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Research on Health Effects of Environmental Chemicals — New Developments through Introduction of Epigenetics —

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1 Introduction

In the last several decades, the physical constitution of people in advanced countries has changed apparently, and drastic increases in some kinds of disorders have been reported. For instance, the number of people having allergies, such as pollen allergies or bronchial asthma, has increased 10 times in 50 years since the end of World War II. One in every three people has some sort of allergy in Japan. Such a rapid change cannot be brought by a genetic variation. We believe it can be attributed to a recent change in living environment and lifestyle.

The Chemical Abstracts Service (CAS), which started chemical database services in 1965, reported that the number of chemicals registered with CAS topped 50 million in 2009. CAS reported that it took only nine months for the number of registered chemicals to increase from 40 million to 50 million, indicating that the number of chemicals discovered or synthesized by human beings has been increasing at a rapid pace. Japan and other advanced countries have been benefiting tremendously from such chemicals. Meanwhile, the number of chemicals taken in by human beings as medicines, cosmetics or foods has also been increasing, making it necessary to ensure the safety of such chemicals. In fact, safety evaluations, including adverse effects, have been performed.

However, in line with the development of new scientific technologies and the increased use of chemicals, a wide variety of chemicals, including non-regulated chemicals and unintentionally-generated chemicals, have been released into the environment, raising concerns about their adverse effects on human health. Particles and chemicals discharged by automobiles are suggested to be involved in the increase in the number of people having an allergy of some sort These chemicals in the living environment (i.e., environmental chemicals) are thought to be behind the recent change in human physical constitution.

When considering ways to reduce or prevent adverse effects of chemicals on health, it is essential to have scientific knowledge about the effect of each chemical on health and its mechanism. In this report, with regard to the mechanism of the health effect of chemicals, we would like to discuss the current state and problems involved in "epigenetics," which has recently been drawing attention as one of the new approaches in life science. "Epigenetics" is expected to hold the key to elucidating the mechanism of "late-onset effects" of environmental chemicals, that is to say, the effect of exposure to environmental chemicals during the fetal period and childhood emerging in adulthood.

This report introduces epigenetic effects of environmental chemicals. The Science & Technology Trends Quarterly Review has already introduced epigenetics as a new research area in the field of cancer research in its May 2003 edition.[1] Epigenetic research has been progressing at an accelerated pace in a wide sphere in life science and its overall picture has been presented in the June 2009 edition of the Quarterly Review.[2] Please refer to the edition for better understanding.

2 Epigenetic Regulation of Genetic Information

2-1 Regulation of genetic information by epigenetic modification

Epi- is a prefix meaning "above" or "over." The birth of an organism and all life phenomena are based on genetic information written in genes. Genetic information is also associated with maintenance, improvement and disturbances of human health. In molecular genetics, the mechanism for regulating

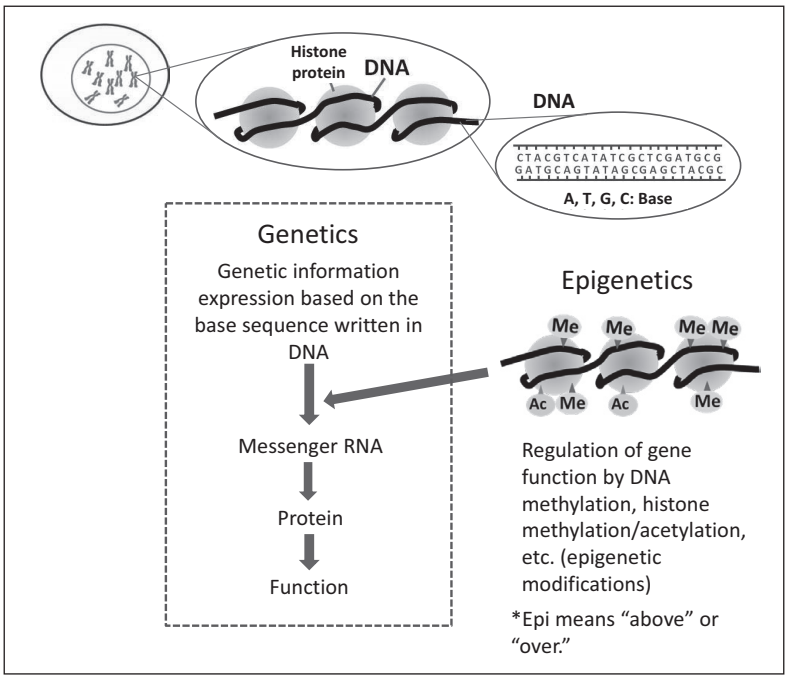

Figure 1 : Regulation of Genetic Information by Genetics and Epigenetics
In genetics, messenger RNA is created based on DNA sequence information, and the messenger RNA creates protein. On the other hand, epigenetics is a mechanism to regulate gene functions not dependent on DNA sequence. Gene function is regulated by mechanisms of both genetics and epigenetics.

Source: Prepared by the STFC

genetic information, such as the genetic ON/OFF switch, has been studied based on DNA sequences. On the other hand, "epigenetics" focuses on "the mechanism for regulating gene functions not dependent on changes in DNA sequence." Specifically, it is a mechanism to regulate the ON/OFF switch of genes not by DNA sequence but by "epigenetic modifications," such as DNA base methylation and histone protein methylation and acetylation (Figure 1).

2-2 Characteristics of epigenetics

Among the characteristics of epigenetic modifications are that they are, first of all, susceptible to environmental factors and that, secondly, they occur more frequently than mutation (change in DNA sequence). These characteristics have drawn attention to the possibility that environmental factors may change genetic information via epigenetics and thus affect human health and physical constitution.

As an example showing the relationship between epigenetics and environment, there is a study on monozygotic twins.[3] Monozygotic twins share common genetic information. However, it is reported that differences between the twins in epigenetic modifications, such as DNA methylation and histone acetylation widen as they grow. These results suggest that environmental factors affect the patterns of epigenetic modifications, which leads to phenotypic discordance, such as differences in susceptibilities to disease.

The third characteristic of epigenetics is the accumulative nature of epigenetic modifications. This accumulative nature is suggested to be closely associated with late-onset effects. In the mechanism based on genetics, if mutation occurs as a result of DNA sequence alteration, it could directly cause a change in protein alignment and cripple the function of the protein. On the other hand, in the case of epigenetic modification, its effect appears when cumulative modifications, such as histone modifications and DNA methylation, reach a certain level (Figure 2).

The fourth characteristic is that epigenetic alteration is reversible. By taking advantage of this reversible nature and by undoing DNA methylation change, drugs to prevent the progression of cancer have been developed.

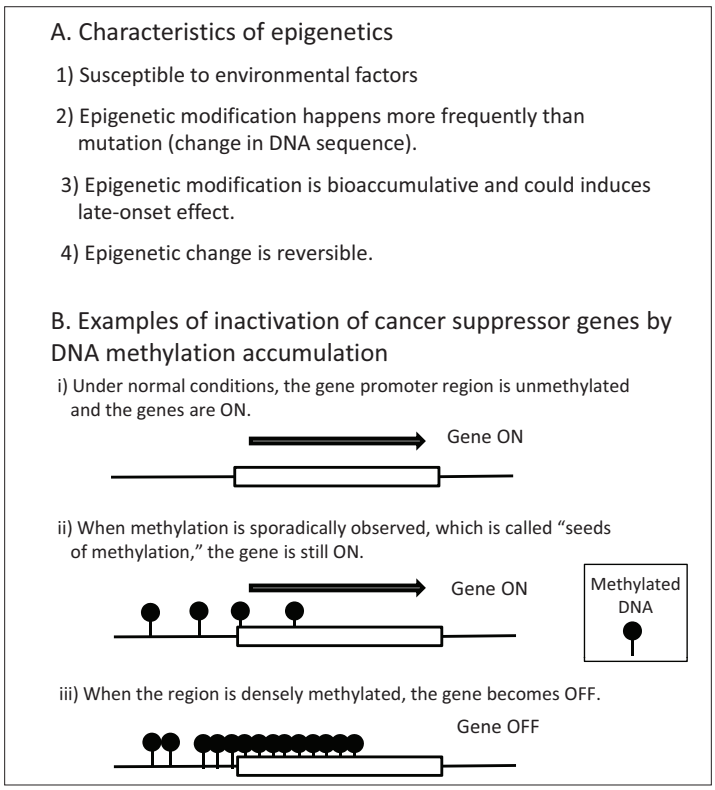

Figure 2 : Figure 2 Characteristics of Epigenetics (B is modified version based on Reference[4])

Source: Prepared by the STFC

3 Biological/Health Effects and Mechanisms of Environmental Chemicals

3-1 Health effect of environmental chemicals

Minamata Disease (Kumamoto Minamata Disease) in the 1950s was a disease caused by methyl mercury discharged by a chemical plant near Minamata Bay in Kumamoto Prefecture. People who had ingested methyl mercury-contaminated fish and shellfish developed neurological symptoms, such as numbness and pains in the hands and feet, impairment of language and ataxia. Moreover, methyl mercury ingested by mothers is transmitted across the placenta from mother to fetus and is accumulated there, resulting in serious damage to the development of fetus' cerebral nervous system.[5] After it was recognized that diseases like Minamata disease had been caused by human-induced environmental pollution, a concept of "*kogai* (public nuisance)" gained currency and prompted the establishment of the Ministry of the Environment and systematic implementation of pollution control and environment protection measures as national efforts.

Many countries, mainly advanced countries, have implemented measures to regulate and control the release of large quantities of poisonous chemicals into the environment. In 1973, Japan led the world in establishing the "Law Concerning the Examination and Regulation of Manufacture, etc. of Chemical Substances" to control and regulate manufacture and import of chemicals. In reality, however, a survey conducted under the PRTR (Pollutant Release and Transfer Register) system[6] shows that a large variety of poisonous chemicals have been released into the environment. We do not have enough knowledge about the harmfulness of not only new chemicals that are produced one after another but also many existing chemicals. The problem of environmental pollution caused by residual chemicals remains unsolved.

Polychlorinated biphenyls (PCBs), which began to be produced in around 1930, were once widely used as insulating material, cooling mediums and plasticizing agents. Meanwhile, the manufacture and use of PCBs have been banned by many countries in the world since the early 1970s, as the outbreak of so-called "*yusho* disease" in Japan and Taiwan revealed that PCBs have strong harmful effects. However, PCBs that were released into the environment as waste still remain in the environment, raising concerns about their adverse effects on immunity and the learning

function of humans and wild animals.

With regard to highly poisonous persistent organic pollutants (POPs), such as PCBs, the Stockholm Convention on Persistent Organic Pollutants (POPs Convention) was adopted in 2001 with the aim of eliminating or reducing the release of POPs into the environment under international cooperation. The convention was later signed by more than 150 countries and entered into force in 2004. Among POPs are those that are manufactured for intended use, such as aldrin, chlordane, dieldrin and DDT, and those that are generated in the process of production of agricultural chemicals or released in the process of garbage incineration, such as dioxin and dibenzofuran.

In recent years, there are concerns about "endocrine-disrupting chemicals," such as chemicals having the same action as the female hormone estrogen or interfering with the reproductive function by disrupting hormone action. Bisphenol A, which is widely used as a material for synthetic resin, as well as the abovementioned PCBs and dioxin, are also reported to act as endocrine disruptors.

Meanwhile, the hazardous property of naturally occurring chemicals or elements that exist in the environment also poses a problem. A typical example is inorganic arsenic.[7] The intake of arsenic-contaminated well water has been causing disorders including skin disorders and cancers in such countries as China, Taiwan, India, Bangladesh and Argentina. It is reported that over several thousands of people are suffering from such symptoms in the world. A new technology is being developed to remove inorganic arsenic from well water. However, as it has yet to be put to practical use due to costs and technical problems, exposure to inorganic arsenic is going on around the world.

3-2 Functional mechanism of environment chemicals and epigenetics

Thanks to the progress of genomics technology, it has become clear that environmental chemicals cause various biological effects by affecting "on-off" switching of gene expression (Figure 3). A group of proteins called transcription factors plays a pivotal role in switching or regulating gene expression. It has been revealed that various types of chemicals alter gene expression by acting on specific transcription factors, resulting in causing various biological effects.[8] For instance, dioxin activates the transcription factor, Aryl hydrocarbon receptors (AhR) binds to it, and alters the expression of target genes, which is believed to lead to adverse biological effects.

In addition to these findings, there is a growing number of reports that chemicals exert biological effect by inducing epigenetic changes. One famous example showing that environmental chemicals alter DNA methylation is a study on the agouti mouse.[9] The coat color of the agouti mouse changes from yellow to brown depending on the methylation status in the DNA region that regulates the expression of the agouti gene. It is reported that if exposed to bisphenol A, an environmental hormone, during the fetal or prenatal period, the coat color of the mouse turns yellow, and that if the mouse is given foods associated with DNA methylation metabolism, such as folic acid, the color turns back. The research has revealed the gene that becomes the target of epigenetic action and that it is linked to expression of coat color. This is one of a few examples showing a clear causal relationship wherein chemical substances change phenotype via epigenetic modification.

As for inorganic arsenic actions, it was found that if pregnant mice are freely given water containing inorganic arsenic for only 10 days, the incidence of liver cancer is increased in their male offspring.[10] DNA methylation in the promoter region of ERα gene,,, which is associated with cell proliferation and carcinogenesis, was found to decrease in the liver of those male offspring. .Lower level of DNA methylation is favorable for genes to work. In fact, gene expression was found to be increasing, suggesting that inorganic arsenic increased cancer by enhancing the expression of carcinogenic ERα gene via epigenetic modification[10] (Figure 4). Another study reported that when a strain of mice which are prone to develop lung cancer are fed with water containing inorganic arsenic for a long time, it caused DNA methylation changes of tumor suppressor genes in the lung, inhibited the expression of these genes, and increased lung cancer.[11]

There is also a report that administration of vinclozolin (insecticide) to pregnant rats for one week decreased the spermatogenic capacity of their male descendants down to the 4th generation and increased the incidence of male infertility. These male rats inherited the DNA methylation change of reproductive cells. These results suggest that exposure to chemicals in fetal life causes changes in programming via

epigenetic change of reproduction cells and that its effect is transgenerational.[12]

Epigenetic effects are reported in many other chemicals, including diethylstilbestrol (DES), a synthesized hormone, phenobarbital, a hypnotic, anxiolytic and anti-epileptic drug, dibromoacetic acid, a byproduct in the process of drinking-water disinfection, and benzopyrene, a highly-carcinogenic combustion product. Nickel, some other metals, cigarette smoke and diesel particulate are also reported to have epigenetic effects. However, the causal relationship between the biological effect of these chemicals and epigenetic changes remains mostly unexplained.

Figure 5 shows the concept of the causal relationship between DNA methylation change and cancer. When a cancer and epigenetic change are observed as a result of exposure to a chemical, there are two cases. One is that the epigenetic change caused the cancer and the other is that the cancer is caused by some actions of the chemical induced epigenetic change closely related to carcinogenesis. Further studies are necessary to clarify if and how deeply the chemical-induced epigenetic changes are related to the alteration of biological and physiological functions.

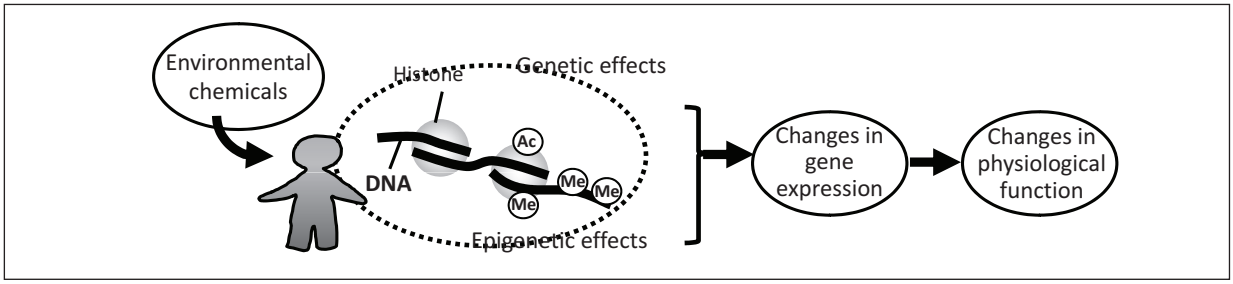

Figure 3 : Mechanism of Action of Environmental Chemicals via Alteration of Gene Function
In recent years, a variety of environmental chemicals are reported to be influencing biological functions by altering gene expression.

Source: Prepared by the STFC

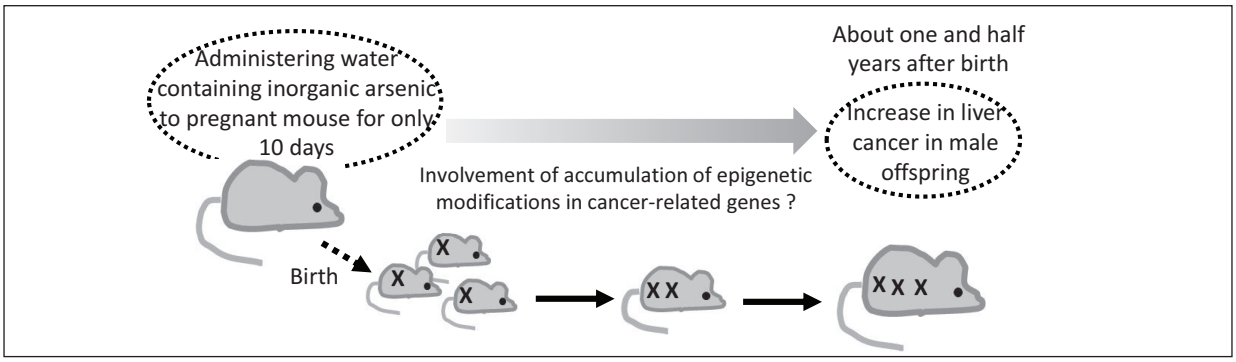

Figure 4 : Example of Late-Onset Transgenerational Effect of Environment Chemicals
The epigenetic effect is suggested in the experimental system in which giving inorganic arsenic to the pregnant mice increased the cancer in their male offspring in adulthood (Reference[9]).

Source: Prepared by the STFC

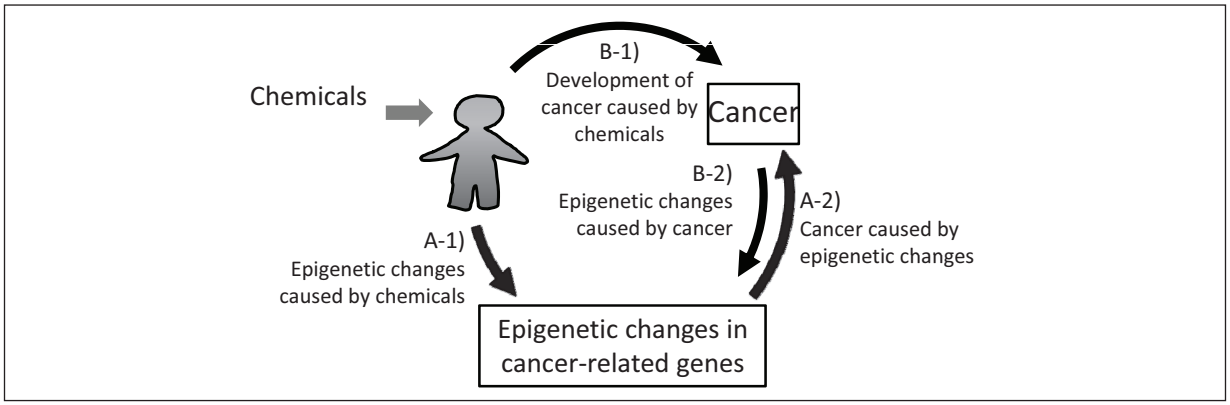

Figure 5 : Relationship among Exposure to Chemicals, Biological Effects, and Epigenetic Changes

Source: Prepared by the STFC

4 Effect of Exposure to Environmental Chemicals during Developmental Stage and Epigenetics

4-1 DOHaD hypothesis and epigenetics

In epidemiology study, the "DOHaD (developmental origins of health and disease) hypothesis" has recently come to draw attention. This hypothesis holds that the nutritional environment in the fetal and infant periods affects the risk of lifestyle-related diseases in adulthood. These periods have plasticity and are believed to be highly sensitive to chemicals. Epigenetics is suggested to play an important role in maintaining the changes in gene function caused by chemical exposure during perinatal periods, and the changes may exert adverse effects in later life. Thus, epigenetics is thought to be associated with DOHaD as a plausible molecular mechanism.

4-2 Japan Children's study

In conjunction with the DOHaD hypothesis, there has been growing international concern about environmental risks on children's health. With regard to the world research trend on children's health and the environment, please refer to the March 2009 edition of the Science and Technology Trends.[14] Under these circumstances, the Ministry of Environment started the "Japan Environment & Children's Study," an unprecedentedly large-scale national survey in FY2010. The survey is designed to elucidate the effects of exposure to chemicals and living environment on children's health. The ministry plans to recruit about 100,000 pregnant women from across the country in three years starting in FY2010 in order to survey and track children continuously from birth to around age 13 by conducting questionnaire surveys and performing physical measurement as well as environment survey, including chemical measurement.[15]

In such epidemiological research, biomarkers that are informative of the relationship between environmental influences and health effects would become powerful tools in analyzing research results. Changes in epigenetic modification can cause health effects, where some of the modifications would begin to change before their effects appear. In this regard, such epigenetic modifications would become biomarkers, or "epigenetic markers," that can detect the environmental effects at early stages. Since DNA is relatively stable, epigenetic markers such as DNA methylation are thought to be useful for retrospective study.

An epidemiological survey was conducted on umbilical cord blood DNA of about 700 children in New York City. The study reported that DNA methylation of the upstream region of gene ACSL3 is associated with transplacental exposure to polycyclic aromatic hydrocarbons derived from car exhaust emission and childhood asthma, suggesting that the change in DNA methylation in ACSL3 upstream may become an epigenetic marker.[16] Further study needs to prove the usefulness of the epigenetic marker proposed in the epidemiological survey in New York, including if the marker can be used for the Japanese, who have different genetic backgrounds.

5 Ways to Advance Future Research

Epigenetic modifications are thought to be influenced by environmental factors and alter the function of genes, and thus have an impact on human health. For this reason, environmental deterioration may have transgenerational adverse effects on human health. In fact, some of the epigenetic modifications of genes are reported to be inherited in the next generation. At the same time, it would be possible to maintain and enhance health condition via epigenetics by establishing a sound living environment. Therefore, research on biological effects that takes into account the epigenetic effects of chemicals in the environment is important for establishing a healthy life environment. Such research is growing in the United States and other countries. As of now, however, there are some discrepancies among the results on the epigenetic effects of environmental chemicals. The research is still in an early stage and needs to be further promoted.

Since epigenetics is closely related to developmental biology, epigenetic research has made progress in this field. Significant progress has also been made in the field of cancer research. The genetics technology and knowledge that have made dramatic progress since the 1990s have also been effectively utilized in epigenetics research. The research in these fields in Japan has made a great contribution to the progress of epigenetic study. However, with regard to epigenetics research on environmental chemicals, Japan's contribution

would be limited compared with the United States and European countries. Since Japan experienced a number of pollution issues, such as Minamata disease caused by methyl mercury, Itai-itai disease caused by cadmium and Yokkaichi asthma caused by air contamination, the country has led the world in the research on biological effects of these heavy metals and hazardous chemicals in the air and environment.[17] In recent years, however, the number of young researchers has been decreasing in these research areas, raising concern that the research in these fields may taper off in Japan. At a time when environmental health research requires the epigenetic point of view, Japan is facing a challenge of securing and fostering human resources in the area.

The National Institute of Health (NIH) of the United States has been implementing "the Epigenome Roadmap Project" (total budget: $190 million) since 2009, which is one of the largest epigenetics projects in the world. Recently, the NIH released an epigenome map charting epigenetic modifications of various cells and tissues.[18] Last year, the International Human Epigenome Consortium (IHEC) was inaugurated in Paris. The IHEC is set to start a "1,000 Epigenome" project (total budget: $130 million) designed to map 1,000 reference epigenomes.[19] Japan is looking into taking part in the project.

These projects are mainly focused on basic epigenomes in normal cells and do not sufficiently deal with epigenetic modifications induced by exposure to chemicals. International collaboration and exchange of knowledge would be useful to establish a common view of epigenetic effects of chemicals. In order to nurture an environment to protect human health and make a significant contribution to the international community, it would be necessary for Japan to play an active role in establishing a global research system. .

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Profile

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Dr. Nohara is concurrently serving as professor at the Graduate School of Life and Environmental Sciences, University of Tsukuba. She has been engaged in research with young researchers and students, believing that it is important to understand the "action mechanism" of the environment in order to prevent adverse effects of the environment on human health.

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