

Trends in RNA Research

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1.1 Introduction

Deoxyribonucleic acid (DNA), ribonucleic acid (RNA), proteins, lipids and carbohydrates are the five principal biological macromolecules constituting living organisms (Figure 1).

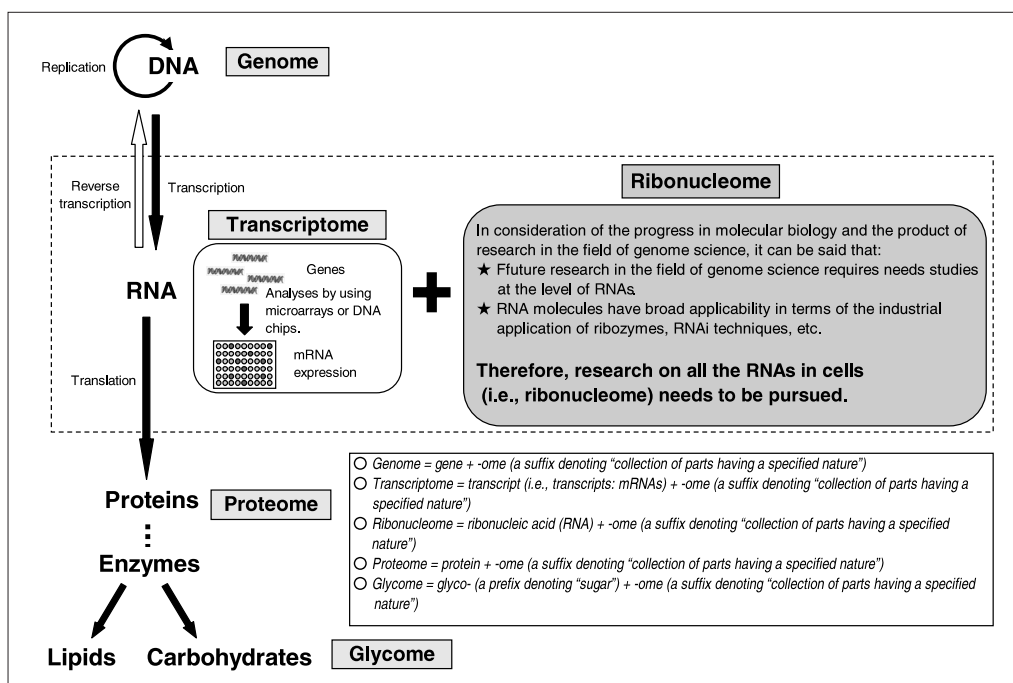
DNA is the material carrying genetic information, and proteins are produced based on the information through by RNA molecules. Carbohydrates and lipids, which play important roles in maintaining life together with proteins, are secondarily produced in the process in which proteins act as enzymes. Therefore, in order to understand the true nature of life phenomena, it is especially important to clarify the mechanisms underlying the genetic information flow from DNA to proteins.

Genetic information contained in DNA, which is

read by messenger RNAs (mRNAs) (this process is called “transcription”), and the resulting transcripts serve as the templates in the biosynthesis of proteins (this process is called “translation”), and is reflected in the characteristics of organisms. The word “genome” represents the whole genetic information in DNA, and the series of studies conducted by observing the flow of genetic information are called genome science research, etc.

Since the publication in February 2001 of the fruits of the Human Genome Project, in which sequencing of all the human genes had been attempted, proteins have received the greatest attention because they may form the next target of life science research. All the intracellular/extracellular proteins is called “proteome,” and “proteome analysis” involves global analysis of changes in the quantities and post-translational

Figure 1: Position of RNA research from the viewpoint of the genetic information transfer process



Source: Authors' compilation based on references by Professor Kimitsuna Watanabe of the Graduate School of Frontier Sciences, The University of Tokyo.

modifications of all the proteins. Various analytic methods are used in proteome analysis, including transcriptome analysis in which expression levels of all the mRNAs coding for the proteome as well as those not coding for proteins are measured. Not only due to the fact that proteins, which are directly involved in a very wide variety of vital phenomena, serve as enzymes to facilitate chemical reactions *in vivo* and as major essential components of tissues of organisms, but also because proteins are functional molecules that may have direct industrial applications, proteome analysis represents the field where fierce international competition in research and development has been fought.

Besides research from the viewpoint of the genetic information flow mentioned above, spotlight has also centered on carbohydrates (sugar chains, in particular) that are involved in intercellular recognition or interaction, with glycome analysis, which is the analysis of expression patterns of the whole set of carbohydrate components, becoming another focus of attention.

On the other hand, with regard to RNA, transcriptome analysis has only been conducted to quantitatively assess the change in mRNA expression levels. However, against the backdrop of recent progress in molecular biology and the product of genome science research conducted so far, the significance of RNA functions has drawn researchers' attention. For example, it has been shown that mRNA expression patterns observed in transcriptome analysis do not always correlate with protein expression, indicating that functions of RNAs themselves as well as phenomena at the level of RNAs such as posttranscriptional modification of RNAs have great significance in genetic information transfer. In addition, the fact that many other functional RNA molecules than those in the translation system including mRNA have been discovered as well as the progress in research on, for example, ribozymes, RNAs with enzyme-like functions, and on the phenomenon called RNA interference (RNAi), which recently gained attention as the gene expression inhibition mechanism via double-stranded RNA, have led to the expectation that RNAs may have broad industrial applicability.

Since the functions of RNAs have great significance in the process of genetic information transfer, further studies related to RNAs need to be incorporated in future research on the field of genome science. In consideration of such necessity as well as the facts that RNAs are functional molecules and that possibilities of industrial application of RNAs as functional molecules have opened up, the need for research on RNA molecules themselves will further intensify.

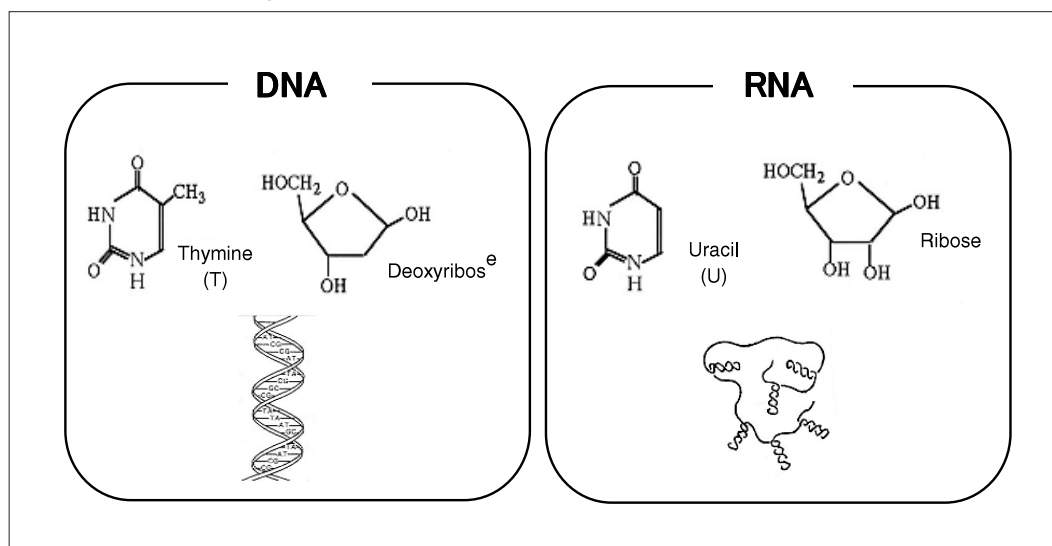
This article provides an overview of RNA research, and discusses strategies for promoting ribonucleome research, which is the global analysis of all the RNAs in cells. The word "ribonucleome" is a combination of the word "ribonucleic acid (RNA)" and the suffix "-ome" and means all the RNAs in cells in an organism.

1.2 Differences between DNA and RNA

RNA in general is the molecule transcribed from DNA by using the base sequence of DNA as a template, and, like DNA, is formed as a chain of unit structures comprising [a nitrogenous base + a pentose sugar molecule + a phosphate molecule]. RNA is different from DNA in that, while four types of bases including adenine (A), guanine (G), cytosine (C) and thymine (T) can occur in DNA, uracil (U) is used instead of thymine (T) in RNA as well as that the constituent sugar molecule is deoxyribose in DNA as compared with ribose in RNA (Figure 2).

Due to these differences, three-dimensional structures of RNA also greatly differs from that of DNA. First, while DNA molecules take a double helix structure, which is formed through hydrogen bonding between the bases of two DNA strands, RNA molecules, which are originally synthesized as single strands, exhibit diverse conformational structures where single and double strands coexist in one RNA molecule because they often partially fold themselves to form double strands. In addition, ribose, the constituent sugar molecule in RNA, has one more hydroxyl group (OH group) as compared with its counterpart in DNA, i.e., deoxyribose, and has much higher chemical reactivity. Therefore, RNA is an unstable molecule

Figure 2: Structural differences between DNA and RNA



Source: Provided by Professor Kimitsuna Watanabe of the Graduate School of Frontier Sciences, The University of Tokyo.

susceptible to degradation, leading to greater difficulty in artificial synthesis of long RNA chains and to the necessity of higher costs for research activities as compared to DNA.

Another characteristic of RNA differentiating it from DNA is that bases in RNA molecules may undergo various modifications. The most widely known example of such modification is the addition of a structure called “cap” to the 5’ end of eukaryotic mRNA, which carries out important functions in the stabilization of mRNA, splicing, transfer of mature mRNA molecules from the nucleus to the cytoplasm, and so on. Many types of modifications to RNA molecules that directly affect their functions have been reported recently, and approaches for studying RNA molecules as well as modifications to them need to be established.

In light of these principal characteristics of RNA molecules, it may be necessary to: (1) develop simple techniques for chemically synthesizing RNA molecules with modifications; (2) develop methods for isolating trace amounts of RNA with high efficiency; and (3) increase the sensitivity of mass spectrometry, in order to pursue future research on RNA. At present, development of research techniques for RNA lags far behind that for DNA and proteins, so development of research techniques is essential for RNA research from now forward.

1.3 Life science literacy and education

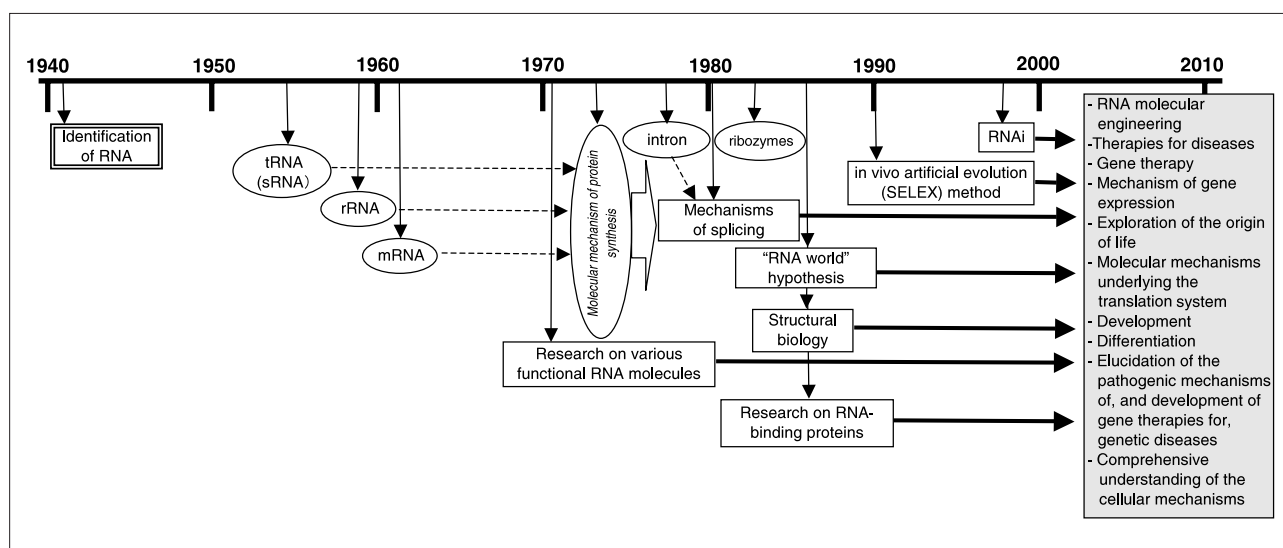
Key events in the progress of RNA research are shown in Figure 3.

During the period from identification of RNA in around 1940 to about 1970, research on RNA focused on RNA molecules in the translation system, with regard to RNA as the intermediary that transfers genetic information on DNA to synthesize proteins. RNA molecules in the translation system include messenger RNA (mRNA) carrying the genetic information copied from DNA, transfer RNA (tRNA) that conveys amino acids, and ribosomal RNA (rRNA) carrying ribosomes.

In the late 1970s, introns (regions on DNA that do not carry genetic information and are not transferred to mRNA) gained the spotlight, and studies were vigorously conducted on the mechanisms of splicing, by which introns are excised from precursor mRNA and only the remaining exons, regions on DNA with genetic information, are connected to create mature mRNA molecules.

Since 1970, studies aiming at identifying RNA molecules other than those in the translation system had been pursued, and ribozymes (RNA with enzyme-like activity) were discovered during the early 1980s, resulting in dramatic progress in

Figure 3: Key events in the progress of RNA research



Source: Authors' compilation based on references by Professor Kimitsuna Watanabe of the Graduate School of Frontier Sciences, The University of Tokyo.

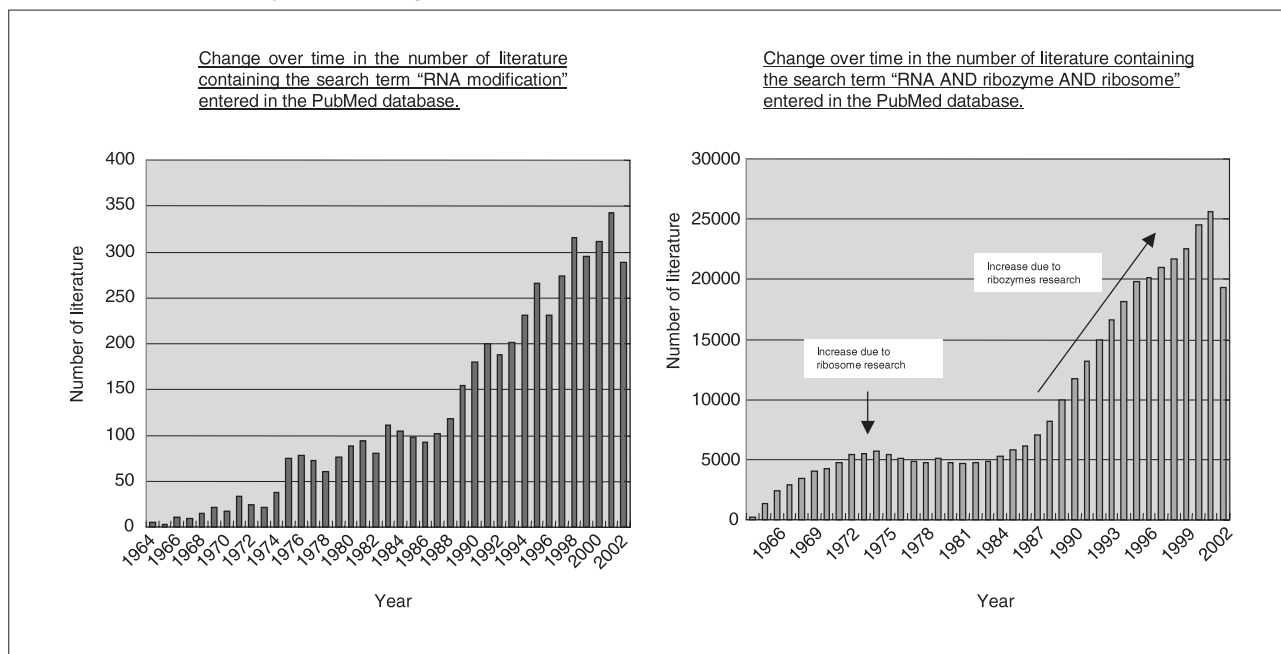
RNA research. The discovery of ribozymes has led to wide recognition that RNA has dynamic functions (functions in active reactions such as catalytic reaction) and has renewed the traditional common notion that all the *in vivo* reactions were catalyzed by the enzymatic activities of proteins. In addition, in the 1980s, the "RNA world" hypothesis that the chemical evolution leading to cellular life on earth likely passed through a stage where RNA alone performed all of the functions of the modern macromolecules RNA, DNA and protein, was proposed (Gilbert, 1986) and gained the spotlight.

Since the 1980s, it has become evident that RNA and RNA-binding proteins are also deeply involved in meiosis, development and differentiation as well as such life phenomena of higher order as those in the nervous system and pathogenesis. Moreover, in the middle of the 1980s, the concept of structural biology was introduced into the field of RNA research, which aims to clarify the functions of biological macromolecules based on their three-dimensional structures revealed by X-ray crystallography or nuclear magnetic resonance (NMR) imaging. In the field of structural biology, rapid progress has been made in recent years, and the fruits of research have been published one after another, including the publication in 1998 by several study groups of X-ray crystallographs of ribosomes themselves on which proteins are synthesized.

Furthermore, in 1990, the *in vitro* selection (artificial molecular evolution) (SELEX) method, by which the RNA with a function of interest can be selected and isolated from among RNAs with random sequences, was developed, and a field called "RNA molecular engineering" emerged. This engineering is expected to find various industrial applications including drug discovery and the development of therapies for diseases utilizing RNA molecules that specifically bind to enzymes and amino acids (RNA aptamers), ribozymes, and others.

In addition, in 1998, a phenomenon called RNA interference (RNAi) was first discovered in the nematode *Caenorhabditis elegans* in which introduction of long double-stranded RNA led to the degradation of mRNA with homologous sequences and, in turn, to the specific inhibition of expression (silencing) of selected genes. The technique utilizing RNAi promptly came to attention as a useful method for gene function analysis in the post-genome era. In 2001, it became evident that similar phenomenon can be observed when a specific small double-stranded RNA (small interfering RNA: siRNA) is introduced into the cells of such mammals as humans. The technique utilizing this phenomenon is of great value as a method for the functional analysis of human genes (knock-down technique), and large-scale studies are already underway aiming at the practical application of the technique.

Figure 4: Changes over time in the number of literature on RNA Curriculum.



Source: Provided by professor Kimitsuna Watanabe of the graduate school of frontier sciences, the university of Tokyo.

As has been discussed, the focus of RNA research has become diversified.

The trend over time in the themes of RNA research as reflected in the number of literature published in each year on the PubMed (the on-line version of the international database containing biological and medical literature constructed by the U.S. National Library of Medicine [NLM]) is shown in Figure 4. The literature search was conducted by using "RNA modification" or "RNA AND ribozyme AND ribosome" as search terms, and revealed that the number of literature searchable by using the respective search terms has increased dramatically since the late 1980s. The results of this literature search indicate that RNA research has gained strong momentum in this decade.

1.4 Trends in RNA research in Japan and other countries

With the progress in RNA research, there have been new moves focusing on this area including the foundation in 1996 of the international RNA Society under the initiative of the U.S. as well as the foundation in 1999 of the RNA Society of Japan. As can be seen from the fact that the average age of the members of these societies is said to be mid-thirties or so, the field of RNA research is characterized by contributions from younger

researchers.

In Japan, thanks to support for RNA research activities in the form of, for example, Grants-in-Aid for Scientific Research for priority research areas, provided since 1989, many excellent results have been obtained mainly in basic research on RNA.

In countries other than Japan, RNA research aiming at practical application has been actively conducted under the exclusive initiative of the U.S. In the U.S., venture capital companies have been established aiming at the business application of the results of research on RNA, ribozymes and RNAi, in particular. Marked progress has been seen in research especially on the application of RNAi, and 30 or more venture capital firms targeting the business application of RNAi techniques are said to have been formed since the report that RNAi is also applicable to humans was published.

1.5 Strategies to promote future RNA research (Ribonucleome Project)

Currently, RNA research is just in the formative stage, and the field still contains many unknown factors. Therefore, Japan will be able to effectively carry out original RNA research by strategically pursuing future research activities in this area.

Japanese RNA researchers have accomplished

globally recognized, highly valued achievements including the discovery of modification (cap structure) added to the 5' end of eucaryotic mRNA as well as RNase P (an enzyme involved in tRNA processing), which triggered studies on ribozymes, and have great potential to conduct RNA research of high quality.

Professor Kimitsuna Watanabe of the Graduate School of Frontier Sciences, The University of Tokyo said, "While the number of literature concerning RNA has been rapidly increasing on a worldwide basis (Figure 4), the number of literature published by Japanese researchers has not grown in proportion, and the number of Japanese researchers in this field has not increased either. In other words, it can be viewed that the potential of Japanese researchers has been fulfilled in the form of high quality contributions to the progress in RNA research by a limited number of study groups. Factors behind this trend include the fact that, while many younger researchers tend to be attracted to fields other than RNA research including genomics and proteomics under the current circumstances where the scale of genome science research on the whole has become gigantic, research on RNA has, as before, been independently pursued by separate laboratories at, for example, universities, national/public research institutions and private companies by conducting studies in their respective laboratories in the field where each laboratory has the advantage or studies on themes that are likely to give practical interest."

From now on, in view of the potential for progress in RNA research as has been discussed as well as the necessity of conducting RNA research in order to pursue comprehensive genome science research, RNA research in Japan should be further promoted.

The first thing to do to promote RNA research in Japan may be to incorporate more researchers having great potential. This may require continual support such as the provision of Grants-in-Aid for Scientific Research as well as the establishment of a research support system in the form of the preparation of a research project under which a considerable amount of funds are to be provided for research.

The comprehensive and synthetic research

system in the field of genomics and proteomics adopts a top-down approach, which, in the field of life science, was originally introduced in the Genome Project. Conducting research under a project is a very effective strategy for setting themes to be urgently pursued and for gaining adequate research funds to allow the vitality of researchers including younger ones to be concentrated on RNA research. From the viewpoint of pursuing research projects effectively, simple implementation of just local joint research has limitations, and a beachhead (a research center, etc.) that can function as the central engine is required. The reason is because pioneering research to be conducted by researchers gathered at such a beachhead may lead to the rise of the overall levels of RNA research in Japan.

In light of such current realities, it may be helpful to draw up and carry out a ribonucleome project for pursuing research on all the RNA molecules in cells. If such a project is initiated in Japan now, it could be a pioneer attempt because very few ribonucleome research projects have been launched in the world.

Challenges to be tackled in such a project may include those in the three areas listed in Figure 5.

The first area containing challenges to be tackled may cover the development of simple RNA synthesis methods as the technical foundation on which to pursue ribonucleome analysis and RNA three-dimensional structures analysis.

The second area containing challenges to be tackled may cover RNA functional analysis. Such challenges may include comprehensive analysis of the splicing, processing, modification and degradation in the genetic information transfer process in addition to RNA functions at the stages of intracellular transport and localization, as well as alteration in reading frames (Figure 6); and scientific verification of the RNA world hypothesis that there was a world in which RNA catalyzed all the reactions necessary for a precursor of life's last common ancestor to survive and replicate by using artificial ribozymes or RNA aptamers (RNA molecules that bind to specific molecules such as enzymes and amino acids).

The third area containing challenges to be tackled may cover gene function analysis to which the results of RNA research are applied, and

Figure 5: Overview of the themes of the ribonucleome project

Area (1): Synthesis and structural analysis of RNA

- ◆ Development of RNA molecules containing modified bases.
- ◆ Development of sensitive methods for structural analysis of trace amount of RNA.
- ◆ Structural analysis of such RNA-protein complexes as ribosomes and development of antibiotics (with inhibitory effects on translation) by utilizing the data obtained in such an analysis.

Area (2): Functional analysis of RNA

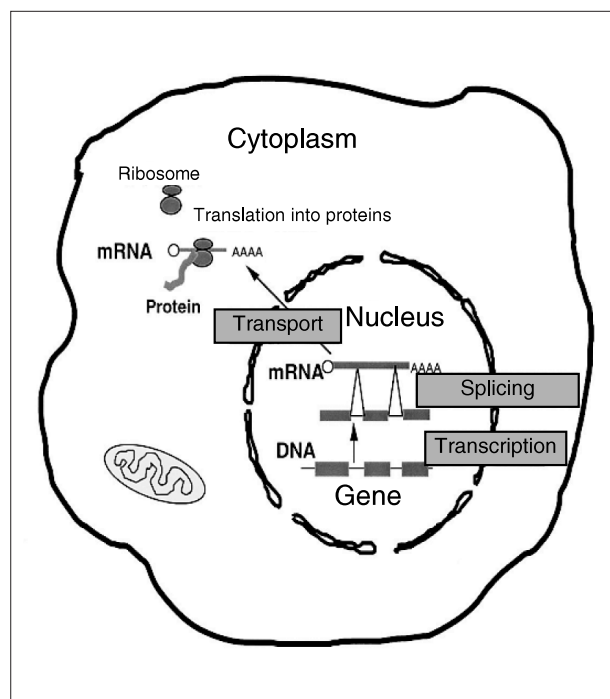
- ◆ Comprehensive functional analysis of all the RNA molecules in cells (ribonucleome) and genome mapping.
- ◆ Construction of an RNA editing database through bioinformatics approaches.
- ◆ Scientific verification of the RNA world hypothesis by utilizing artificial ribozymes and aptamers.

Area (3): Gene function analysis and investigation of the involvement of ribonucleome in pathogenesis

- ◆ Gene function analysis by utilizing knock-down mice created by the RNAi technique.
- ◆ Analysis of causal relationships between genetic diseases and alterations in the modifications to RNA molecules.
- ◆ Gene therapy utilizing RNAi and ribozymes.

Source: Authors' compilation based on references by Professor Kimitsuna Watanabe of the Graduate School of Frontier Sciences, The University of Tokyo.

Figure 6: Intracellular transmission of genetic information mediated by RNA molecules.



Source: Provided by Professor Kimitsuna Watanabe of the Graduate School of Frontier Sciences, The University of Tokyo.

analysis of the involvement of RNA in pathogenesis. As shown by the active establishment in the U.S. of venture capital companies aiming at the business application of the results of RNA research, increasingly greater expectation has

been placed on such industrial application. Under such present circumstances, the challenges to be tackled in this area may include functional analysis of human genes in knock-down mice created by using the RNAi technique, as well as the development of gene therapies for diseases such as cancer by utilizing ribozymes.

Especially in the field of RNA research, a research center that can function as the central engine may be helpful in consideration of the facts that no beachhead in research and development has been established and that it is thought to be necessary to gather more researchers having vitality to be put into RNA research activities. By making initiative-taking efforts in RNA research at such a center in conjunction with separate research activities performed with funds such as traditional Grants-in-Aid for Scientific Research at laboratories of universities, national/public research institutions, etc., it can be expected that RNA research will be pursued more actively in the future.

1.6 Conclusion

In recent years, RNA molecules have been found to be highly functional, and future progress in RNA research especially on ribozymes and RNAi, that

are likely to have broad industrial applicability, is expected to greatly facilitate the advancement in this field. Moreover, the significance of the functions of RNA molecules in the genetic information transfer process has been recognized, resulting in greater necessity for broadening the scope of RNA research activities in view of, for example, the promotion of comprehensive genome science research. Currently, RNA research is just in the formative stage, and, by strategically pursuing ribonucleome research from now on, Japan will be able to effectively carry out original RNA research.

The first thing to do to promote RNA research in Japan may be to incorporate more researchers having vitality, thereby making it necessary to launch a ribonucleome research project. In order to effectively run such a project, it is desired that a system should be developed to conduct, for example, the development of fundamental techniques, comprehensive RNA functional analysis, and studies on RNA molecular engineering with industrial application in view, in a comprehensive manner under the initiative of a research center, etc., which can function as the central engine. By making such initiative-taking

efforts under a project in conjunction with a wide variety of research activities performed with funds such as Grants-in-Aid for Scientific Research at laboratories of universities, national/public research institutions, etc., we can expect not only the qualitative and quantitative promotion of RNA research in Japan but also the facilitation of comprehensive genome science research on the whole.

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References

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