising an RNase III enzyme named Drosha and a dsRNA-binding protein known as DGCR8. This processing yields a 70-nucleotide stem-loop structure termed a pre-miRNA. Subsequently, Dicer, an enzyme, binds to and cleaves the dsRNA portion of the pre-miRNA, generating the mature miRNA molecule. This mature miRNA is then capable of integration into the RNA-induced silencing complex (RISC), thus sharing downstream cellular machinery with siRNAs.

Shi *et al.* (2015) observed that knockdown of fatty acid elongase1 in rapeseed (*Brassica napus*) plants to evaluate the relative fatty acid contents in seeds of wild-type (cy2) and *fae1* transgenic lines (BnFAE1-Ri 1, BnFAE1-Ri 2). Knockdown of BnFAE1 sharply decreased the content of erucic acid (< 3 %), largely increased the oleic acid content (>55 %) in transgenic plants. F1 seeds derived from reciprocal crosses between BnFAE1-Ri lines and high erucic acid cultivars showed increased the oleic acid content (>52 %) and sharply decreased the levels of erucic acid (< 4 %), demonishing that the RNAi can effectively interfere with the target gene in F1 seeds.

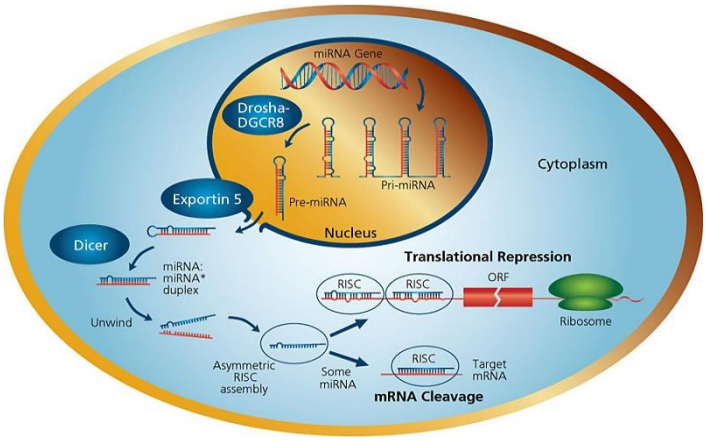


**Fig. 2 Schematic diagram of the mechanism of miRNA in targeting mRNA for gene silencing**

Guanghui *et al.* (2018) analysed the function of a wheat-specific micro RNA 9678 (miR9678), which is specifically expressed in the scutellum of developing and germinating wheat seeds. They observed an over expression of miR9678 delayed germination and improved resistance to pre-harvest sprouting in wheat through reducing bioactive gibberellic (GA) levels; however, miR9678 silencing enhanced germination rates. The result showed that abscisic acid (ABA) signalling proteins bind the promoter of miR9678 precursor and activate its expression, indicating that miR9678 affects germination by modulating the GA/ABA signalling.

Zhou *et al.*, (2020) conducted microarray assays to analyse miRNA expression levels in seeds of the rice (*Oryza sativa* L.) cultivar kasalath. In artificially aged seeds osa-miR164c was transcriptionally upregulated, while osamiR168a was downregulated. Further, they reported that under the same ageing condition, osa-miR164c over expressed in OE164c transgenic seeds and osa-miR168a silencing in MIM168a transgenic seeds of the rice cultivar kasalath led to lower germination rates, whereas osa-miR164c silencing in MIM164c and osa-miR168a over expression in OE168a

resulted in higher seed germination rates compared with wild-type seeds.

**Conclusion**

Antisense technology, an innovative methodology gaining increasing traction within the realm of agricultural science, holds promise for significantly advancing both the quality and quantity of crop yields. This cutting-edge approach has garnered acclaim as a premier tool for selectively deactivating individual genes, positioning it as a cornerstone for delving into the functions of genes whose roles remain elusive. Moreover, its utilization of relatively diminutive transgene constructs enables the simultaneous suppression of multiple targeted genes, further accentuating its versatility and efficacy. The field has witnessed substantial advancements, chiefly propelled by the refinement of modified nucleotides, which confer heightened specificity to target sites, bolstered stability, and diminished toxicity. Notably, the prevalence of RNAi-based antisense technology has been notable, particularly in its widespread application across various agricultural sectors. Its deployment, encompassing a broad spectrum of crops including frequently applied (93%) especially on vegetables (41%), cereals (33%), cash crops (26%) to improve biotic resistance (29.6%) and also to enhance nutritional values (18.5%). It is predominantly aimed at fortifying resistance against biotic stressors and augmenting nutritional profiles, reflecting its multifaceted utility and potential impact on agricultural practices.

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