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Trends in Recent Research of Epigenetics, a Biological Mechanism that Regulates Gene Expression

Yuko ITO
Life Science Unit

1 Introduction

The term "epigenetics" is starting to become a frequently used term, though it is not yet familiar to the general public. Epigenetics is defined as "phenomena which regulate gene expression through a mechanism other than genome mutation and produce changes in cells and the body" [Note 1, 2] (Figure 1).

The increased use of the term "epigenetics," a field under research, is due to the recent shift in major life science research from genome sequencing to the understanding of the mechanisms and regulation of genomes. Subsequently, people researching translation regulation mechanisms, the behavior of chromosomes and RNA, genes that cause disease, and development and regeneration started to exchange information so as to reveal the mechanisms that regulate genetic expression, resulting in a whole new research field. In other words, it was as if after putting some pieces of a jigsaw puzzle together, researchers started to see the big picture of a continent.

This new continent, called epigenetics, covers many areas of research in life sciences, including biological

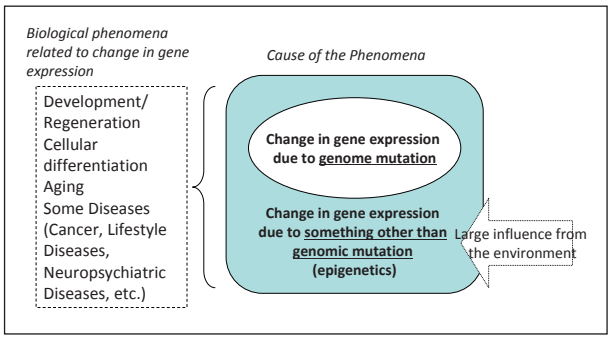

Figure 1 : Epigenetics in Biological Phenomena
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[NOTE1] : Definition of Epigenetics
The definition of epigenetics is still under debate; however, I will adopt the following definition for this report.
*From Purpose of Founding, on The Japanese Society for Epigenetics website: *It is known that the expression of genetic information, which the genome possesses, is not regulated by only the base sequence and transcription apparatus. In organisms, gene expression is also regulated by the chemical and structural modification of chromatin, which is composed of genomic DNA and proteins, such as histones. These kinds of regulation are called" epigenetics," and are established during development and are known to work as cellular memory.*[2]
For reference, the following is the first stated definition of epigenetics.
* "Changes in genetic function, which is transmitted to offspring and daughter cells, caused by mechanisms other than changes in the underlying DNA sequence, and the study therof."[4]

[NOTE2] : Words Derived from Epigenetics
"Epigenetic" is used as an adjective as opposed to the research area of "epigenetics." In Japanese, "epigenetic" is sometimes used as the name of the research area as well. In addition, "epigenome" means epigenetic changes on the genome, and "epigenomics" is the study of epigenomes. "Epigenomic" is the adjective.

development/differentiation, the effect of pollutants, revealing the mechanisms of lifestyle diseases and developing drugs for treatment.

We wrote about epigenetics research in the field of cancer research in 2003 in this journal, Trends in Science & Technology.[1] This time we decided to focus on the trends in epigenetics research once again, since epigenetics research has branched into various research areas in the past six years and the research has advanced to the point where there is a great need for international collaboration. Therefore, I will introduce recent topics in epigenetics research and show the meaning, importance and expectations for the future in epigenetics research.

2 Examples of Epigenetics

Identical twins are known to have different body features, personalities and preferences, as well as differences in the onset of disease and the severity of symptoms, despite possessing identical genes. Moreover, these differences are small when twins are young and increase with age. Also, in the case of cloned animals, the exact same fur pattern is not passed down from the original animal to the clone, even though their genomes are identical. This means that even if a clone of your beloved calico can be created, the clone may turn out to have bi-colored fur, which does not at all look like the calico[5,6] (Figure 2). [Note 3] These are all familiar examples of epigenetics.

In addition, organisms operate strict regulation of gene expression, whereby necessary genes of the genome are expressed and unnecessary ones are terminated, at each step of development and differentiation. This regulation of gene expression allows cells with identical genomes to differentiate into tissues and organs with different shapes and functions, such as the heart, lungs and neurons, and stay that way in the body for a long time. This is also an example of epigenetics.

Moreover, epigenetics is associated with many diseases. Epigenetics is pliable and the condition of genetic expression changes in response to external stimulation, such as environment and lifestyle as well as aging, which may alter healthy genetic expression. This is called “epigenetic breakdown” and is deeply related to various diseases, including cancer.

The genome of this cat clone (right) is identical to that of the calico (left) but the fur pattern and personality are different.

Photo courtesy: TAMU College of Veterinary Medicine

[NOTE3] : Color of the Clone’s Fur

When the cat clone was created, the cellular nucleus (derived from the calico) used for nuclear transplant was not fully initialized. This suppressed some gene expressions on the X chromosome, where the gene that determines the brown color of the fur is located, and as a result, the clone’s fur lacked brown and was only bi-colored. However, recent research indicates that fur color and patterns are also influenced by the uteral environment during the fetal period as well as by the postnatal nurturing environment (this is also epigenetics). However, detailed mechanisms are still unclear, and it will take a little more time to create a true “copy animal.”

On the other hand, this pliability is believed to be applicable for the treatment of diseases, and basic research in epigenetics is underway on how to return abnormal gene expression to normal. In addition, people involved in regenerative medicine are awaiting progress in epigenetics research since more advanced knowledge of epigenetics is required to freely create (customize) target cells and organs from cells possessing pluripotency (cells that can differentiate into any tissue or organ), such as ES cells and iPS cells.

Genomic DNA methylation is the most well known mechanism for genetic expression, but there are many others mechanisms. [Note 4] Much needs to be clarified about the mechanisms, and active investigation is underway.

3 Epigenetic Research is looming

Epigenetics research is blooming, with more academic papers being published and activities being carried out by the research community.

3-1 Number of Academic Papers on Epigenetics

How much did epigenetic research expand in the past 10 years? What is the state of research in Japan and throughout the rest of the world?

In order to answer these questions, I accessed the Web of Science, part of the ISI Web of Knowledge (THOMSON REUTERS) database, and conducted a search, using "epigen*" as the keyword, of the period from 2000 to 2008. Research articles (Articles or Reviews) published during this period were included in the search. I organized secular changes in the number of research papers, the number of research papers by country, the proportion of research papers on various research areas, and the number of research papers by research area of the authors' affiliation into graphs using the Analyze Results function of the ISI Web of Knowledge.

(1) Changes in the Number of Research Paper Publications

There were 10,110 published research papers in total related to epigenetics between 2000 and 2008 (as of February 23, 2009). Chronological change shows the significant increase in publications following 2004 (Figure 3).

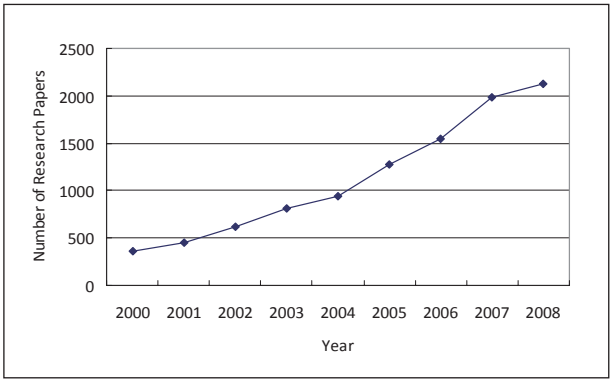

Figure 3 : Changes in the Number of Research Papers Related to Epigenetics

Prepared by the STFC

[NOTE4] : Major Mechanisms of Epigenetic Genetic Expression

Examples of Major Gene Expression by Epigenetics	Mechanisms
Methylation and Demethylation of Genomic DNA:	Cytosine base gets methylated and the methylation causes suppression of genetic transcription.
Chromatin Remodelling:	Genomic DNA in the cell forms a complex with histone, and sequences of these complexes form a structure called chromatin. Structural changes of chromatin cause activation and suppression of genetic trascription.
Methylation and Acetylation of Histones:	Methylation and acetylation of histone changes chromatin structure.
Genomic Imprinting:	The phenomena where genetic expression differs due to the discrimination of paternal or maternal genes due to difference in mehtylation pattern of DNA.
X-chromosome Deactivation:	The phenomena where mammalian female XX deactivates one of the X-chromosomes to balance out the genetic size with male XY.
Non-coding RNA Function:	Among the RNAs which do not code protein, RNAs involved in gene expression and regulation such as RNAi are involved in the mechanisms of genomic imprinting and X-chromosome deactivation.

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(2) Number of Published Research Papers by Country

Research paper publication by country (Figure 4) shows that the US published the most: close to half of the total number of research papers. On the other hand, though Japan is second only to the US, it publishes only about 10% of the total (1,072 papers), which is a similar volume to those of Germany and England.

(3) Proportion of Research Papers on Various Research Areas

Figure 5 shows the top ten research areas for publication in the top four countries of publication shown in Figure 4: the US, Japan, England and Germany.

The result shows that in all four countries, most published papers were written by authors working in the fields of Biochemistry & Molecular Biology, Oncology, Cellular Biology and Genetics. In particular, the US and Germany had similar proportions of fields with large numbers of publications, including Biotechnology & Applied Microbiology, which was not seen in Japan or England. On the other hand, Oncology had a higher proportion in Japan compared to the other three countries, and Biophysics and Reproductive Cell Science were the characteristic areas found only in Japan. In England, Plant Science and Endocrinology & Metabolism were characteristic areas. As shown, epigenetics research is covered by a wide range of research areas and each country has a slightly different portfolio.

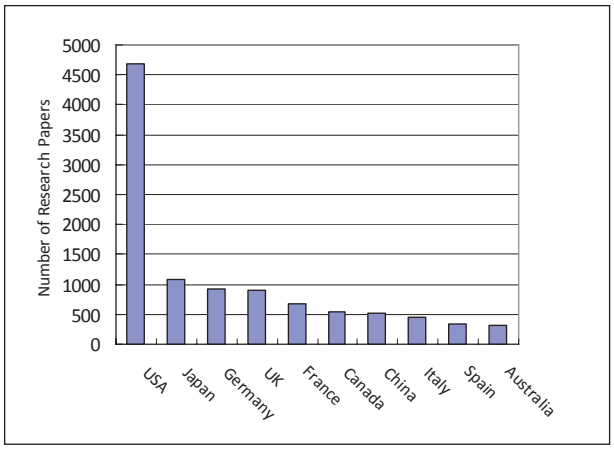

Figure 4 : The Number of Research Papers Related to Epigenetics by Country

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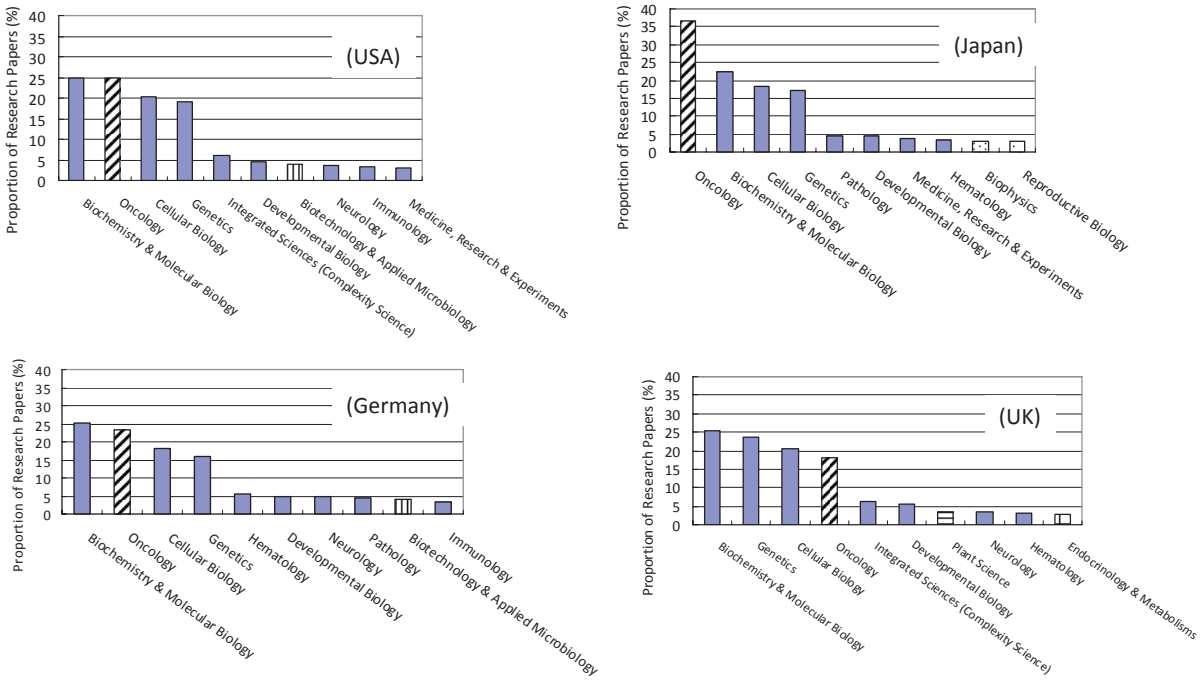

*Only the top ten research areas are shown
*Papers that cover more than one research area are counted as one paper in each applicable area, therefore, the sum of the proportions may not equal 100%

Figure 5 : Proportion of Research Papers on Various Research Areas by Country (2000–2008)

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(4) Research Institutes in Japan

Which university/public research organizations in Japan are undertaking epigenetics research?

Table 1 shows the research institutions in which the authors of 1072 research papers published between 2000 and 2008 belonged to in Japan. Among universities, the University of Tokyo, Kyoto University and Sapporo Medical University had the highest number of publications, and public research organizations with high numbers of publications included the National Cancer Institute, RIKEN, the National Genetic Research Institute and the Aichi Prefecture Cancer Center. There were generally no regional differences and epigenetic research was undertaken by various universities and research institutions in all regions.

Table 1 : The Number of Research Papers by Japanese Institutions (2000–2008)

Organization to which the authors of the paper belong	Number of papers
University of Tokyo	117
Kyoto University	91
National Cancer Institute	84
RIKEN	79
Sapporo Medical University	63
Japan Science and Technology Agency	51
Nagoya University	50
Osaka University	46
Kyushu University	44
Tohoku University	44
Tokyo Medical & Dental University	42
Chiba University	35
National Institute of Genetics	35
Okayama University	34
Hiroshima University	29
Gunma University	25
Aichi Cancer Center	24
Hokkaido University	23
Kumamoto University	22
Tottori University	22

*When authors belong to more than one institution, each institution is counted as one.

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3-2 State of Research Community

The epigenetics research community is becoming more active in Western countries and in Japan. The following are examples of its activities.

(1) Large Scale Research Conference in the US—From the US to International Collaborative Projects—

One example of a large research conference related to epigenetics held in the US is the 69th Cold Spring Harbour Symposium[Note 5] entitled, "Genome of Homosapiens."[7] The cover of the program was a photograph of identical twin girls as if to emphasize that epigenetics differs even with the same genome, and received much attention from many researchers. Though the name of the symposium hints that it targets research on humans, most presentations were related to epigenetics of yeast, C. elegans, fruit flies, plants (Arabidopsis) and mice.

In addition, Gordon Research Conferences[Note 6] with the title of "Epigenetics" have been held every other year since 1995. The publicized program of the 2007

[NOTE5] : Cold Spring Harbor Symposium

Cold Spring Harbor Laboratory is a world renowned NPO research institute that provides frontier research and education in Biology (especially genetics), and is located in Long Island, New York. It has produced many Nobel Prize laureates, such as Barbara McClintock (maize transposons). Symposiums on the latest research topics have been held there every year since 1933.

[NOTE6] : Gordon Research Conferences

This organization has held research conferences since 1931 to facilitate free discussion and interaction for researchers of Biology, Chemistry and Physics. It has international authority and there are different section meetings (over 400) for each research topic. The conferences are financed by individual participation fees as well as financial support by governments, corporations and financial groups. The contents of the meetings are not publicized, and the participants are prohibited from citing contents from the meetings in research papers.

conference shows that there have been reports about "epigenome analysis,"[Note7] analyzing which part of the genome is causing epigenetics, and epigenetics related to human diseases in addition to the traditional main reports about gene regulation mechanisms using model animals.[8] Another conference is scheduled for August 2009, with a subtitle of "Role of the Environment and Epigenetics on Behavior, Health and Disease," suggesting there may be an increase in research on human diseases and the effect of the environment.[9]

In addition, the "Human Genome Task Force" of the American Association for Cancer Research hosted the "Human Epigenome Workshop" in 2005 as well as a conference with a selected target to plan international collaborative projects related to human epigenome mapping as a follow up in 2006.[10] At the conference, the importance of founding an international group of specialists, AHEAD (The Alliance for the Human Epigenome and Disease), was discussed. There were 29 members in the Task Force in 2006, including specialists from the US, and European countries such as England, Spain and the Netherlands, and from Asia including Japan, China, Korea and Singapore. Compared to the Human Genome Project, which was more centered on Western countries, this group has involved Asian countries from the start and has the potential to grow into a more international project. Since the analysis of epigenomes requires much more information compared to that of genomes, it requires collaborations involving many countries.

(2) Building a Large Scale Research Network in the EU

The EU founded the Epigenome Network of Excellence (NoE) as a part of Framework FP6 (2002–2006) in 2004 with funding of 12.5M Euros.[11] The NoE was founded solely to make academic profit for the epigenetics research community by supporting academic conferences, workshops, training and shared resources. The implementation period is from 2004 to 2009, however, FP7 (2007–2013) keeps it financially supported.

Recently a huge epigenetics research network was formed with the participation of 46 Universities and research institutions (83 research groups) from 12 countries: England, France, Germany, Spain, the Netherlands, Belgium, Switzerland, Italy, Austria, Croatia, Denmark and Sweden. In addition, 350 research groups from all over the world are participating in on-going research projects of the EU through the NoE website.

(3) Epigenetics Research Organizations in Japan

The Japanese Society for Epigenetics was founded in December 2006, and has been hosting annual meetings since 2007. The purpose for founding the society is as follows.

Since experimental organisms used in epigenetics research vary so widely, from yeast and plants to mammals, researchers have been split into many different specializations with many different conferences (including The Molecular Biology Society of Japan, The Japanese Biochemical Society, Japanese Cancer Association, Japanese Society of Developmental Biologists, The Genetics Society of Japan, The Botanical Society of Japan, The Japan Society of Human Genetics, The Japanese Society for Neurochemistry and Japan Society for Cell Biology). The Society was founded to promote communication, from the perspective that a place where researchers from various societies under the category of epigenetics research can gather and exchange information is absolutely necessary if progress is to be made in the research. (From The Japanese Society for Epigenetics website.) [2]

Moreover, volunteers from The Japanese Environmental Mutagen Society founded the Environmental Epigenomics Society in December 2008 as a place to discuss various toxicological phenomena with epigenetics as the keyword. The reason for its foundation was that the importance of research in the field of environmental epigenomics concerning the correlation between environmental factors and genetic expression was starting to be recognized in toxicology and clinical medicine.[12]

In addition, in order to clarify technological challenges for the industrial application of epigenetics, the New Industry and Industrial Technology Development Organization commissioned the Japan Biological Informatics Consortium to

[NOTE7] : Epigenomic Analysis (mapping)
Epigenomic analysis is the analysis of the epigenetic time course and the locus on the genome.

research "Investigation about the trends in research related to epigenetics and challenges for industrial application" in 2007, and the report was published in February 2008.[3] For this research report, an investigation committee consisting of academic-industrial committee members (including researchers representing the epigenetics field) was established, and considerations and suggestions were made based on lectures and debates held during the committee meetings. As a result, technological challenges of epigenetics were summarized in seven academic and applied areas, and the distance from practical application and the future importance were shown for each of them (Table 2). In the category of "Distance from Practical Application," there were many listed as "(far from practical application)" (10 challenges).

Table 2 : Summary of Technical Topics of Epigenetics by NEDO Investigation

Topics	Distance from practical application	Future importance	International competitiveness	Necessity of national support
1. Application for Prevention, Diagnosis and Treatment of Cancer				
Investigation of epigenetic abnormalities in each type of cancer	◎	◎	○	○
Application for diagnosis (risk, existence and characteristics of cancer)	◎	◎	◎	◎
Search for activation factors, targeting epigenetic modification molecules	◎	○	○	◎
Treatment development targeting epigenetic mutation in individual genes	△	◎	○	○
2. Investigation of Epigenetic Abnormality in Acquired Diseases other than Cancer				
Investigation of the involvement of epigenetic abnormalities in acquired diseases other than cancer (immunological diseases, neurological diseases and lifestyle diseases such as diabetes)	△	◎	○	◎
Application of epigenetic abnormalities in diagnosis	△	○	○	○
3. Factors that Induce Epigenetic Abnormalities				
Investigation of factors and lifestyles that induce epigenetic abnormalities	○	◎	○	◎
Development of prevention method for epigenetic abnormalities, such as functional food	○	◎	○	◎
4. Safety Evaluation of Chemicals, Tests				
Development of detection of potency of environment and chemicals with regard to induction of epigenetic abnormality	○	◎	○	○
Analysis of effect of fetal exposure on induction of epigenetic abnormality	△	○	○	○
5. Regenerative Medicine, Cellular Therapy, Cellular Bank				
Application for cellular evaluation and classification of clones, iPS Cells, ES Cells, organ regeneration	◎	○	◎	◎
Differentiation control of the cell by induction of epigenetic modification	△	○	○	○
Differentiation control by epigenetic regulation of individual genes	△	○	○	○
6. Evaluation, Diagnostic Tools and System Development				
Techniques and tools necessary for research (separating single cells, analysis, etc)	◎	◎	◎	◎
Development of high-sensitivity and high-precision detection equipment for diagnosis of epigenetic modification	△	◎	○	◎
Development of genome-wide analysis equipment of epigenetic modification for research	◎	△	△	○
Methods for analyzing the condition of cellular-level epigenetic modification (including imaging)	△	○	○	○
Analytical method of epigenetic modification at the tissue and individual levels	○	○	○	◎
Foundation of an epigenome database	△	◎	△	○
7. Agriculture, Livestock Farming and Food				
Strain improvement by inserting epigenetic mutation	△	○	○	○
Development of method to prevent epigenetic mutation, i.e. functional food	○	◎	○	◎

* The topics were given ◎, ○ or △ based on discussions with the investigation working group and the investigation committee, which comprises epigenetics researchers and influential members of the Industry and Academic committee.[3]

* ◎, ○ and △ in the figure indicate the level of positivity. Therefore, regarding "Distance from practical application," ◎ means close, ○ means fairly close and △ means far. With regard to "Future importance," "International competitiveness" and "Necessity of national support," ◎ means high, ○ means fairly high and △ means not very high.

Source: Reference[3]

These are considered as topics in epigenetics that are currently at the basic research level. Consequently, progress in basic research will bring about a breakthrough to the next technological development.

4 What can We Learn from Epigenetics?

Here I show what we have learned from epigenetics and what we can learn in the future.

4-1 Biological Research of Epigenetics

(1) Mystery of Mammalian Birth

One of the great mysteries of Biology is why a male and a female are necessary for mammalian reproduction. Some insects, fish, reptiles and birds are known to reproduce through parthenogenesis, the ability to reproduce without fertilization by a male; however, mammals only reproduce sexually and there have been no reports of naturally-occurring mammalian parthenogenesis in the wild.

For mammalian reproduction, genes of both a male (sperm) and a female (oocyte) are necessary, and though embryos (fertilized egg before the formation of placenta) form with only paternal genes or maternal genes, development stops before the embryo grows into an organism. Actually, a normally developed embryo's paternal and maternal genomes have different epigenetic patterns, and the combination of these two genomes, which differ epigenetically, are known to be necessary for mammalian development. This was revealed by a report in 2004[13] of two female mice (ova) that bore offspring when one of the ova was changed into a paternal epigenetic pattern. In the future, research will move ahead regarding why mechanisms that prevent parthenogenesis developed in mammals.

(2) Evolution of Mammals

The methylation of genomic DNA is conserved in a wide variety of organisms, including plants, insects, fish, birds and mammals, though genomic imprinting that differentiates paternal and maternal genes is only found in viviparous mammals. This means that genomic imprinting is not found in egg laying mammals such as the platypus.

In addition, different types of viviparous mammals, including marsupials such as kangaroos and koalas, for example, and other types such as humans and mice (eutherian), have different genes involved in genomic imprinting, which indicates that the genes of the common ancestor of marsupials and eutherians became involved in genomic imprinting after splitting off from the platypus in the evolutionary journey, and subsequently marsupials and eutherians split away and evolved to have their own genomes.[14] As shown, the process of mammalian evolution can be traced through epigenetic analysis.

(3) Essence of Plants

Though I have mainly introduced research on animals, epigenetic research on plants (Arabidopsis) is also progressing. Since the mutation of genes involved in plant epigenetics is not as fatal as it is in animals, a variety of morphology (with morphology of leaves and pollen different from normal) created through gene mutation is being studied in Arabidopsis to advance the research. In addition, morning glory, rice and wheat are used in research.

4-2 Research of the Role of Epigenetics in Disease

(1) Understanding the Pathogenesis of Cancer

Since the 1990s there have been many reports about the abnormality of the methylation of DNA in genes related to multiple cancers in various cancerous cells, bringing attention to the relationship between epigenetics and the onset of cancer.

In particular, gastric cancer is believed to be deeply related to epigenetics. One of the factors that increase the risk of gastric cancer is *Helicobacter pylori*, the infection of which was recently discovered as inducing epigenetic mutations.[15] When gene 7 on locus 8, known for its DNA methylation in gastric cancer, was compared between the gastric mucosa of subjects testing positive for *H. pylori* infection and those testing negative, positive subjects had methylation increases of 5 to 303 times those of negative subjects. In addition, non-cancerous mucosa of gastric cancer patients had methylation increases of 2 to 32 times those of gastric mucosa of healthy subjects. Moreover, the level of methylation of specific genes is reported to decrease after the eradication of *H. pylori*, indicating that knowledge of epigenetics may be useful for detecting risks of and preventing gastric cancer in the future.

The types of cancer that can be detected through epigenetics include gastric cancer, colon cancer, breast cancer and kidney cancer.[16]

(2) Relationship between the Pathogenesis of Mental Disorders and Behavioral Disorders

Recently, progress has been made in research based on the hypothesis that epigenetic breakdown is involved in neuropsychiatric disorders and neurodevelopmental disorders.

As for neuropsychiatric diseases, research includes: 1) research on the involvement of the epigenetic changes (change in histone modification) in the mechanisms of the effects of antidepressant and electric convulsive treatment, 2) research on the relationship between genomic imprinting and bipolar disorder, since the symptoms and the age of onset differs depending on whether the bipolar disorder is paternal or maternal, 3) comparative research on the methylation of DNA, which is thought to be related to the onset of bipolar disorder and schizophrenia, in postmortem brains of patients and the control group, and 4) the relationship between the variance of the onset of bipolar disorder in identical twins (cases where only one of them develops the disorder) and DNA methylation.[17] However, such research is all still heavily based on estimation, and the involvement of epigenetics is still to be clarified.

Regarding neurodevelopmental disorder, there are about nine disorders that are known to have congenital abnormality in genes related to epigenetics, such as DNA methylation, chromatin remodeling (conformational changes of chromatin), histone modification and X chromosome inactivation.[18] In addition, there was a report in 2004 that showed postnatal abuse decreases the gene expression of the glucocorticoid receptor due to methylation, which subsequently causes behavioral disorders in animals,[19] suggesting that epigenetics is involved in acquired neurodevelopmental disorders as well.

In the future, the importance of environmental factors on neurodevelopment and behavior will be understood based on scientific evidence like epigenetics. Furthermore, progress in research in this area will bring a great contribution to the prevention and treatment of behavioral disorders.

(3) Relationship with Lifestyle Diseases

Recently a hypothesis arguing that factors leading to lifestyle diseases form during the fetal period (Fetal Origins of Adult Disease) was introduced.[20] According to this hypothesis, exposure to poor nutrition or hypernutrition during the critical period of organ and metabolic formation in the fetal period induces epigenetic changes, such as DNA methylation, and disease develops as a result of postnatal hypernutrition and little exercise. In addition, the epigenetic changes caused by the fetal environment are passed down for a few generations. However, these hypotheses are not yet proven and the mechanism is still to be clarified.[21]

A report in 2005 investigated the effect of exposure to poor nutrition in the pregnant mother and normal feed to the offspring after birth on genes related to fat metabolism in the liver at postnatal day 50 in animals (adult). The result showed that DNA methylation was decreased and expressions of multiple genes were increased 3 to 10 fold, indicating that the effects of changes in the fetal environment are still observed in adults.[21,22]

In 2009, a knockout mouse lacking Jhdm2a, a gene involved in epigenetics, developed obesity and hyperlipidemia.[23]

The information above shows there is a number of indications that epigenetics is involved in the development of adult diseases.

4-3 Drug Development and Epigenetics

The development of drugs for cancer treatment targeting epigenetics is underway. In particular, inhibitors of DNA methyltransferase (DNMT) and Histone Deacetylase (HDAC) are reported to be effective.[Note 8] Table 3 shows that as of May 2009 there are three epigenetic medicines on the market, all approved in the US. In addition, there are drug candidates that are going through phase I and II of clinical trials in the US that are not listed in the figure (Belinostat, MGCD-0103, Panobinostat, Romidepsin).[24]

Decitabine, a DNMT inhibitor, is reported to be more effective when administered at a low dose for a long period compared to a high dose for a short period.[25] Since DNMT is necessary in healthy cells, its inhibitor may cause cell death at high doses and there are fewer side effects at lower doses. However, the mechanism of the effect of the long-term administration of a low dose is still unclear. In addition, a clinical trial of combined administration of

Table 3 : Current Status of Drug Development Targeting Epigenetics

Type of Inhibitor	Name of Drug	Applicable diseases	Status (May 2009)
DNA Methyltransferase (DNMT) Inhibitor	5-azacytidine (Trade name: Vidaza)	Myelodysplastic syndromes	Approved by US FDA in 2004
	5-aza-2'-deoxycytidine (decitabine) (Trade name: Dacogen)	Myelodysplastic syndromes	Approved by US FDA in 2006
Histone Deacetylase (HDAC) Inhibitor	Suberoylanilide hydroxamic acid (SAHA) (Trade name: Vorinostat, Zolinza)	Cutaneous T cell lymphoma	Approved by US FDA in 2006
	MS-275 (SNDX-275, Entinostat)	Under consideration for melanoma, hematological cancer, non-small cell lung cancer, etc.	Phase II of clinical trial in the US
	FK228 (Romidepsin)	Under consideration for cutaneous T cell lymphoma	Phase II of clinical trial in the US
	Valproate	Approved as an anti-epileptic drug Under consideration for expansion of application to include hematological cancer	Phase II of clinical trial in the US

Prepared by the STFC based on Reference [24, 25]

decitabine and various HDAC inhibitors is underway.

In Japan, there are not yet any approved epigenetic drugs. However, FK228, shown in Table 3, was discovered by a researcher at Fujisawa Pharmaceutical Co., Ltd (now Astellas Pharma Inc.) during an investigation of natural products of fermentation. It was subsequently developed into a drug by Gloucester Pharmaceutical, Inc., an American anti-cancer drug development venture company, which licensed out the product.[26]

4-4 Quality Evaluation of iPS cells with Epigenetics

Since stem cells possess the ability to differentiate into various types of cells, their application in regenerative medicine to regenerate tissues and organs damaged by disease has been long awaited. In particular, since iPS cells are produced by inserting genes into somatic cells, there are few ethical issues compared to ES cells produced by a fertilized embryo. The technology used for iPS cell generation has brought the practical use of regenerative medicine a step closer. However, current iPS cell generation technology produces inconsistent genetic expression signatures, so further technical progress is required to produce uniform and high quality iPS cells.[27] This inconsistency is caused by the variety of epigenetics of the somatic cells used to produce iPS cells.

Therefore, for practical application of iPS cells in clinical settings, the further establishment of methods to standardize iPS cells and also an epigenetic quality evaluation system to provide safe iPS cells to patients is required. For these reasons, the importance of research in epigenetics that targets iPS cells will increase in the future.

[NOTE8] : DNMT inhibitor and HDAC inhibitor

DNA methylation of DNA in epigenetics occurs when DNA methyltransferase (DNMT) causes the methyl base of S-Adenosyl-L-methionin to transfer to DNA. When DNMT is inhibited methylation decreases and genetic expression is induced.

On the other hand, during the acetylation of histone, which activates genetic transcription, the actions of histone acetyltransferase (HAT) and histone deacetylase (HDAC) oppose each other. When HDAC is inhibited, histone is acetylated and genetic transcription is activated.

As is shown, inhibiting DNMT and HDAC activates genetic expression and genetic transcription, so these two types of drugs are expected to possess therapeutic effects on diseases that are caused by suppression of genetic expression and transcription.

4-5 Development of Epigenetics Detection Equipment

It is important to detect which DNA base sequence in the genome have been methylated in order to know the epigenetic conditions as well as the diagnostic marker for diseases such as cancer in the future. However, ordinary genetic tests can not detect methylated regions.

In recent years, various methods have been developed to detect methylation. Currently, the most well known method is bisulfite sequencing, which determines the DNA sequence through bisulfite preparation of a DNA fragment to change the base.[28] Traditionally, such detection was time consuming; however, efficient high speed sequencers are starting to make exhaustive methylation analysis possible. For example, there has been a report about methylated genes of Arabidopsis thaliana detected by shotgun sequencing.[29] The size of the Arabidopsis genome is around 130 million bases, which is small compared to the 3-billion-base human genome. Nevertheless, this showed that we are a step closer to analyzing methylated genomes in mammals.

However, detection of other types of epigenetics, such as histone modification and genomic imprinting, still employ time-consuming traditional methods, including the chromatin immunoprecipitation method and the FISH method.[30] Therefore, the development of new methods and equipment that will enable high throughput (quick analysis) and hence facilitate the clinical application of epigenetics is awaited. In addition, as shown in the previous paragraph, equipment for the detection of epigenetics at the single cell level is necessary for the epigenetic quality evaluation of iPS cells. Therefore, one of the most important research areas is the development of analytical equipment for epigenetics, considering the clinical application of the findings from epigenetics research.

5 Progress of Epigenetics Research in the US and Europe

5-1 The US

NIH plotted the NIH roadmap in 2002 to promote more effective and productive medical research and it presented research topics that were the most important across the entire NIH (27 institutions).[31] Since then, there have been little changes made to the selected research topics included in the road map.

In 2007, epigenetics research was added to the NIH road map and project funding of $19 billion over 5 years starting in January 2008 was provided.[32] The funding was implemented to support the research community discussed in section 3-2.

For reference, major epigenetics research programs included in the road map as of May 2009 are indicated in Table 4.

In addition, the direction of American epigenetics research is rapidly heading toward targeting humans, as evidenced by the name of the March 2009 research achievement debriefing session related to the NIH

Table 4 : Major Epigenetic Research Program on NIH Roadmap (May 2009)

Content of Research Program	Universities in Charge
・Mapping of genomic region where epigenetics takes place in various human cells ・Development of the technology to utilize this knowledge as a reference	MIT, UCSF, Ludwig institute for cancer research, Washington University (Seattle)
Cooperate in the above projects	Baylor College of Medicine
・Epigenetic profiling, in vivo imaging of intracellular epigenetic changes	Stanford University, Rockefeller University, Fred Hutchison Cancer Research Center, Chicago University, University of North Carolina
	Cornell University, Washington University (Saint Louis), UCSD, Arkansas University Faculty of Medicine
・Discovery of new epigenetics in mammalian cells	University of North Carolina, Mount Sinai School of Medicine
・Research of epigenetics related to human health and diseases	Emory University, University of Virginia, Institute for Cancer Research, Massachusetts General Hospital, University of Texas

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road map: "Emerging evidence for epigenomics changes in human disease."

5-2 Europe

Support for epigenetics research in Europe started around 2000. A team participation project, EPITRON (Epigenetic Treatment of Neoplastic Disease)[33] is funded with 10.9M Euros under FP6, and seven countries participate, including France. In addition, another team project, HEROIC (High Throughput Epigenetic Regulatory Organization in Chromatin), is funded with 12M Euros also by FP6, and eight countries participate, including the Netherlands (2005-2010).[34] In addition, both projects receive additional, ongoing funding under FP7.

5-3 Japan

The activity of the research community does not seem to be reflected in the financial support, as there is yet to be a promotion of large projects (collaboration of multiple research institutions, etc.) for epigenetics in Japan. As a result, the majority of public funding goes toward individual research, such as Grant-in-Aid for Scientific Research. Starting in 2007, the "Generation Cycle and Epigenome Network of Germline" (Leader: Hiroyuki Sasaki, President of National Institute of Genetics) will be conducted for 5 years as a part of the Specific Area of Research of Grant-in-Aid for Scientific Research (approximately 2.3 billion yen).

There are some projects that include epigenetics as part of their program, for example, the "iPS Cells and Biological Function," of the Sakigake Strategic Creative Research Promotion Project by Japan Science and Technology Agency (JST). JST has stated that "epigenetics is strongly related to cancer research, but it is also influential in stem cell research" in their G-TeC report published in January 2008 and entitled, "Stem Cell Homeostasis."[35] In addition, they noted that they "believe that epigenetics related programs will be strongly promoted in Japan following the Western countries in the future," indicating that large scale epigenetics research will be funded in the near future.

6 Conclusion—Expectations for Epigenetics Research in the Future—

What determines the outcome of organisms, including humans, is a question that has been debated since ancient times. Is it nature (genes) or nurture (environment), or both? Common sense tells us that it is a little bit of both. However, there has been no scientific evidence to support this belief.

As opposed to the genome, which does not change over a lifetime, epigenetics is constantly changing, right from the moment of fertilization. This means that epigenetics adds the two variables of the external influence of environment and the passage of time to the genetic information of an organism, called the genome.

Through the Human Genome Project, we became familiar with the idea that humans' future behavior and susceptibility to disease is fully determined at birth. However, from the perspective of epigenetics, humans keep changing in response to external influences, and though the base is already determined at birth, the details are uncertain and possess much potential.

Epigenetics research has just begun and future progress is eagerly awaited. In particular, both 1) the accumulation of scientific knowledge about gene expression regulation for biological homeostasis, and as a result, 2) the development of technology to artificially regulate biological gene expressions are highly expected. This may bring about much discussion about gene control in society.

In addition, though I have only briefly touched upon it in this paper, epigenetics is known to change with the process of aging. Therefore, in the future, epigenetics research may contribute to the development of treatment for diseases and disorders specifically related to the elderly. Also, achievements in epigenetics research may help us understand how organisms are damaged by their environment or stress and how to measure the damage accurately, which may help us plan policies to protect us from such damage, by improving our lifestyles, for example.

As shown here, epigenetics is a research area that has the full potential for future achievements and is the subject of much international attention. Therefore, research on epigenetics needs active support in Japan as well. In addition, one of the most important research topics is the development of analytic equipment for epigenetics.

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Profile

Yuko ITO
Life Science Unit Leader,NISTEP

PhD in Pharmacology. She has participated in the research of human chromosome structure and function.
She is currently a science and technology policies specialist. She is interested in the trends in frontier science in the field of life sciences and competitive research funding policies and how scientific knowledge is applied in society.

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