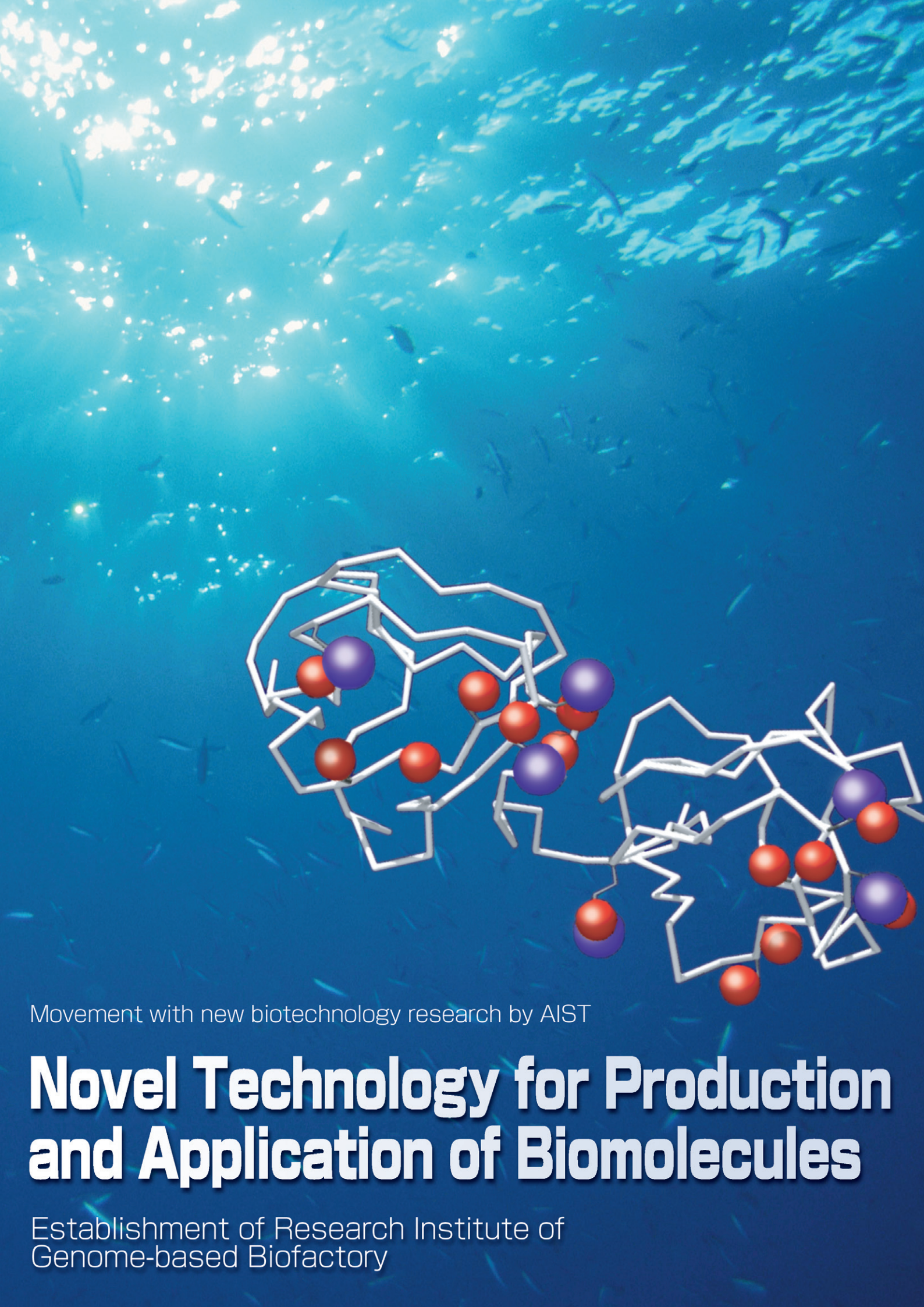


Movement with new biotechnology research by AIST

Novel Technology for Production and Application of Biomolecules

Establishment of Research Institute of Genome-based Biofactory

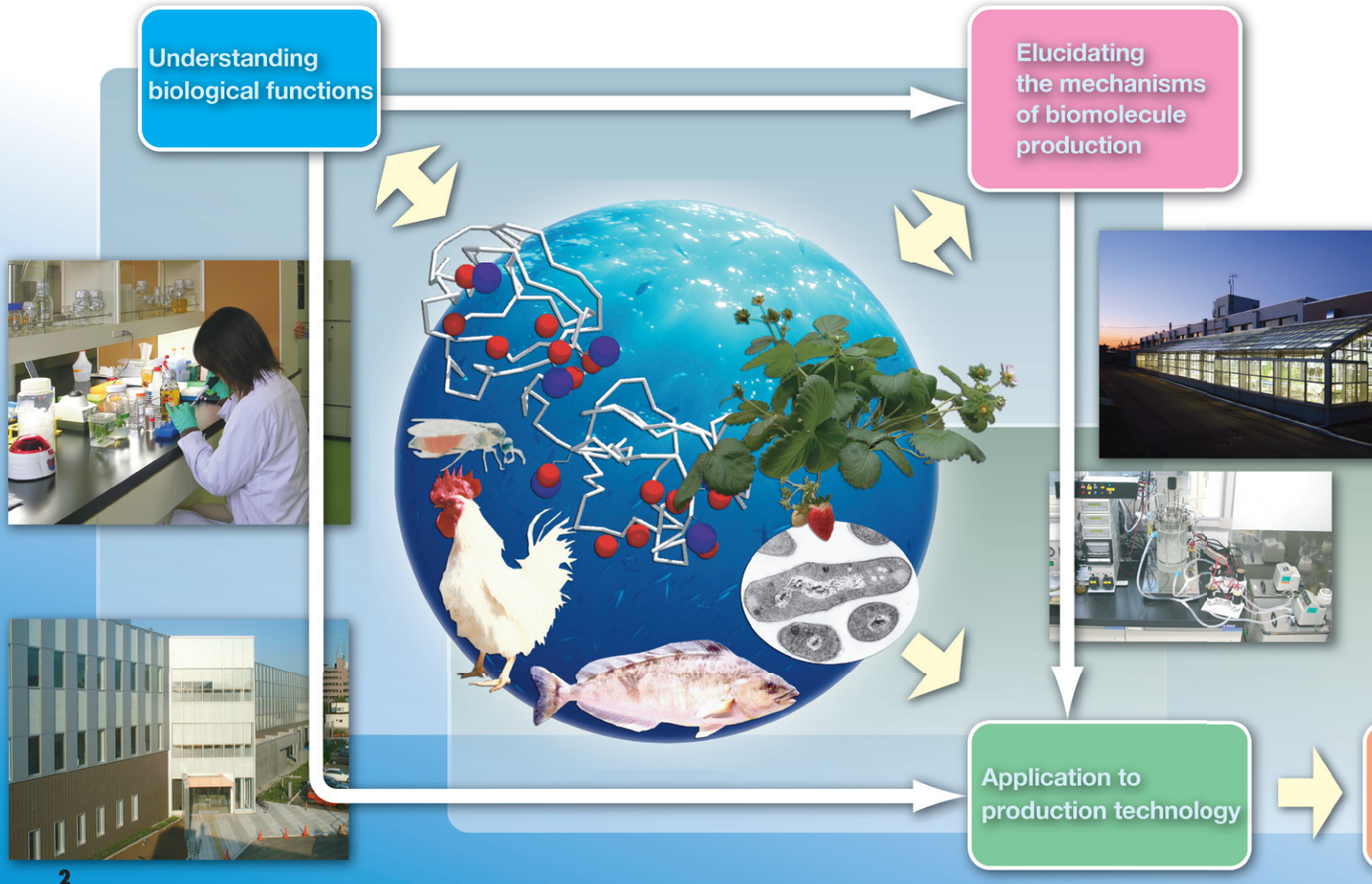
Research and Development of Biomolecule Production Technologies at the National Institute of Advanced Industrial Science and Technology (AIST)

Hiroshi Kuriyama, Research coordinator (life sciences)

Expectations for Biotechnology

For thousands of years, mankind has benefited from production technologies that have utilized the functions of living organisms. Such benefits can be traced back to the use of microorganisms (e.g., yeast and lactic acid bacteria) to produce fermented food products such as soy sauce, vinegar, yogurt, and alcoholic beverages (beer, wine and Japanese *sake*). More recently, living organisms have been used to produce antibiotics such as penicillin and streptomycin, amino acids for use as seasonings and nutritional supplements, and various enzymes as key components in detergents and medicinal drugs. The main compounds produced by conventional fermentation industries are metabolites derived from natural processes in microorganisms, such as ethanol and lactic acid. On the other hand, biomolecules with complex structure, such as antibiotics and enzymes, are requisite for the survival of an organism.

In the past several decades, significant research efforts have been made to advance technologies whose goals are to modify the useful functions of organisms and to improve the production efficiency of biomolecules. These technologies have benefited considerably from basic research in the field of life sciences, which have undergone major advancements in the latter half of the 20th century. In particular, genetic engineering techniques, which can significantly alter the functions of an organism, have been utilized to create organisms that effectively produce biomolecules having improved functions. Today, development of biomolecule production system is under fierce global competition, as numerous research institutes and corporations engage in such research. In the coming years, development of effective production technologies for new and safe biomolecules that can be used as clinical, diagnostic, and veterinary drugs will be of importance.



and Application of Biomolecules

Biotechnology Research at AIST

New findings in basic scientific research related to biotechnology have broad applications to a variety of industry sectors (e.g., agriculture, livestock, and fisheries industries). They are also expected to make considerable contribution to the development of new food products, clinical and diagnostic drugs, diagnostic methods, and measures to counter environmental problems. We, at AIST research centers located throughout Japan, are carrying out research and development in order to create new industrial technologies.

Biotechnological research projects currently being carried out at our research bases of AIST in Tsukuba, Tokyo Waterfront, Kansai are; 1) search for microorganisms or enzymes having novel functions, 2) modification and improvement of functions of microorganisms and enzymes, and 3) analyses of functional and structural properties of human genes and proteins, which lead us to the development of new clinical and diagnostic drugs.

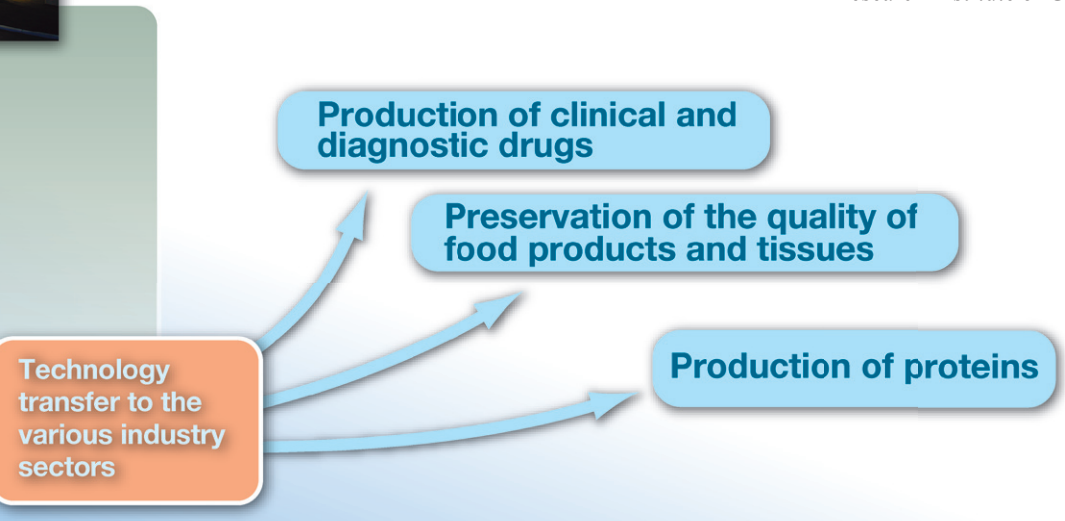
In the future, we believe it is of great importance to meet the broad demands of biotechnology-related industries by taking an advantage of the unique characteristics of the research bases and units within AIST. Additionally, it is important to incorporate new findings in basic research in the life sciences (e.g., genome analyses) into the development of biological production technologies, as it will facilitate the application of biotechnology to the various industry sectors.

Establishment of the Research Institute of Genome-Based Biofactory

In April 2004, AIST established Research Institute of Genome-Based Biofactory at AIST Hokkaido in Sapporo, Japan. This research unit was formed to focus research on the application of biotechnology to production technology. At AIST Hokkaido, we have recruited young and excellent research scientists, and have also been engaged in unique research and development activities for the past decade including a period when AIST Hokkaido was named as Hokkaido National Industrial Research Institute. As a result, our original and promising technology fields have effloresced: such novel technologies include effective production of heterologous proteins in recombinant microorganisms and production of animal vaccines using transgenic plants. We are also promoting technology transfer and collaborative research projects with our industrial partners; number of such collaborations is currently on the rise.

At this new research institute, we will strive to develop biotechnology for providing added value to the biomolecules by incorporating new findings from leading biological research, such as those arising from genome analyses. The research topics include the development of DNA chips, analyses of functional properties of proteins, and the advancement of efficient methods to produce biomolecules that were once deemed difficult to produce. In addition, we are improving our personnel and the facilities at the research institute; in summer 2003, biotechnology research building (floor area: 7000 m^2) was completed, and a greenhouse for production of functional proteins in transgenic plants, was also constructed.

It is also our mission to contribute to the promotion of industrial development in Hokkaido; in particular, we will strongly support the Biotechnology Industrial Cluster Plans launched and promoted by the Organization of the Hokkaido Bureau of Economy, Trade and Industry. We will do so through our research activities and through the promotion of active exchange programs with the regional industries. In this special issue, we will introduce some representative research being conducted at this newly established Research Institute of Genome-Based Biofactory.



Plant-based Vaccines

Takeshi Matsumura

Group Leader, Plant Molecular Technology Group, Research Institute of Genome-based Biofactory

Current Status of Transgenic Plants

Today, transgenic plants are cultivated on an area of approximately 67,700,000 ha worldwide (data for 2003), which is roughly 1.8 times as large as the land area of Japan (approximately 37,000,000 ha). This value is currently increasing steadily. Most of the transgenic crops grown in these fields are soy, maize, cotton, and canola (for oil production) that demonstrate herbicide-tolerance and/or pest- and pathogen-resistance. Because these transgenic crops are edible plants, there is a misconception among the public that transgenic plants are synonymous with GMO food products. It is less known, however, that plant genetic engineering technology is used for purposes other than GMO food development.

Production of Drugs Using Plant Functions

Progress in plant genetic engineering contributes to the agricultural industry by developing new varieties of crops that have increased production and by reducing the labour needed for cultivation. However, plant biotechnology can have much broader applications. For example, development of plants that produce mammalian medical proteins is currently under way. These proteins, which include substances such as edible vaccines, anticancer agents, and antibody substances are not naturally found in plants. Until recently, cultured mammalian cells or recombinant microorganisms were used to produce these proteins. So, why are we now attempting to produce them with plants? Here are several reasons why we may want to do this:

1. Plants do not become infected with mammalian pathogens. Unlike the problems related to the BSE issues, the risk of pathogens mixing in is considerably low when transgenic plants are used. This is because reagents are not used during the manufacturing process.

2. Unlike the production system based on cell or microorganism culture, there is no need for massive culturing facility (i.e., culture tank). Recently, the demand for these culture tanks has far exceeded their supply; this is causing serious problems in the production of the proteins.

3. The cost of production is low. For example, the production of antibodies with transgenic plants was predicted to be 1/3000 the cost of that done with cultured mammalian cells (reported in a scientific journal).

4. By utilizing edible crops, these functional proteins can be taken up simply by "eating". This frees us from the "agony" of injections. This will also result in less demand for injection syringes and needles, which leads to a reduction in medical waste.

Development of "Edible Vaccines" for Chicken Leucocytozoonosis

Here, we describe an example of the use of a transgenic plant in medical protein production technology. We have developed, in collaboration with a company that specializes in veterinary medicine, a transgenic plant that has been introduced with genes encoding vaccine substance for chicken leucocytozoonosis. Leucocytozoonosis is an infectious disease, similar to that of the better-known human malaria. This disease is an economically important for poultry industry, causing reductiion of egg production, anemia, discharge of green feces,and sometimes death. (Fig. 1). Today, approximately 4-5 billion yen are being spent on sulfa drugs on a yearly basis in Japan alone. Significant labour is required when injections are used to administer the vaccine for this disease, as a single farm could be raising tens and hundreds of thousands of chickens. There is also a concern that pain due to injections could lead to a reduction in egg production (Fig. 2). Therefore, development of easily administered and painless oral vaccine was awaited.

We introduced a gene encoding a vaccine for chicken leucocytozoonosis to potatoes (this vaccine is already on the market). Leaves of these transgenic potatoes were freeze-dried (Fig. 3), and were administered to the chickens by mixing it with feed. The results showed that the introduction

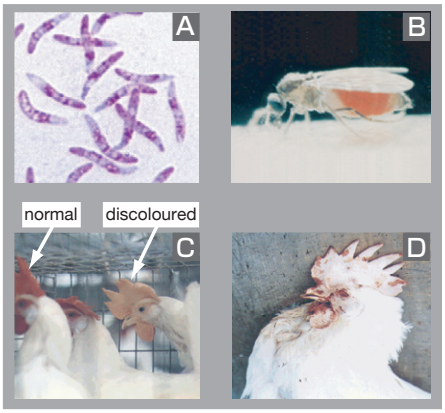


Fig.1 : Chicken leucocytozoonosis (Chicken protozoan disease)
A: Leucocytozoonosis protozoan
B: Vector insect: biting midge (C.arakawae)
C: 18 days after infection: anaemia (discoloration of cockscomb)
D: 13 days after infection: died after spitting blood

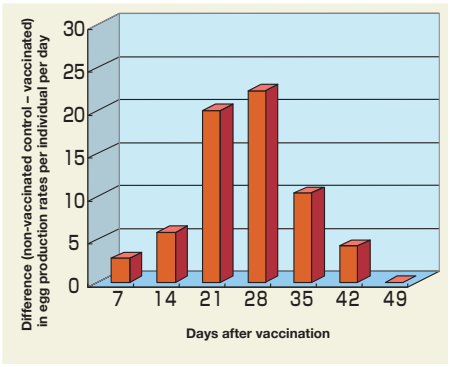


Fig. 2 : Reduction in egg production rates due to vaccine injection.

Fig. 3 : Vaccine feed for chicken.
A: Feed for raising chicken.
B: Transgenic potato leaves (freeze-dried).
C: Mixture of chicken feed and freeze-dried transgenic potato leaves.

of transgenic potatoes increased the serum antibody levels of the chickens, which implies that the duration of their defence against the disease has been extended (Fig. 4). This example shows that plant genetic engineering technology can be used to produce vaccine substances without extractions or purifications. They can also be easily administered, without pain.

This transgenic potato cannot be considered as being equivalent to the potatoes that are found on the shelves of the grocery shops. In other words, it must not be considered "food"; throughout the stages of cultivation, harvesting and processing (e.g., freeze-dry), it will have to be treated as a "material" to produce veterinary medicine.

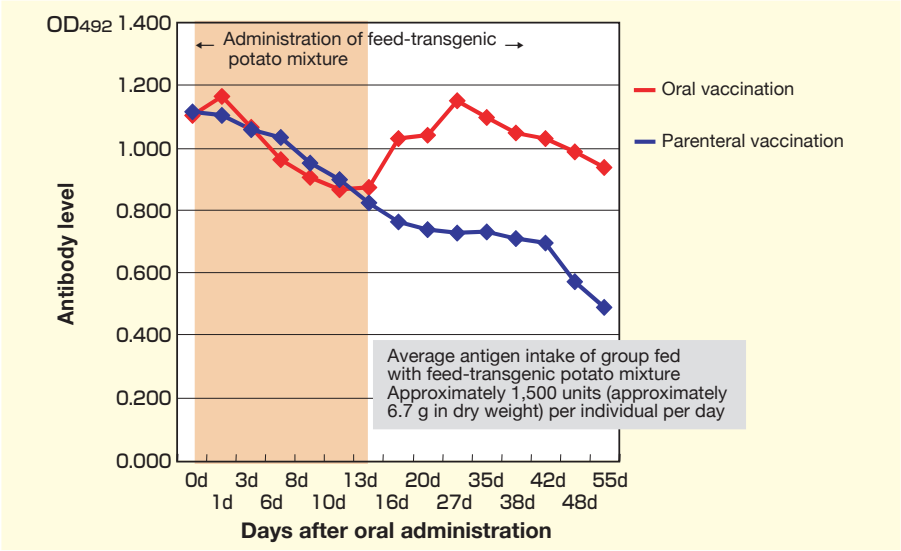


Fig. 4 : Changes in chicken serum antibody levels after oral administration of transgenic potatoes.

Future Prospects

The objective of our research is to use plants not only as agricultural products but also as a "device" that can produce useful substances utilizing the genetic information of various biological species. Looking ahead, we believe this technology, which produces medical drugs using transgenic plants, can open up new markets, potentially forming a new industry sector.

Expectations for Plant Biotechnology

Masatoshi Tanida, Vice President
Hokkaido Green Bio Laboratories Corporation

Currently, as an alternative to the traditional hybridization breeding methods, plant biotechnology is receiving wide attention as a new technique that can create valuable new plants (crops) for both producers and consumers. However, plant biotechnology can point to a variety of techniques that are used in the plant sciences; it includes techniques that provoke a desired modification by culturing a tissue, remove pathogenic viruses, or create valuable plants by recombining genes. This technique of recombining genes can create plants (crops) that were previously thought impossible to produce with the traditional hybridization breeding techniques. Because of this exciting feature, numerous scientists are conducting basic and applied research on this technology.

One of the topics in this area of research includes the development of plants that create beneficial material or substances that are not naturally found in plants. Herbicide-tolerant soy can only be sold as soy, however, if it can produce substances that have new functions, it would create an added value to this product. For example, the development of crops that produce substances that can stimulate the human or animal immune systems or vaccines that prevent diseases would fall into this category. Although most of these compounds have to be extracted from the plants before use, there are some substances that can be used simply by eating them; these are the so-called "edible vaccines".

This new use of crops, also known as "molecular agriculture", will provide direct benefits to the consumers, and will also promote a new industry based on agricultural products. Some believe Hokkaido is not good at processing or adding value to the products gained through the primary industries. However, if we can make the crops produce beneficial material with the help of plant biotechnology, it will lead to a whole new array of marketable products. Research and development of such products will create closer ties between the agricultural and the manufacturing industries. We have great expectations for AIST, which can take on a leading role in this effort through their agricultural and manufacturing knowledge and know-how. We also look forward to collaborative projects with AIST. By doing so, we hope to advance the applied use of this promising technology.

Antifreeze Proteins Found From Fishes Living in Northern Waters

Sakae Tsuda

Group Leader, Functional Protein Research Group, Research Institute of Genome-based Biofactory
Visiting Professor, Division of Biological Sciences, Graduate School of Science, Faculty of Science (cross-appointed)

Using Special Features of Biological Organisms for Human Benefits

Organisms that reside in extreme environments where humans are unable to survive can potentially have special and extremely userful functions. For example, there are organisms that can transform deadly substances into non-toxic compounds or species that can degrade crude oil spilled from a stranded oil tanker. The goals of our research group are to elucidate these beneficial functions of various proteins, and to contribute our findings to the industries and to our daily livelihoods.

Special Ability of Organisms Inhabiting the Freezing Waters: AFP

Antifreeze proteins (AFP) are protective biomolecules that prevents intracellular freezing when an organism is exposed to subzero temperatures. At these temperatures, AFPs function by binding to the ice crystals that are being formed, which effectively prevents the growth of the crystals. The AFPs can therefore protect the internal structure of products containing water (hydrated substances; e.g., meat, vegetables, processed food, blood, cells, tissues, and organs) at temperatures around the freezing point. This feature of AFPs is bileived to delay reductions of the product quality, flavor and smell during preservation. They also allow living organisms to maintain fully functional activities at low temperatures. By putting AFPs into practical use, costs that are associated with the existing cold storage technology and facilities are expected to decrease considerably.

Although 30 years have passed since the discovery of AFPs, they have not yet been put into practice. The main reason why the development has taken so long is due to the rarity of the substance; it was believed until recently that AFPs could only be collected from fishes inhabiting the Arctic and the Antarctic Seas. To put AFPs into practical use for medical purposes or for food production, manufacturing firms require AFPs in the order of grams and kilograms. This was a major hurdle that must be jumped in order to put AFPs into applied use, as technology to obtain enough quantities of AFPs from such fish supplies was unavailable. This technical limit, otherwise known as the "quantity limits", is one of the major problems biotechnology research scientists face every day when attempting to put their findings into practice.

Faced with this limitation, we had, at one point, decided to examine whether or not AFPs are found in small fishes that inhabit the Ishikari River, which flows through the city of Sapporo. To conduct the test, we captured fishes and collected their blood, which is a general procedure that is used to assess the existence of AFPs; for individuals that had a body length of less than several centimeters, we grounded the fish meat (muscle) and examined its extract. To our surprise, we found significant amounts of AFPs in these local fishes such as Hypomesus olidus (Osmeridae, pond smelt). Since then, we have measured AFP activity levels of over 160 species of fish, which were collected in the coastal areas of Hokkaido and in the local grocery shops. As a result, we developed a technique that can purify non-limiting amounts of AFPs from fishes that are caught locally. This technique has two major advantages; first, we do not have to be too conscious about the freshness of the fish we use, and second, we do not have to send fishing vessels out to the Arctic or the Antarctic.

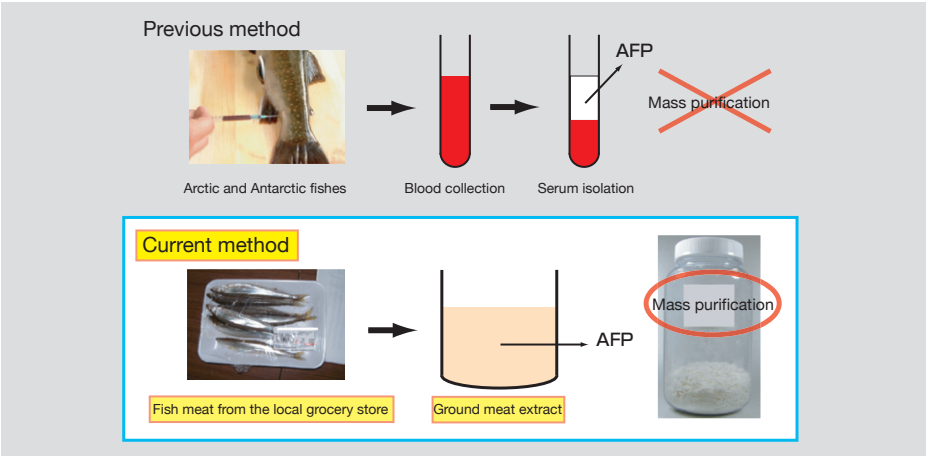


Fig. 1 : Old and current techniques of purifying AFPs.
Previously, we had to draw blood immediately after catching the fish in the Arctic or the Antarctic Seas. The AFPs were then purified from the serum. With the new method we developed, AFPs can be purified in mass quantities from the muscles of small fishes that are found in the coastal areas of Japan.

Industrial Application of AFPs

When we first mention that we may be able to use AFPs for medical purposes or for food products, the first reaction we get will generally be, "Is it safe?" At this stage, we have no concrete answer to this question. We still require significant amount of work before putting AFPs into practice in the respective industries. We must perform safety (toxicity) tests, examine AFP's stability and recyclability, and search for any reactions with the coexisting substances. We must also check the length of time it can preserve the quality of the products and evaluate any secondary uses. When performing these tasks, we must bear in mind that there are three types of AFPs that possess structurally and functionally different characteristics. Moreover, within each of these three types, numerous isomers exist. These different types and isomers of AFPs were found among the various fishes that we had examined. Therefore it is important that we conduct gene mapping (antibody staining) and examine

the effective concentrations for each type of AFPs, and also determine which AFP is most suitable for specific purposes. These tasks have significant implications on the industrial application of AFPs, such as for construction of production facilities (GMP compliance) and quality control. Even before our investigations on AFPs began, our research group had done considerable work identifying the structural and functional properties of proteins under cold environments using NMR and X-ray methods. As I look back, it is very satisfying to see what we have achieved in our work to elucidate the functions of AFPs.

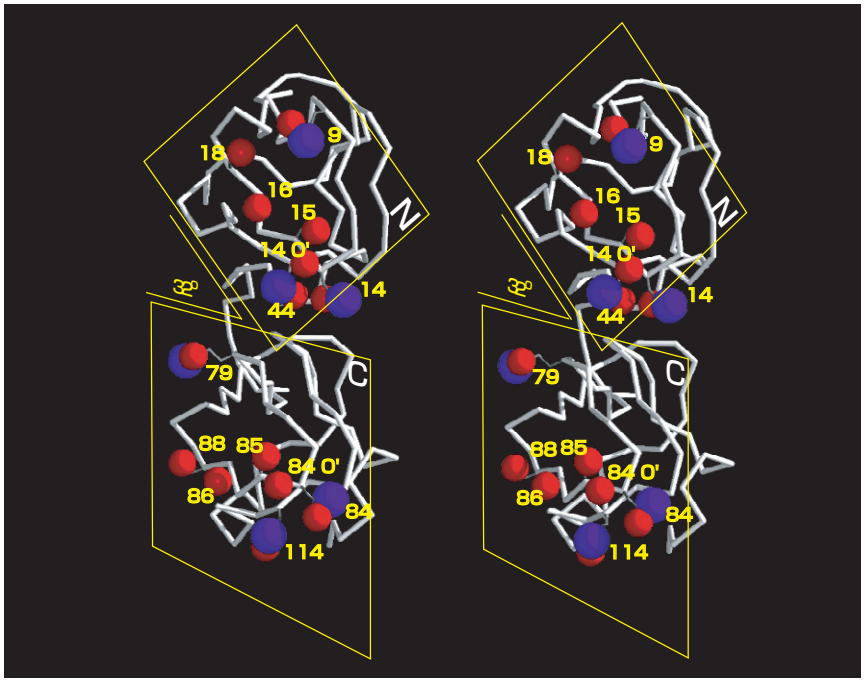


Fig. 2 : Three-dimensional molecular structure of fish-derived AFP (dimer) using heteronuclear multidimensional nuclear magnetic resonance (NMR) method (stereo figure). On the molecular surface of both N-domain and C-domain, multiple polar amino acid residues are found as if they were bonding to a plane that is formed by the ice crystals' specific oxygen atoms (shown inside the square).

Solving the Mysteries of AFPs

On one occasion, I asked a colleague of mine some very fundamental questions, such as, "When did AFPs come into existence? Was it after the ice age? How many species on this planet possess AFPs?" Although he had no answers to these questions, he replied that my questions must be answered because they have direct consequences to our ability to secure the AFP resources. Therefore any kind of question related to the AFPs could potentially have direct implications on the industrial use of this protein. On this planet, there are over 20,000 species of fish alone; given the number, there is no knowing when our questions will be fully answered.

New prospects for protein research

Isao Tanaka
Professor, Graduate School of Science, Faculty of Science,
Hokkaido University

In this post-genome era, it is clear that we must elucidate the functions of over 20,000 genes that are encoded in the human genome. Research on proteins is expected to be one of the core research topics in the field of biological sciences. In particular, the analyses of human proteins are expected to be under fierce global competition, as they have direct implications on the research and development of new drugs.

Many countries have already initiated national projects on protein research; in Japan, the Ministry of Education, Culture, Sports, Science and Technology has funded a project coined National Project on Protein Structural and Functional Analyses starting in the fiscal 2002 year. Two main research groups within this project, which will specialize in "gene expression" and "intracellular signal transduction", respectively, were housed in the Next Generation Post-Genome Research Building at Hokkaido University (North Campus).

These projects will, no doubt, contribute to the advancement of basic technologies in protein research, and indeed, technologies for the production and analyses of proteins are advancing very rapidly. Presently, there are very little differences in the proliferation of basic technology of protein research in Japan, Europe and in the U.S. However, the U.S. and Europe has significant advantages in terms of global leadership and the ability to develop new basic technology. In Japan, it is therefore important to develop knowledge and technologies that are not mere imitations of those produced elsewhere; we must develop original and world leading research.

As national universities in Japan are transformed into corporations, the nature of research being conducted at universities is bound to change. In particular, research scientists in the field of life sciences will have to become more conscious of their research output. However, in addition to conducting research, universities have social responsibilities as educational institutions, and because of this, the ultimate objectives of AIST and the universities may not match perfectly. However, I believe the collaborations between the institutions will bring two different scientific cultures together, and thereby help bring about original and world leading scientific discoveries in Japan. Through the research on proteins, I hope there we will be variety of new and exciting developments in Hokkaido.

Development of Protein Production Technologies at Low Temperatures

Satoru Ohgiya, Takehiko Sahara

Expression and Molecular Regulation Research Group, Research Institute of Genome-based Biofactory

Expression Systems in the Post-Genomic Era

The Human Genome Project was completed in 2003, and we are now in the post-genomic era. Japan possesses approximately 20,000 full-length human cDNAs as a valuable gene resource, and it is now of importance to characterize functionally unknown proteins encoded by the cDNAs, as such genetic information can be used for the development of new drugs. Big projects for determination of three dimensional structures of various proteins are also being conducted.

However, a major obstacle that faces us in this area of research is the production of proteins by genetic engineering; such systems to produce proteins are called an "expression system". To date, *Escherichia coli, Saccharomyces cerevisiae* (yeast), insects, and plant and animal cells have been used as hosts for various expression systems. However, each of these expression systems has its advantages as well as disadvantages from the viewpoint of expression levels, production costs, and time required for the construction of the expression system. The most widely used expression system is that of *E. coli*, however, it is well known that many proteins produced in *E. coli* are insoluble. In the post-genomic era, there is a growing need for the expression systems that can produce a variety of proteins in a soluble form with a high efficiency. Additionally, these systems must be applicable to a large scale production of useful proteins, as this is a critical factor in the expression system for industrial or applied purposes.

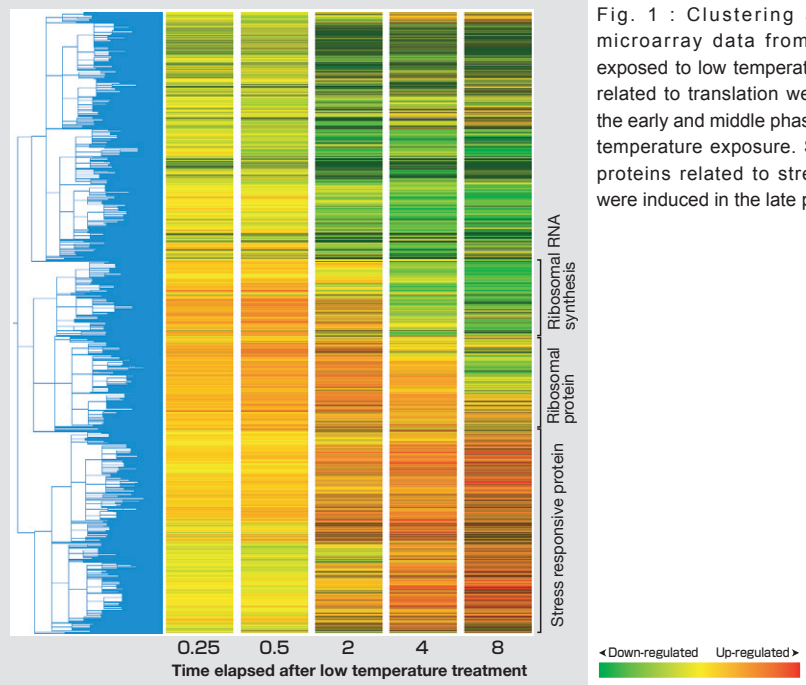


Fig. 1 : Clustering analyses of microarray data from yeast cells exposed to low temperatures. Proteins related to translation were induced in the early and middle phases during low-temperature exposure. Subsequently, proteins related to stress response were induced in the late phase.

Low-Temperature Expression System Using Yeast

Expression systems using *S. cerevisiae* is thought to be suitable for expression of human cDNA, because this microorganism is an eukaryote and thus has intracellular structures similar to humans. Another advantage of this microorganism is that it can be cultivated in a large scale at a low cost. However, a major disadvantage of yeast-based expression system is its relatively low expression efficiency. On the other hand, yeast genome database is among the best organized; this will be a major advantage when attempting to improve the yeast-based expression system.

Another aspect of yeast that can potentially be an advantage when designing an expression system is its tolerance to low temperature. Low temperatures have been often used to solve the problem of target protein insolubility in expression systems

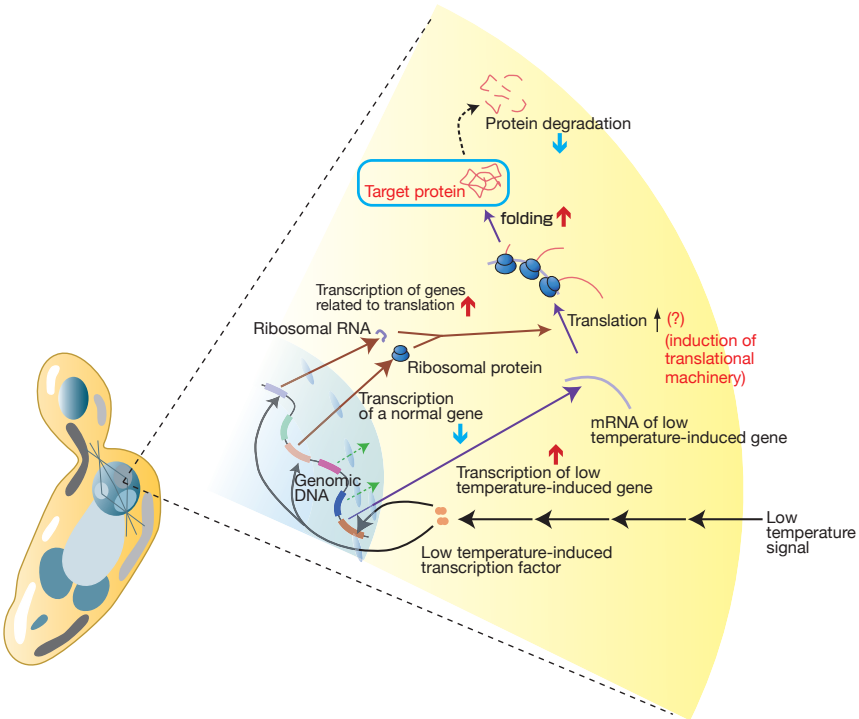


Fig. 2 : Effects of low temperature on yeast expression systems. Low temperature is a favorable environment for protein synthesis; in addition to the initiation of selective transcription of low temperature-induced genes, low temperature induces components of translational machinery, increases protein folding efficiency, and suppresses protein degradation. The effects of low temperatures are shown in red (increased) and light blue (decreased) arrows.

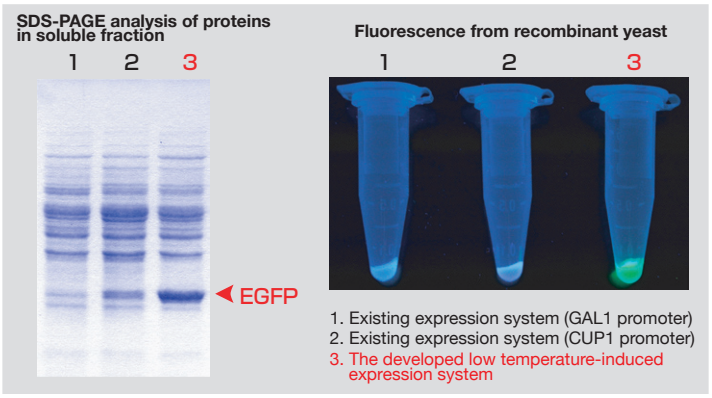


Fig. 3 : Comparison of existing expression systems and low temperature-induced expression system. Fluorescence from enhanced green fluorescent protein (EGFP) was used to assess the expression efficiencies in these expression systems.

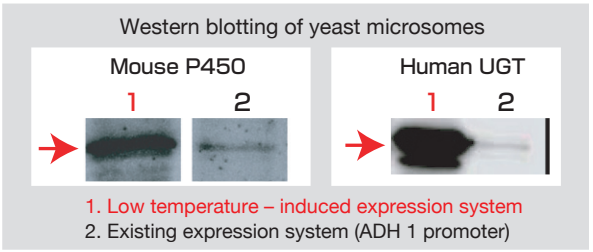


Fig. 4 : Comparison of existing expression systems and low temperature-induced expression system. Drug metabolizing enzymes produced in the endoplasmic reticulum were indicated by arrows.

using *E. coli*. Although *E. coli* ceases to grow at 15°C, yeast can grow at 10°C or at even lower temperatures of 4°C. Given the facts, we thought that we could overcome the problems encountered in the previous yeast expression systems by designing a system using yeast's tolerance to low temperatures and genomic information.

To achieve this goal, we first identified genes that are induced at low temperatures (i.e., possesses a promoter that is induced at low temperatures) through an experiment using yeast cDNA microarray, and found that a battery of translational machinery components is induced at low temperatures (Fig. 1). Through this analysis, yeast expression system by the low temperature-induced promoter was found to be more efficient than the existing yeast expression systems at moderate temperatures in various stages of protein synthesis (Fig. 2). These results suggest that low-temperature expression system using yeast could be used for effective production of proteins.

Development and Use of Low-temperature Expression System in Yeast

In an experiment using enhanced green fluorescent protein (EGFP) as a model protein, we demonstrated that our low-temperature expression system in yeast was far more efficient in producing the target protein than the yeast expression systems already on the market (Fig. 3). Through an analysis of the band distribution of SDS-PAGE, we found that EGFP accounted for up to 50% of the total amount of soluble proteins in yeast cells. This high expression efficiency was also found when mouse cytochrome P450 and human UDP-glucuronosyltransferase (UGT) were used as model proteins (Fig. 4). Because these drug metabolizing enzymes are targeted to the endoplasmic reticulum (ER), one must alter the N-terminal amino acid sequences to produce them in organisms that does not possess ER (i.e., *E. coli*). However, in our expression system using yeast, proteins can be expressed without such modification of these cDNAs.

A distinct characteristic of this expression system is that it does not require a chemical inducer. In the system we have developed, simply lowering the culture temperature can induce expression in yeast. This feature enables us to automate a protein expression apparatus (Fig. 5).

Perspectives of Low-temperature Expression System Using Yeast

The use of low temperature-induced expression system would be favorable when target proteins tend to form insoluble aggregates in *E. coli*-based expression systems or when expression levels of target proteins are not enough in the existing yeast expression systems. To date, we have produced approximately 40 different proteins (mainly human proteins) using our system, and the success rate of producing these proteins as a soluble form was quite high, while most of these proteins formed insoluble aggregates or could not be produced in the *E. coli* expression system. We have also succeeded in crystallization of a protein produced in our expression system. Today, this technology has been licensed to a biotechnology company, and services for outsourcing protein expression has started.

We are currently using various approaches to improve our low temperature expression system in order to produce various proteins in greater quantities. We believe this low temperature expression system will allow us to obtain proteins that have been impossible to produce using the existing *E. coli* and yeast expression systems. We hope that this technology will contribute to the expanding field of structural biology, and to the functional analyses of unknown proteins, which can be applied to new drug development. We also hope that this technology will be applied to various industrial production.

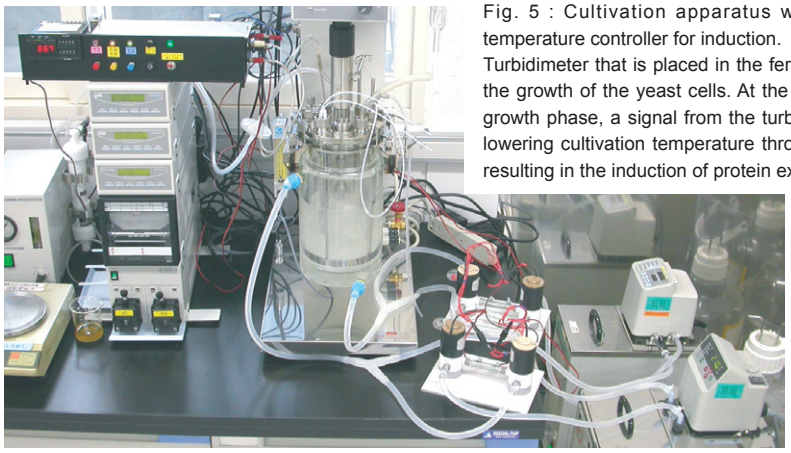


Fig. 5 : Cultivation apparatus with automated temperature controller for induction.
Turbidimeter that is placed in the fermenter monitors the growth of the yeast cells. At the mid-exponential growth phase, a signal from the turbidmeter initiates lowering cultivation temperature through a controller resulting in the induction of protein expression.

Using Actinomycetes to Create Beneficial Proteins

Tomohiro Tamura

Group Leader, Protelysis and Protein Turnover Research Group, Research Institute of Genome-based Biofactory

Development of a New Protein Expression System

As we continue to collect genome information of various species, the production technology of recombinant proteins is likely to become more and more important. Today, various expression systems are developed using variety of organisms as hosts (e.g., *Escherichia coli*, yeast, insects, plants). Despite the recent developments, not all proteins can be synthesized in these expression systems, which calls for a need to diversify the expression systems.

In the existing expression systems, production temperatures and host genome GC content is limited to values within a certain range. We therefore decided to test an actinomycete, *Rhodococcus erythropolis*, whose production temperatures and GC content lies outside of this "usual" range, as a host for our expression system (*Rhodococcus* can reproduce at temperatures between 4°C and 35°C and has a high GC content). Species in the genus *Rhodococcus* has considerable tolerance to organic solvents, and also has a strong *in vivo* catalytic activity converting aliphatic series, aromatic series and heterocyclic compounds. These characteristics make *Rhodococcus* one of the ideal candidates as "next generation host", and the development of host-vector system using this genus has already begun. By using this species as host, we can provide a new production environment that is different from the existing expression systems. The aim of our research group is to develop a new expression system for proteins that were difficult to produce with the existing systems.

Production of Proteins That Inhibit Cell Reproduction

The production temperatures of recombinant proteins depend on the growth and the reproductive temperatures of the hosts. For example, in order to produce functional proteins that inhibit reproduction of host cells, it is best to produce them when the functional activity (inhibitory effects) of the proteins is low. Although this can be achieved by lowering production temperature, it is very difficult to lower the temperature below 10°C in the existing expression systems. In these systems, high functional activity of the host cells has to be maintained throughout the production of the proteins. In contrast, using the developed expression system, we were able to produce this type of proteins (e.g., DNase I) at temperatures that are usually used for storage (4°C).

Protein production at low temperatures not only suppresses the functional activity of the proteins (enzymes), but also prevents the proteins to become insoluble (prevents the formation of inclusion body). Proteins become insoluble regardless of its type, but several factors can cause proteins to become insoluble.

1. High protein production rate; concentration of proteins become too high at some localities within the cell.
2. Differences in intracellular reduction-oxidation (redox) environment.
3. Lack of protein modification after translation.
4. Inappropriate interactions with molecular chaperones and/or foldase.
5. Nonspecific disulfide binding.

The first factor described above can be avoided by producing proteins at low temperatures, as production rate of proteins slows down at low temperatures. Use of *Rhodococcus* is expected to affect factors 1 and 2. Codon usage frequency and the intracellular environment in *Rhodococcus* change the production rate of proteins, and the intracellular redox environment is expected to be different from that of *E. coli*. These characteristics of *Rhodococcus* may be the reason why the expression system

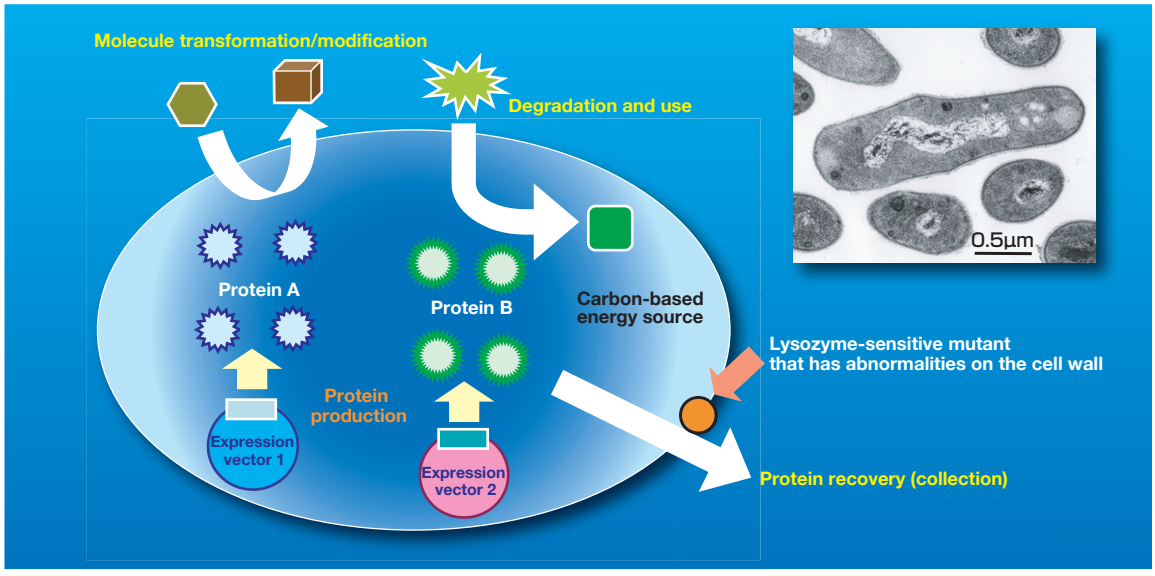
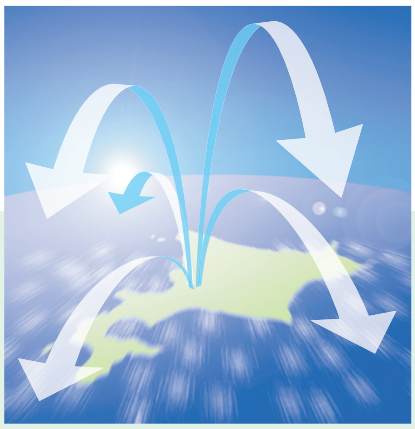


Figure : The development of bio-factory using actinomycetes.



Together Towards Our Goals and Dreams! Hokkaido's Bioindustry is Evolving Rapidly.

Takutomo Samukawa
METI Hokkaido, Economic Policy Department, Bio-industry Division

In a special feature that appeared in the journal Nikkei Bio Business (December 2004 Issue), Hokkaido was ranked second in the overall standings in a comparison of 36 biotechnology-clusters nationwide. In 2002, Hokkaido was named by the government's "Biotechnology Strategy Guidelines" as one of the three regions where biotechnology-cluster formation will be promoted. Since then, we have been engaged in a relentless effort to develop this cluster site. It is very satisfying to see our accomplishments and the reputations we have gained, particularly given that we had started from "scratch" three years ago.

At our organization, we have strived to get Hokkaido out of the economical downturn by promoting biotechnology-related industry, which is a sector that has bright prospects for the future. We hope to challenge the world in this biotechnology industry by taking advantage of our cool-dry climate, tradition that dates back to the times of Sapporo Agricultural College, and the nation' s largest supply of agricultural and fisheries resources.

Hokkaido's biotechnology industry is developing in a way where players and supporters of the industry are working together. This partnership has helped startup over 30 university venture businesses and has contributed to maintaining annual growth rate of over 10% for the past 5 years. This success has led to large contracts, increased infusion of investment capital, and development of new products. Our success has been recognized worldwide, which is symbolized by the numerous business awards we have received inside and outside of Japan.

C7 (collaboration 7) Hokkaido, which is one of the "supporters" in the development of biotechnology-related industries in Hokkaido, conducts active focused and intermediary support to the related 9 institutions at the recommendations of our organization. Among the related institutions is AIST, which has now become an important supporter and a player in the development of biotechnology industries in Hokkaido.

In this land of the north, there are many who are looking for opportunities to start up or expand venture businesses with the support of AIST. The Research Institute of Genome-based Biofactory, which takes advantage of the features of Hokkaido, is a valuable asset to the region and is something that we can be proud of as residents. We will conduct various collaborative projects with AIST to work towards commercialization of new biotechnology.

With the support of AIST Hokkaido and Research Institute of Genome-based Biofactory, we hope the seeds of the biotechnology industry that we have sown in this great land of the north will bear fruit someday and provide benefits to the world and to mankind. We thank you for your continued understanding and support, and together we shall move towards a brighter future!

using *Rhodococcus* shows different protein production capabilities from those of the existing systems.

Using *Rhodococcus* as a Biofactory

Expression system using *Rhodococcus* has succeeded in producing substantial quantities of proteins with a high recovery rate. This has been made possible by developing variety of expression vectors and by modifying the function of the host cell (e.g., lysozyme-sensitive mutant). The development of this technology has also proved that it is possible to create highly functional cells by adding new functional features to *Rhodococcus*.

Although I will not go into details, it has been confirmed that proteins can be produced under the existence of organic solvents in this expression system, which was a difficult task in the *E. coli* systems. Additionally we have succeeded in creating cells that have enhanced capability to degrade slowly biodegrading aromatic compounds. Therefore, this system can be used and applied to bioprocessing, which has its foundations on fermentation technologies.

There are many species in actinomycetes that can produce antibiotics. By introducing a gene cluster of antibiotic drug synthesis system to our expression system, we may be able to create cells that can produce mass quantities of antibiotic drugs. Additionally, depending on the proteins that are expressed, production of various compounds such as pharmaceutical intermediates may be possible. Thus, the creation of biofactory based on *Rhodococcus* may become a reality in the future not so far away.

Genome GC contents: Gene information encoded in the genome consists of four bases (A: dATP, C: dCTP, G: dGTP, T: dTTP), and the composition of these bases differs by species. Species-specific base composition is reflected in the codon usage frequency of the genes that encodes the proteins. Production efficiency of proteins may decrease considerably if proteins that are derived from genes with a high AT content are expressed in an organism that has a high genome GC content.

Novel Technology for Production and Application of Biomolecules

Establishment of Research Institute of Genome-based Biofactory

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