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INSIGHT

The discovery of tiny RNAs that have crucial roles

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On October 7, the Nobel Assembly at Sweden's Karolinska Institute announced the awarding of the 2024 Nobel Prize in Physiology or Medicine to American scientists Victor Ambros and Gary Ruvkun for their discovery of microRNAs and their role in posttranscriptional gene regulation.

This award is well-deserved. The discovery of microRNAs has elucidated another significant life-regulating mechanism beyond the central dogma, marking not only a conceptual breakthrough in the history of science but also providing a new perspective on the process of life evolution. RNA was once thought to mainly function as a 'template' for protein translation. This work was the first to discover that very tiny RNAs can play regulatory roles, rather than acting solely as templates for protein translation.

Ambros' contributions were initially ignored for a long period of time. As an assistant professor at Harvard University, Ambros was engaged in genetic research, mainly studying gene mutations and looking for genes that affect the development of *C. elegans*. During this process, he discovered a gene, *lin-4*, that exhibited unusual characteristics: it did not encode any proteins but was crucial for the development of *C. elegans* (Lee et al., 1993). This marked the first discovery of microRNA genes, establishing Ambros as a pioneer in the field. However, due to the novelty of his work and the limited understanding of the gene's function at the time, Ambros faced significant isolation following his discovery of microRNA, as the scientific community had not yet recognized its importance. Fortunately, Ambros and Ruvkun almost simultaneously discovered the regulatory mechanism by which the microRNA *lin-4* inhibits the 3′ UTR (untranslated region) of *lin-14* in *C. elegans* (Lee et al., 1993; Wightman et al., 1993). Given that microRNAs had been identified only in *C. elegans*, as a single instance (*lin-4*), they were initially thought to be species-specific, leading to limited interest from the broader scientific community. It was not until 2000, when Ruvkun published the second microRNA, *let-7*, in *C. elegans*, that a wave of microRNA research began, as this microRNA was also conserved in other organisms, including human (Pasquinelli et al., 2000; Reinhart et al., 2000). Later, Ambros and colleagues discovered a vast number of new microRNAs in various organisms.

In fact, microRNAs are a class of small RNA molecules that play critical regulatory roles in gene expression despite their small size. They are crucial regulators of many biological processes, such as embryonic development, blood cell differentiation, muscle function, viral infections, and cancer. As widely expressed regulatory molecules, microRNAs have been proven to be of great scientific significance and high potential for clinical application in the future. Pharmaceutical companies are actively designing and developing therapies that target microRNA mechanisms for various human diseases.

To date, thousands of microRNA genes have been discovered and extensive research has clarified the production and function of microRNAs. The mechanisms of microRNA biogenesis and their role in regulating gene expression are now largely understood (For comprehensive reviews, please refer to (Bartel, 2018; Chen and Kim, 2024; Shang et al., 2023). Most microRNAs are transcribed into primary microRNA (pri-microRNA) transcripts, which are several hundred to several thousand nucleotides long, by RNA polymerase II (Figure 1). These pri-microRNAs are first cleaved by the RNase III Drosha and its cofactor DGCR8 into hairpin structures, known as precursor microRNAs (pre-microRNAs). After the first cleavage step in the nucleus, the transport protein XPO5 (exportin 5), with the help of the GTP-bound protein Ran-GTP, exports the pre-microRNAs from the nucleus to the cytoplasm. In the cytoplasm, pre-microRNAs are recognized and cleaved by another RNase III enzyme, Dicer, to produce a dimer of approximately 22-nucleotide-long microRNAs, i.e., microRNA:microRNA* duplex. The microRNA:microRNA* duplex then binds to AGO proteins to form a precursor of the RNA-induced silencing complex (RISC). The microRNA* strand is rapidly removed and degraded, leaving the mature microRNA to form a functional miRISC complex. Subsequently, through perfect or imperfect base pairing with the target mRNA, the miRISC can elicit the degradation of the target mRNA or repress its translation. Although the first microRNA *lin-4* was demonstrated to silence target genes via the inhibition of translation, later work demonstrated that the main effect of microRNAs in mammalian cells is

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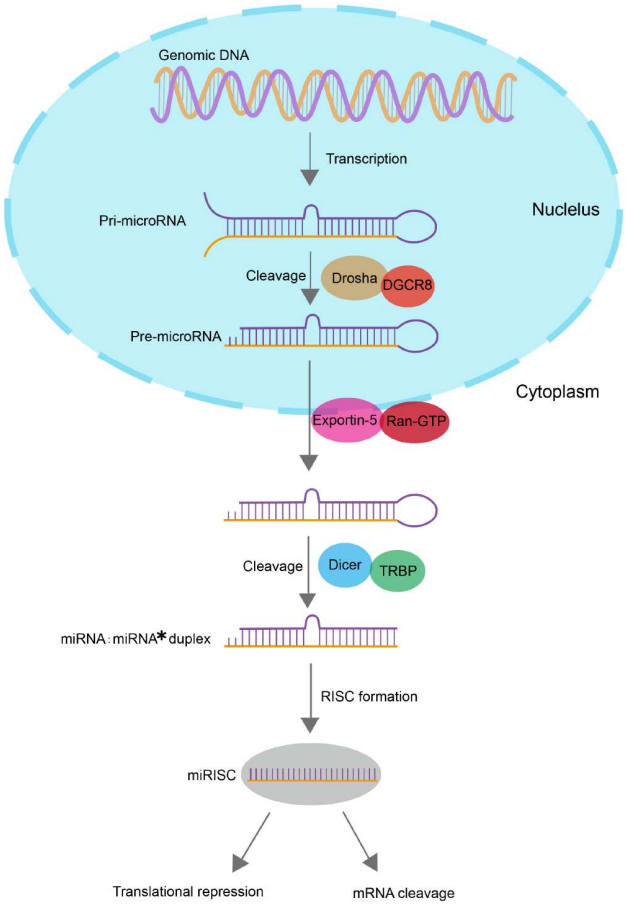

Figure 1. The biogenesis and functions of microRNAs. The maturation of microRNAs includes the production of pri-microRNA transcripts by RNA polymerase II or III. Then, the microprocessor complex Drosha-DGCR8 (Pasha) cleaves pri-microRNAs in the nucleus. The resulting precursor hairpin, pre-microRNA, is exported from the nucleus into the cytoplasm via Exportin-5-RAN-GTP. In the cytoplasm, the RNase Dicer/TRBP complex cleaves the pre-microRNA hairpin to its mature length. In the cytoplasm, the functional strand of the mature microRNA is loaded onto Argonaute (Ago2) protein into the RISC, where it directs RISC to silence the target mRNA by mRNA cleavage or translational repression.

induction of mRNA degradation (Shang et al., 2023). How each microRNA is sorted and directed to distinct silencing pathways is ambiguous. The binding of microRNAs to different AGO proteins may play different roles, indicating AGO proteins in determining the functional specificity of microRNAs. However, how microRNAs first arose and then are maintained and how they are mutually selected with AGO proteins during evolution remains a mystery.

Interestingly, microRNAs have also been reported to transport back to the nucleus to regulate gene expression or be released from cells via exosomes modulating gene expression in neighboring cells (Chen and Kim, 2024; Shang et al., 2023). Moreover, microRNAs have been reported to activate gene expression in the nucleus, rather than their well-documented repressive roles. Remarkably, they are also implicated in the inheritance of parental acquired traits to progenies. Yet, the mechanisms underlying the specific selection and translocation of certain microRNAs to their target locations require further investigation. It remains unclear regarding what extent canonical microRNA machines are involved in these processes. Although depleting many individual microRNA results in only subtle phenotypic alterations, developing new techniques to spatiotemporally track microRNAs in living cells could help resolve some of these debates.

The impact of microRNA regulation on genes is substantial and widespread. microRNA regulation follows a 'one-to-many' model, rather than a 'one-to one' model, where a single microRNA can regulate multiple genes simultaneously, influencing many biological processes, such as developmental timing, cell death, cell proliferation, hematopoiesis and nervous system patterning (Shang et al., 2023). The abnormal expression of microRNAs, whether due to gene amplification, deletion or epigenetic changes, can enhance or diminish their function, thereby affecting the expression of target proteins and causing the occurrence of diseases. Meanwhile, other noncoding RNAs, such as lncRNAs or circRNAs, may act as sponges to diminish the activity and function of microRNAs (Chen and Kim, 2024). Conversely, microRNAs may also abrogate the activity of other functional noncoding RNAs (Chen and Kim, 2024).

The relationship between microRNA and diseases is indeed very close, including cardiovascular diseases, neuronal diseases, and cancers. Numerous microRNAs have been demonstrated to participate in the regulation of various cardiovascular diseases, including myocardial hypertrophy, heart failure, and atherosclerosis. microRNAs play a crucial role in neural embryonic development, neuronal differentiation and plasticity, as well as in learning and memory processes (Meza-Sosa et al., 2014). They also exert significant influence on the onset and progression of neurological conditions, including neurodevelopmental, neuropsychiatric, and neurodegenerative disorders. Abnormal expression of microRNAs in cancer cells can lead to changes in the expression of cancer-related target genes, thereby endowing cancer cells with malignant characteristics, such as unlimited replicative capacity, sustained angiogenesis, tissue invasion and metastasis, abnormal cellular energetic metabolism, and genomic instability and mutation (Iorio and Croce, 2012).

MicroRNAs (microRNAs) hold significant potential for medical applications, with research making substantial progress in various areas, including disease diagnosis, treatment, and prognosis assessment. For example, microRNAs can be used as tumor markers for the early detection of cancers. Certain types of cancer cells abnormally express particular microRNAs. Detecting these markers enables early cancer diagnosis, significantly improving the survival rate of patients. As cancer progresses, microRNA expression changes dynamically. Continuous monitoring of microRNA levels in patients offers real-time insights into cancer progression, providing crucial insights for

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treatment adjustments (Peng and Croce, 2016). In the past few years, a number of microRNAs or their combinations have been successfully utilized in cancer diagnosis clinically.

Inhibiting oncogenic microRNAs or enhancing tumor-suppressive microRNAs is a potential cancer therapy approach. Moreover, microRNAs can complement traditional treatments like surgery, chemotherapy, and radiation, boosting their effectiveness. For example, studies have shown that altering the expression of specific microRNAs in patients during chemotherapy can increase the sensitivity of cancer cells to chemotherapeutic drugs, thereby improving treatment efficacy. Additionally, microRNAs are crucial in regulating vascular endothelial function, indicating their potential as therapeutic targets for cardiovascular diseases (Laggerbauer and Engelhardt, 2022). In neurodegenerative diseases, including Alzheimer's disease and Parkinson's disease, aberrant microRNA expression has been observed, indicating their potential as therapeutic targets as well (Sharma et al., 2024).

Research on microRNA has rapidly progressed from the laboratory to the clinical stage, with numerous successful Phase I clinical trials and ongoing Phase II clinical trials (Chakraborty et al., 2021; Kim and Croce, 2023). As they can stably exist in circulating blood, microRNAs possess the potential to serve as biomarkers for the diagnosis of cardiovascular diseases (Laggerbauer and Engelhardt, 2022). The biggest challenge currently for microRNA-based drugs is identifying the best microRNA therapeutic targets for each disease (Tani, 2024). Additional challenges include drug stability, delivery efficiency, target specificity, and off-target effects. Chemical modifications such as methylation and LNA technologies, can enhance drug stability and extend their half-life. Encapsulation techniques, like lipid nanoparticles, can also enhance the delivery efficiency of drug molecules. However, given the successful development of siRNA drugs, finding more specialized application scenarios might be the next endeavor for microRNA researchers.

The discovery of microRNAs is significant and is set to become a crucial tool for detecting and treating various diseases. Ongoing research continues to reveal new applications, driving advances in personalized and precision medicine. As our understanding of microRNAs deepens, more targeted therapies and diagnostic tools are anticipated to emerge, leading to more effective treatments and improved patient outcomes. The integration of microRNA-based therapies with current medical practices represents a promising area that could revolutionize healthcare. Notably, the Nobel Prize has also acknowledged the discovery of small interfering RNAs in 2006, underscoring the crucial roles of tiny RNAs in various biological processes and fostering the development of RNA-based treatments for diverse disorders.

New technologies including high throughput assays, ultra-resolution microscopy, single-molecule imaging and CRISPR-Cas9 screening continue to uncover fundamental information on the structural and molecular dynamics of microRNA machinery. Advanced RNA enrichment and sequencing methods will promote the discovery of new microRNAs and their modified variants, unveiling additional hidden genetic and biochemical information. How miRNA substrates and targets are selected from the transcriptome and subjected to multi-level regulation of biogenesis and turnover will continue to shed light on the understanding of the regulation, function and application of microRNAs.

Compliance and ethics

The authors declare that they have no conflict of interest.

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