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News & Views

Non-coding nucleotide of small size grabbed the Nobel Prize: A new level of gene regulation affecting health condition

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New information on gene regulation

Already last year, the subject of messenger RNA (mRNA) based Covid 19 Vaccine grabbed the Nobel Prize in physiology and medicine and now another year another Nobel Prize on the subject of RNA science. The discovery of microRNA (miRNA) which regulates new level of gene expression in eukaryotic cells has been considered for Nobel Prize in 2024. To be precise, miRNAs are tiny non-coding single-stranded RNA molecules comprised of nearly 22 nucleotide-long that bind with (base pairing) complementary sequences of mRNA and finally decide which mRNA to be translated to protein. Usually, messenger RNAs (mRNA) are translated into proteins. MicroRNA, which is a hundred times smaller than mRNA, blocks gene expression by binding to regulatory segments in their target mRNAs. Victor Ambros, from the University of Massachusetts and Gary Ruvkun, working at Harvard Medical School, have been declared to share the Nobel Prize in Physiology or Medicine for the year 2024 due to their contribution to the discovery of miRNA and its role in the post-transcriptional gene regulation. Nearly three decades past, due to scientific curiosity, the first insight into miRNA came to light by these two researchers while working together with *Caenorhabditis elegans*. The worm *C. elegans*, a eukaryotic, free-living soil nematode that contains many specialized cell types such as nerve and muscle cells like that of complex higher animal species, was used as a model for this precious discovery. In biological systems, DNA makes RNA and RNA makes proteins, so the flow of genetic information is unidirectional from DNA to RNA, and then RNA to protein is the central dogma of life. Of course, it differs in RNA viruses. Have we ever shown curiosity to know when all the somatic cells of our body contain the same chromosomes and precisely harbour the same set of genes even then lot many functional differences are exhibited by muscle cells as compared to nerve cells and intestinal cells? How such functional difference is controlled in multicellular organisms? It may be a tricky question with leaky answer. It is evident that different cells in our body synthesize and express specific protein as per the functional requirements of that cell in time specific manner. Similarly, due to environmental change and change in body condition, gene activity needs fine tuning to adapt the diverse situations. This is possible due to high precession in gene regulation activities so that only the correct set of genes is active in each specific cell type at a specific time point. If any technical slang or anomaly arises in gene regulation, it may lead to cancer and metabolic disease and even leads to autoimmune diseases. Obviously, the question arises how the gene activities are controlled; who are the master regulators? Initially it was speculated that transcription factor is the key controller of gene expression. The transcription factors are specialized proteins that can bind to specific stretch of DNA and regulate the mRNA synthesis thus decide which mRNA to be generated that ultimately translated into protein. In 1993, Ambros and Ruvkun were able to find out another new level of gene regulation mechanism due to the pivotal role played by small-sized RNA molecules in *C. elegans*; that was the beginning of micro-RNA discovery. Such an unusual device of gene regulation unique in *C. elegans* has never been considered relevant to humans or other multicellular organisms. Subsequently, over time, this assessment was found to be no longer true, as several genes coding for different microRNAs in metazoan, including humans, are universal. The gene coding for microRNA has been detected in phylum porifera, nematode, arthropod, reptile, marsupia, and mammalian species.


Victor Ambros

Professional carrier of Nobel Prize winners

Victor Ambros was born in 1953 in Hanover, New

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Gary Ruvkun

Hampshire, USA. Under the mentorship of David Baltimore (Nobel prize recipient 1975) at Massachusetts Institute of Technology (MIT), he worked on the poliovirus genome to complete his graduateship in 1979. From the same institute he completed his PhD and postdoctoral research. Ambros was initially a professor at Dartmouth Medical School, and presently, he is Silverman Professor of Natural Science at the University of Massachusetts Medical School, Worcester. The other recipient, Gary Ruvkun, was born in Berkeley, California, USA, in 1952. He holds a bachelor's degree in Biophysics from the University of California at Berkeley and a PhD in Biophysics from Harvard University in 1982. From 1982-1985, he was a postdoctoral fellow at MIT, Cambridge. He became a Principal Investigator at Massachusetts General Hospital and Harvard Medical School in 1985, where he is now a Professor of Genetics.

Certain genes are not for protein

Readers should be clear in their mind that *lin* gene and *lyn* gene are two different genes. Unlike *lin* gene, the *lyn* (LYN Proto-Oncogene, Src Family Tyrosine Kinase) is a protein coding gene enzymatic in function. The *lyn* protein is one of several Src-family tyrosine kinases involved in signal transduction that regulate information transfer within cells (Xu *et al*., 2005). We must keep in mind that miRNAs are non-coding RNA with remarkable diversity, and there must be some genes that code for miRNA; those are known as mir genes (each denoted mir, pronounced 'meer'). Thus, mir genes produce tiny transcripts and functionally act as antisense regulators of other RNAs. The gene pool of worms, flies, humans and several animals contain plenty of mir genes that code for miRNAs. Few of them are conserved across species, and many of these genes are expressed only at specific times or places (Moss, 2002). Contrary to presently available vast information on several miRNAs only two such miRNA genes were known earlier. According to Victor Ambros "*C. elegans* has taught us a lot about microRNAs, the first identified microRNAs, are *lin-4* and *let-7* genes" (Ambros, 2010). The contributing role of these two microRNA genes was perceived with visible morphological phenotypes during the developing larval stages of the worm. The earliest discovery of microRNAs was detected in *C. elegans*. Mutant of *C. elegans* carrying a mutation in *lin-4* gene displayed several phenotypic deformities due to major disruption of worm during postembryonic development. Developmental absurdity like lack of certain morphological structures, absence of many cell types, developmental abatement, mired development of vulva leading to accumulation of eggs, failure in cell division and cell differentiation as per time plan in some cells was prominent in those mutants (Horvitz and Sulston, 1980). In this direction, another mutant affecting the *lin-14* gene exhibiting various temporal developmental was detected (Ferguson *et al*., 1987). Already, Ambros established that the *lin-4* gene negatively regulates the *lin-14* gene (Ambros, 1989). Two mutant strains of *C. elegans* affecting the *lin-4* and *lin-14* genes that displayed defects in development were screened by these two Nobel laureates to understand the gene function. However, how the blocking effect takes place was not explored. Cloning of the gene led to an unexpected finding. The *lin-4* gene produced an unusually short RNA molecule that is lacking a code for protein production. These surprising results suggested that this small RNA coded by *lin-4* did not block the activity of *lin-14* through the protein molecule. Rather, it was responsible for inhibiting *lin-14* by direct interaction between the two genes' RNA strands (Fig. 1). The *lin-4* RNA was also much smaller (22 nucleotides long) than any other previously studied RNA. In 1993, Ambros and Rosalind Lee (Ambros' wife), along with his lab colleague Rhonda Feinbaum, published their findings in the journal Cell that the regulatory product of *lin-4* gene is a tiny strand of RNA (Lee *et al*., 1993). Interestingly, back-to-back in the same journal in 1993, Ruvkun described how *lin-14* was regulated by another gene *lin-4* (Wightman *et al*., 1993). Ambros and Ruvkun were working independently from two different labs located distantly; through data sharing between the two labs, both were able to notice partial complementarity between the *lin-4* encoding RNA and multiple elements in the *lin-14* 3' UTR. Through their discovery, they were able to establish a new gene regulation mechanism that allows each cell to select only the relevant instructions to synthesize the desired protein at a specified point in time. This ensures that only the correct set of genes is active in each cell type. The function of miRNA is to block the expression of its target (mRNA) by binding in a sequence-specific manner that leads to either degradation of mRNA or repression of further translation of that mRNA to protein (Esquela-Kerscher, 2014). It probably acts as an antisense regulator of other RNAs (Fig. 1).

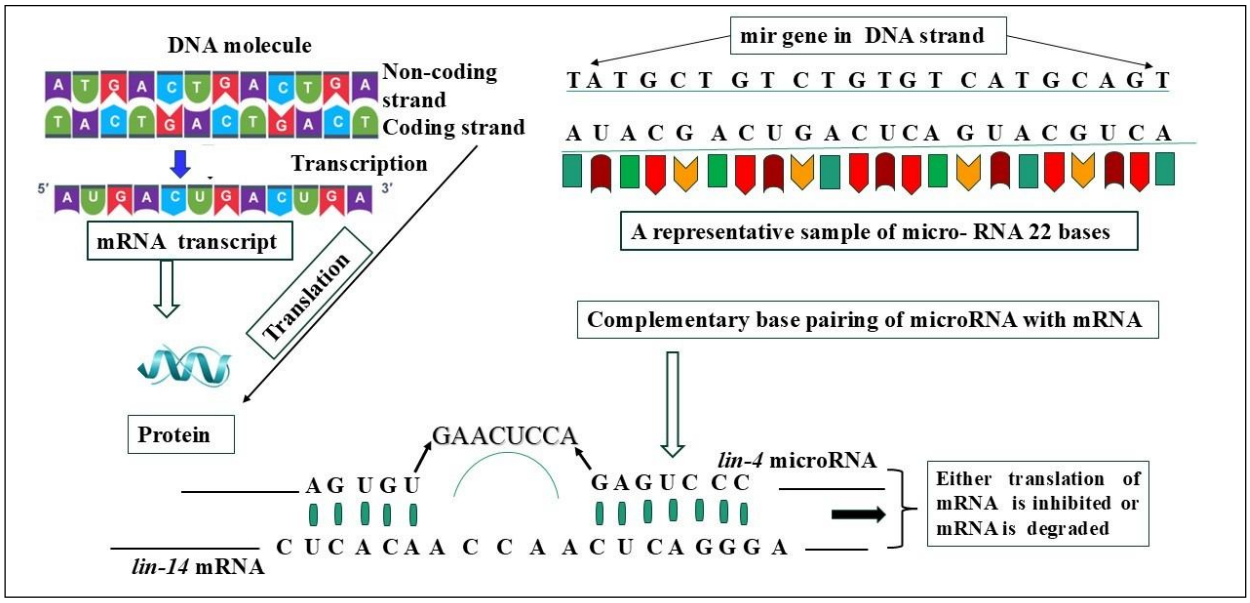

Fig. 1. A complementary base pairing of microRNA with mRNA that inhibits the translation of mRNA

Genetic homepage of larval stage

A swift account of *C. elegans* life cycle and its developmental stages needs to be elaborated for better understanding of the current topic. The life cycle of *C. elegans* can be divided into two major phases: the phase of embryogenesis is the formation of a cellular mass of 550 cells (the initial larva) followed by postembryonic development, which progressively walks through the L1→L4 larval stage to become an adult worm. The development process in multi-cellular organisms demands extreme precision. Therefore, during post embryonic development, the precursor cells (stem cells) exhibit stage-specific cell division for the formation of cell lineages and cell-fate determination. A lot many stage-specific events take place comprise of cell division patterns, cell cycle progress, synaptic rewiring, developmental arrest, stage-specific transcriptional activation, and repression, together with other differentiation and morphogenetic processes. Each stage is separated by a molting. Initially, the mechanism of miRNA function was illuminated by studying the *lin-4* gene in *C. elegans*. Early work conducted by Ambros and Ruvkun revealed *lin-4* as the first gene responsible for the cascade of factors required to govern postembryonic proliferation as well as the differentiation of hypodermal stem cells into specialized skin cells and egg-laying structures in the worm (Ambros, 2000). For example, certain cell lineages, such as the V5 hypodermal cell lineage, follow a distinct pattern of cell division, thereby distinguishing the first larval stage from 2nd larval stage. Likewise, for cell fate determination, the vulval cell lineage undergoes a gust of cell division and differentiation during a specific time slot, which is the third larval stage (Ambros, 2000). It has been observed that certain early-acting genes take part in the progression of time from hatching through the L-3 stage of larva; subsequently, late-acting genes jump into action to push it further from L3 and L4 development. Proper timing of *lin-4* expression is critical to control the transition between the first and second larval stages of nematode life cycle. Mutant worms lacking lin-4 repeat cell fates normally seen in L1, which result in some cell defects, absence of egg-laying structures, long body shape, and uncoordinated movement in adults.

With the support of the above information, the scientific community suggested that *lin-4* may act as a master regulator for gene expression during the developmental process in a time-dependent manner in multicellular organisms. During cloning of the *lin-4* and *lin-7* genes, it was found to represent 22 bases of RNA molecules. Developmental geneticists suggested that *lin-4* is turned "on" at the end of the first larval stages i.e. late L1 stage) and binds to complementary regions in the 32 UTR of *lin-14* and *lin-28* to suppress their expression. Both these RNAs has ability for base pairing with mRNA at 32 untranslated regions (UTR). RNA molecule has a tripartite structure having a UTR region at 52 ends followed by a middle coding region (translated to amino acid) and another UTR region at 32 ends. One may ask, what is the function of those nucleotides already transcribed from DNA but remain silent without being translated to amino acids and persist as UTR segments? It has been clear that the UTRs are known to play crucial roles in the

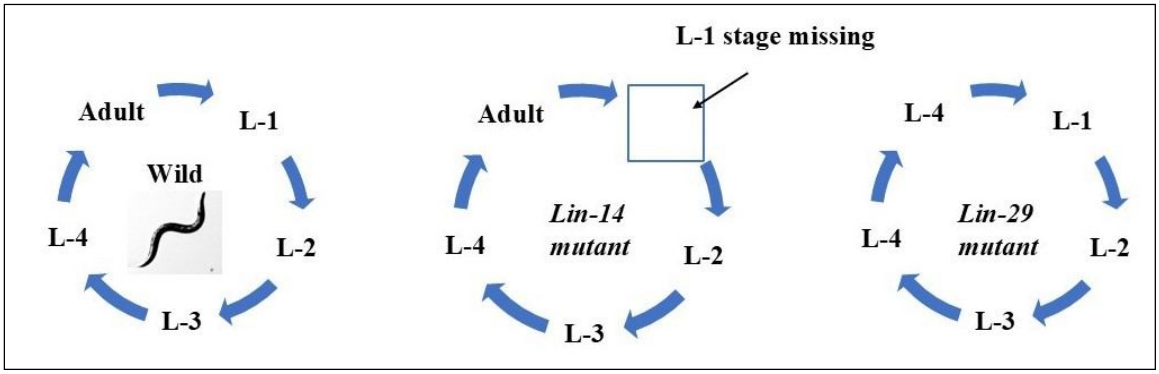

Fig. 2. Development of *Caenorhabditis elegans* progresses through four larval stages L-1 to L-4, and finally attains the adult stage (wild type). In the *lin-14* mutant, the L-1 stage is skipped, whereas, in the *lin-29* mutant, the L-4 stage is repeated and fails to enter an adult stage of worm development (egg stage is omitted here).

post-transcriptional regulation of gene expression, including modulation of the transport of mRNAs out of the nucleus and of translation efficiency, subcellular localization, and stability (Moss, 2002). After discovery of *lin-4* gene next finding is existence of *let-7* gene. The *let-7* gene encodes for a short 21 base nucleotides non-translating RNA that exhibit complementarity to 3' UTRs of various heterochronic genes, including *lin-14*, *lin-28*, *lin-41*, *lin-42* and *daf-12*. Deletion mutant of *let-7* brings repetition of larval cell destinies in the worm lifecycle. For example, mutation in *lin-29* the L4-specific (Larval stage-4) program is repeated, and the adult program is not executed, so the worms remain at the larval stage and do not attain maturity to be adults. Similarly, *lin-14* mutant (lf) (lf: loss of function), the L1-specific developmental program is skipped, so the L-2-specific program is executed earlier than in wild-type (Fig. 2).

Health relation with gene regulation

From fertilization to full-grown babies and in adults, the miRNA guides our development. Once there is an anomaly in miRNA, it can leads to cancer or disease, that is why researchers are keen to study and more concerned about how to revert the anomaly. MicroRNA is a new set of tools for gene regulation; using this tool, our body remains in a healthy state. It has serious implications for heart disease, viral pathogenesis and the regulation of neural function. Therapies based on microRNAs are in clinical trials for heart disease, cancer, neurodegenerative diseases, and more. Once there is mutation in genes coding for microRNAs, it can cause congenital hearing loss, eye and skeletal disorders in human. It has a profound role in development, physiology, and disease conditions. Each microRNA can bind to several mRNAs and is too good at turning cancer cells, so it is useful as a treatment module for cancer.

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