ISSN 0077-4995

NATIONAL INSTITUTE OF GENETICS

JAPAN

ANNUAL REPORT

No. 37 1986

Published by
THE NATIONAL INSTITUTE OF GENETICS
Misima, Sizuoka-ken, Japan
1987

Annual Report of the National Institute of Genetics

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CONTENTS

GENERAL STATEMENT

This report gives an outline of research activities carried out in our institute during the year 1986. The total expenditure for the FY 1986 (from April 1986 to March 1987) amounted to 1 billion 76 million yen, 48% of which was for personnel expenses. In addition, grants-in-aid amounting to 142 million yen were rendered to selected staff members by the Ministry of Education, Science and Culture. Changes in personnel allotments included new positions for three members approved in April; a research staff member (*joshu*) for the DNA Research Center, and a technical and an administrative official for the Department of Administration. However, in accordance with the 6th curtailment plan of the government, positions for two members were cut, so the total number of positions available for regular staff is 93 including 54 for research staff. Concerning facilities, construction work for the DNA Research Center started in July and the 80 per cent of the building was completed by the end of the year; a greenhouse for plant quarantine and three automatic short-day paddy fields equipped with dark chambers were also completed.

I am happy to note that six of our colleagues were honored in the past year. Prof. Akira Ishihama was awarded the Inoue Prize by the Inoue Foundation for the Promotion of Science for his "Studies on the transcriptional control of genes." Prof. Motoo Kimura was conferred an Honorary Doctor of Science degree, from his alma mater, the University of Wisconsin, for his world-famous contributions to population genetics. Prof. Tomoko Ohta won the Avon Women's Grand Prix for her contributions to the promotion of women's social activities through her outstanding achievement in population genetics which serves as encouragment for younger women to find career in science; Dr. Naoyuki Takahata was awarded an Encouragement Award of the Genetics Society of Japan for his "Theoretical studies on population genetics on the molecular level." Prof. Yukinori Hirota won the Fujiwara Award from the Fujiwara Science Foundation for his "Studies on the genes involved in bacterial cell division, and the foundation of a mutant bank of *Escherichia coli*." Finally, Dr. Ei Matsunaga, Director, was awarded a Purple Ribbon Medal in the Emperor's autumn list of national awardees for his contributions to genetic and epidemiologic studies

on congenital anomalies and for his leadership in promoting human genetics in this country.

We mourn the sudden loss of our two colleagues in the past year. Prof. Tsuneo Kada died from esophageal cancer on November 14 at the age of 59, and Prof. Y. Hirota from pneumonia following a stroke on December 23 at the age of 56.

While working in the institute for 19 years, Kada set up the research system for the Laboratory of Mutagenesis and carried out a great deal of pioneering research in his field. Among other things he developed and propagated a simple new method (Rec assay), using repair-deficient mutants of *Bacillus subtilis*, to detect various environmental mutagens such as AF-2 and phloxine which could not be detected by the conventional Ames test. Both AF-2 and phloxine had been used widely as food additives at one time in this country. Kada proposed the classification of antimutagenic substances into "desmutagens" and "bio-antimutagens"; desmutagens refer to those substances which inactivate mutagens outside the cells, while bio-antimutagens relate to factors that inhibit the process of mutation induction within cells. In so doing, he identified desmutagenic factors in various fresh fruits and vegetables and bio-antimutagenic activities in $CoCl_2$ and green tea extracts.

Joining us at his post in 1973 from the Pasteur Institute, Hirota started the K-project, aiming at elucidating all the genes of *E. coli* K-12, by picking up every possible nitrosoguanidine-induced temperature-sensitive mutant. He isolated thousands of independent mutants and characterized a number of genes essential to cell division such as *fts I*. As soon as the recombinant DNA technique was made available, Hirota cloned and determined the sequence of replication origin in *E. coli*. In recent years he initiated a joint study to research all the cellular functions more comprehensively from the view point of cell division. He also had been a key person in the project of nitrogen fixation in the rice rhizosphere.

With respect to personnel changes in the staff, Dr. Jun Kusuda of the Invertebrate Section of the Genetic Stock Research Center, who had been carrying out comparative studies on the molecular structure of the fibroin gene in wild and domesticated silkworms, left in July for the National Institute of Health as a chief researcher to set up a human gene bank there. Dr. Takashi Imamura of the Kyushu University was appointed as professor for the Laboratory of Human Genetics in April, followed by the appoint-

ment of Dr. Hitoshi Nakashima in May as a research staff member in the same laboratory. Dr. Susumu Hirose, Associate professor, the National Institute of Basic Biology, was transferred in June to the newly established DNA Synthesis Section of the DNA Research Center. Dr. Toshihiko Shiroishi, a research staff member, was transferred in July to the Laboratory of Cytogenetics from the Mammalian Section of the Genetic Stock Research Center, his position being filled by Dr. Nobumoto Miyashita, a graduate student of Kanazawa University. Dr. Naoyuki Takahata, Laboratory of Population Genetics, and Dr. Toshitaka Fujisawa, Laboratory of Developmental Genetics, were both promoted to associate professors in August. It is expected that the laboratories with newly appointed staff will resume research activities as promptly as possible.

A working budget for a DNA data bank, which should be available for joint use by researchers all over the country, was appropriated for the first time. Anticipating that the building for the DNA Research Center will be completed by the end of January 1987, the budget for upgrading the electronic computer was also appropriated. As in the previous year, Prof. Takeo Maruyama, Dr. Sanzo Miyazawa and Dr. Takashi Gojobori continued to take charge of the construction and distribution of the DNA data base, the development of analytical programs, and the issue of the DDBJ (DNA Data Bank of Japan) Newsletter (No. 5). Laborious efforts were made on the input of data and for selecting the type of computer. It is expected that the project will get under way next spring with the appointment of a new research staff member.

The Genetic Stock Research Center supplies, as far as possible, upon request of competent researchers in and outside of Japan, specific strains of mouse, *Drosophila*, silkworm, *E. coli*, *B. subtilis*, etc. which are being kept in the Center. The total number of cases supplied in 1985 amounted to 241 (29 sent abroad) and the total number of strains supplied was 4723 (258 sent abroad). Dr. Shin-ya Iyama continued to systematize data-base information about a variety of experimental organisms preserved in universities and research institutes in Japan; in cooperation with other staff, he compiled the "Catalogue of *Escherichia coli* Genetic Stocks in National Institute of Genetics, 1986", updated the "*Drosophila* Genetics Stock List in Japan", and issued "Rice Genetics Newsletter No. 2". These materials were distributed among interested scientists in and outside of Japan.

On April 19th, the institute held its yearly open house for the public.

Some of the research activities of each laboratory were exhibited using panels, microscopes, microcomputers, and molecular models. Scientific films were shown and a lecture, "How did ants become ants?", was given by Dr. Hirotami T. Imai. The double cherry blossoms on the campus were at their best and some 1300 visitors in the neighborhood enjoyed them. On November 1, public lectures were given at the National Science Museum in Tokyo; the titles were "Analysis of molecular mechanisms of cell proliferation in bacteria" by Dr. Seiichi Yasuda, and "Theoretical bases for chromosome evolution" by Dr. H. T. Imai. In spite of it being a Saturday afternoon about 150 eager people from universities and institutes listened to the lectures, which were followed by rather specialized questions and lively discussions. In addition, the 30th meeting of the directors of biomedical research institutes, which are under the jurisdiction of the Ministry of Education, Science and Culture, or attached to national universities, took place in Mishima on October 27–28. Twenty-seven institutes participated and discussed various problems in advancing research activities under the present severe state of administrative and financial constraints.

International personnel exchanges were very active in the past year. Members of our staff went abroad on 33 occasions for the purpose of presenting research results at various scientific meetings, exchanging information, and carrying out collaborative studies or field investigations, three members staying longer than 3 months. On the other hand, 41 scientists visited our institute from abroad, with whom information and views on recent studies were actively exchanged. Some distinguished guests, including Dr. Susumu Ohno of the City of Hope, delivered stimulating lectures at the Biological Symposium. Those who stayed longer than 3 months and carried out collaborative studies with our staff members were: Dr. Pierre Boursot, Université Montpellier II, Miss Pascale Barbier, Université des Sciences et Techniques du Languedoc, Montpellier, France; Dr. Yi-de Huang, Shanghai Institute of Plant Physiology, the Chinese Academy of Sciences, and Dr. Yuan sheng Qiu, Guangdong Microbiology Research Institute, the People's Republic of China; Dr. Lee Won Ho, Pusan National University, Korea; Dr. Josefa Styrna, Jagiellonian University, Poland; Dr. Tjan Kiauw Nio, Bandung Institute of Technology, Indonesia; and Dr. Nguyen Xuan Hong, Hanoi University, Vietnam.

Three years have passed since the administrative category of our institute was switched over to one for joint use by universities. Under the new sys-

tem, we have accepted upon application, 33 collaborative programs, 13 workshops, 7 graduate students, 8 research fellows and 6 scholarships from industries, and 1 entrusted research from a private corporation. Yet, there remain a number of problems to be solved in order to achieve the reality of reform. Among others, construction of the following facilities is of the highest priority; an RI Center, which is indispensable for the expansion of research activities on the molecular level; a second main building to accommodate laboratories for joint use by visiting professors, and lodgings for visiting researchers with welfare facilities. We are all eager to do our best to accomplish the missions of the new institute. We hope to have continued encouragement and support from all persons concerned.

E. Matsunaga

STAFF

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   FUJITA, Nobuyuki, D. Sc.
   NAGATA, Kyosuke, D. Pharm.
   Laboratory of Mutagenesis
   SADAIE, Yoshito, D. Sc., Associate professor
   INOUE, Tadashi, D. Ag.
   TEZUKA, Hideo
   Laboratory of Nucleic Acid Chemistry (*Guest member*)
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Laboratory of Agricultural Genetics

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IYAMA, Shin-ya, D. Ag., Associate professor
MIYASHITA, Nobumoto, D. Med.
INOUE, Yutaka, D. Sc.
SANO, Yoshio, D. Ag.
NISHIMURA, Akiko

DNA Research Center

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IKEMURA, Toshimichi, D. Sc., Associate professor
MIYAZAWA, Sanzo, D. Sc., Associate professor
HIROSE, Susumu, D. Sc., Associate professor

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MIYAZAWA, Akira

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WADA, Bungo; Manager, Emeritus professor of University of Tokyo

TAZIMA, Yataro, Manager

OSHIMA, Chozo; Manager

MATSUNAGA, Ei; Manager, Director of National Institute of Genetics

PROJECTS OF RESEARCH FOR 1986

1. DEPARTMENT OF MOLECULAR GENETICS

Laboratory of Molecular genetics

Studies on regulatory mechanisms of gene expression in *E. coli* (ISHIHAMA, FUKUDA and FUJITA)

Studies on molecular mechanisms of transcription and replication of animal viruses (ISHIHAMA, FUKUDA and NAGATA)

Laboratory of Mutagenesis

Molecular mechanisms of radiation- and chemical-induced mutations (KADA, SADAIE, INOUE and TEZUKA)

Environmental mutagens, desmutagens and antimutagens (KADA)

Biochemical factors involved in cellular repair of genetic damage and induced mutagenesis (INOUE and KADA)

Genetics of *Bacillus subtilis* (SADAIE and KADA)

Molecular mechanisms of unicellular differentiation in *Bacillus subtilis* (SADAIE)

Laboratory of Nucleic Acid Chemisry

Studies on the relationship between nucleic acid conformations and biological activities (MIURA)

Expression of *B. subtilis* genes (YAMANE)

Expression of the foreign genes in *B. subtilis* cell (YAMANE)

2. DEPARTMENT OF CELL GENETICS

Laboratory of Cytogenetics

Studies on species differentiation of mouse from cyto- and molecular genetic view points (MORIWAKI)

Cyto- and immunogenetical mechanisms for regulating tumor development in the laboratory and wild mice (MIYASHITA* and MORIWAKI)

* Genetic Stock Research Center

Immunogenetical studies on the mouse MHC (SHIROISHI and MORIWAKI)
Development of new mouse strains from wild populations (MORIWAKI and SHIROISHI)
Theoretical bases for chromosomal evolution in mammals and ants (IMAI)
Cytogenetical analysis of meiotic mechanisms in mice (IMAI and MORIWAKI)
Cytogenetical studies on Drosophila (YAMAMOTO)

Laboratory of Microbial Genetics

DNA replication in *E. coli* (YASUDA and HIROTA)
Cellular division in *E. coli* (HIROTA, NISHIMURA and HARA)
Penicillin-binding proteins in *E. coli* (HIROTA, HARA and NISHIMURA)

Laboratory of Cytoplasmic Genetics

Peptidoglycan biosynthesis in *E. coli* (SUZUKI and HIROTA)
Studies on cytoplasmic genes during subspecies differentiation of house mouse *Mus musculus* (YONEKAWA)

3. DEPARTMENT OF ONTOGENETICS

Laboratory of Developmental Genetics

Genetic analysis of developmental mechanisms in hydra (SUGIYAMA, FUJISAWA and SHIMIZU)
Studies on transformation and cell differentiation in higher organisms (NAWA and YAMADA)
Mitochondrial DNA organization in male-sterile cytoplasms of rice (NAWA, SANO and FUJII)

Laboratory of Phenogenetics

Genetic studies on insect cells in tissue culture (KURODA and MINATO)
Developmental genetic studies on animal cells in tissue culture (KURODA)
Genetics of somatic mammalian cells in culture (KURODA)
Genetic studies on abnormal fertilization in the silkworm (MURAKAMI)
Mutagenesis in germ cells of the silkworm (MURAKAMI)
Studies on the male-specific killing activity of SRO in *Drosophila* (YAMADA)

Laboratory of Physiological Genetics

Electron microscopic studies on differentiation of animal cells (SHIMADA and KURODA)
Nerve network formation in *Hydra* (KIJIMA)
Genetic analysis of nervous system development in hydra (KIJIMA and SUGIYAMA)

4. DEPARTMENT OF POPULATION GENETICS

Laboratory of Population Genetics

Theoretical studies of population genetics (KIMURA, OHTA, TAKAHATA and AOKI)
Studies on molecular evolution from the standpoint of population genetics (KIMURA, TAKAHATA and OHTA)
Theoretical studies on the evolution of multigene family (OHTA)
Theoretical studies on variation and evolution of extranuclear DNA (TAKAHATA)
Theoretical studies on the evolution of altruism (KIMURA and AOKI)
Population genetical studies on gene-culture coevolution (AOKI)

Laboratory of Evolutionary Genetics

Theory of population genetics and evolution (MARUYAMA)
Studies on molecular evolution (GOJOBORI)
Evolutionary genetics of *Drosophila* (WATANABE)
Radiation genetics in mice (TUTIKAWA)

Laboratory of Theoretical Genetics

Theoretical and experimental studies of transposons in population of Drosophila (MUKAI)
Computer studies on the molecular evolution (MIYATA)

5. DEPARTMENT OF INTEGRATED GENETICS

Laboratory of Human Genetics

Molecular genetics of growth and differentiation of human blood forming cells (IMAMURA and NAKASHIMA)
Studies on DNA polymorphisms in human populations (HORAI and MATSUNAGA)
Genetic studies on retinoblastoma (MATSUNAGA and HORAI)
Mitochondrial DNA restriction analysis of non-human primates (HORAI, MATSUNAGA, HAYASAKA, SHOTAKE and NOZAWA)

Laboratory of Agricultural Genetics

Evolutionary studies in wild and cultivated rice species (MORISHIMA and SATO)
Ecological genetic studies in weed species (MORISHIMA)
Genetic studies on rice reserve proteins (ENDO)
Behavioral genetic studies in animals (FUJISHIMA)

Laboratory of Applied Genetics

Methodology for the conservation of genetic resource populations (YONEZAWA)

6. RESEARCH FACILITIES

Genetic Stock Research Center

Studies and conservation of germplasm resources in rice and wheat species (FUJII and SANO)
Specificity of mutagen tolerance in higher plants (FUJII)
Exploitation of genetic ability of nitrogen fixation in Gramineae (FUJII, SANO and IYAMA)
Studies on genetic differentiation in rice (SANO)
Theoretical studies on breeding techniques (IYAMA)
Genetic studies of trees in natural forest (IYAMA)
Studies on gene transfer in Gramineae (SANO and FUJII)

Documentation of genetic stocks in Japan (IYAMA)
Studies on the management system of genetic stocks information (IYAMA)
Studies on chromosomal polymorphism in *Drosophila* (INOUE)
Analysis of fibroin genes of silkworm and its relatives (KUSUDA)
Molecular studies on the origin of silkworm (KUSUDA)
Coordination of DNA synthesis and cell division in *E. coli* (NISHIMURA)
Synthetic ColEl plasmids carrying genes for cell division in *E. coli* (NISHIMURA)
Development of mouse embryo freezing system (SHIROISHI, MIYASHITA and MORIWAKI)
Molecular mechanism of high frequency recombination in mouse MHC region (SHIROISHI and MORIWAKI)
Genetic mechanisms for regulating tumor development in the laboratory and wild mice (MIYASHITA and MORIWAKI)

DNA Research Center

Regulatory mechanisms of gene transcription (ISHIHAMA)
DNA data analysis (MARUYAMA)
Studies on codon usage (IKEMURA)
Studies on RNA molecules of mouse brain (IKEMURA)
Database design for DNA base sequences (MIYAZAWA)
Control of gene expression in eukaryotes (HIROSE)

RESEARCH ACTIVITIES IN 1986

I. MOLECULAR GENETICS

The Anatomy of *Escherichia coli* RNA Polymerase: Mapping of Structural Domains Involved in Subunit-Subunit Interaction

Akira ISHIHAMA, Robert E. GLASS*, Nobuyuki FUJITA and Teruaki NOMURA

RNA polymerase of *E. coli* is a multimeric enzyme consisting of a core enzyme with a subunit composition of $\alpha_2\beta\beta'$ and one of the sigma factors. The holoenzyme is assembled in a step wise fashion under a defined pathway: $\alpha+\alpha\rightarrow\alpha_2\rightarrow\alpha_2\beta\rightarrow\alpha_2\beta\beta'\rightarrow\alpha_2\beta\beta'\sigma$. Subunit-subunit interaction is absolutely required for the expression of intrinsic activities associated with RNA polymerase. An understanding of protein-protein interaction is therefore of paramount importance to the structure-function characteristics of this complex enzyme.

To detect RNA polymerase subunits and assembly intermediates in whole cell lysates, we empoyed a combination of immunoprecipitation of radiolabeled cell lysates against anti-subunit antibodies and polyacrylamide gel electrophoresis of immunoprecipitates. Using this method, we detected not only intact subunits but also various degradation fragments of RNA polymerase in mutants defective in the assembly of RNA polymerase (Ishihama, A. *et al.* (1987) *Proteins*, in press). The subunit fragments detected differed among the assembly-defective mutants. By analyzing the degradation patterns, we were able to estimate the intermediate species of RNA polymerase assembly accumulated in those mutant cells. In addition, we found that some of the intact subunits were degraded during the stationary-phase of cell growth, presumably due to conformational change of preexisting RNA polymerase as well as to inhibition of the assembly of newly synthesized subunits.

* Department of Biochemistry, Queen's Medical Centre, Nottingham, England.

In order to detect unassembled subunits and assembly intermediates in unlabeled cell lysates, we further developed a rapid and sensitive method using the same collection of anti-subunit antibodies. In brief, whole cell lysates of unlabeled cells are fractionated by glycerol gradient centrifugation under non-denaturing conditions; glycerol fractions are separated on polyacrylamide gel electrophoresis under denaturing conditions; proteins are transferred onto nitrocellulose filters; and, finally, filters are immunoblotted against a series of different anti-subunit antibodies (Ishihama, A. *et al.* (1987) *Proteins*, in press; Fujita, N. *et al.* (1987) *Mol. Gen. Genet.*, in press; Glass, R. E. *et al.*, submitted for publication). The same filters can be reused to immunostain consecutively against a series of different antibodies. Our procedure for studying protein-protein interaction can be generally applied to a variety of studies.

Using this method, we found several pseudo-complexes containing degradation fragments of RNA polymerase. Analysis of these complexes permits the identification of the sites (or domains) involved in subunit-subunit interaction. We then performed a systematic study to identify structural domains on the β subunit involved in subunit-subunit interaction and analyzed the assembly of eleven N-terminal amber fragments of the β subunit ranging in size from 97 to 23% of the length of the inteact β polypeptide (1, 342 amino acids) (Glass, R. E. *et al.*, submitted for publication). In this way, we were able to define regions on the β subunit in binding α, β' and σ.

This method was also successfuly employed for the identification of the heat-shock sigma factor, σ^{32}, and other sigma-related proteins in whole cell lysates (Fujita, N. *et al.* (1987), *Mol. Gen. Genet.*, in press).

RNA Polymerase Sigma-Related Proteins in *Escherichia coli*, I. Detection by Antibodies against a Synthetic Peptide

Nobuyuki FUJITA, Akira ISHIHAMA, Yoji NAGASAWA* and Susumu UEDA*

E. coli RNA polymerase is a complex enzyme consisting of the common core enzyme subunits (α, β and β') and one of the sigma factors. Each sigma factor confers on the core enzyme an ability to recognize a specific group of promoters. So far at least three sigma factors have been identified,

* Nippon Institute for Biological Science, Ohme, Tokyo.

which are designated as σ^{70}, σ^{60} and σ^{32} on the basis of respective molecular weight. The major sigma factor (σ^{70}) is encoded by the *rpoD* gene and the holoenzyme containing σ^{70} recognizes the majority of *E. coli* promoters. The minor species, σ^{32}, is encoded by the *rpoH* (*htpR*) gene and the holoenzyme ($E\sigma^{32}$) recognizes a small group of promoters, known as the promoters for heat-shock genes. Previously, we purified two forms of the holoenzyme, $E\sigma^{70}$ and $E\sigma^{32}$, and compared their promoter selectivities in the *in vitro* mixed transcription system using our collection of *E. coli* promoters (Fujita, N. *et al.* (1987), *J. Biol. Chem.*, 262, 1855–1859). These studies showed that a strict difference exists between the two holoenzymes with respect to promoter selectivity.

In order to detect other sigma-related proteins in *E. coli*, we prepared an antiserum against a synthetic tetradecameric peptide with a sequence, DLIQEGNIGLMKAV, which is present in both σ^{70} and σ^{32}. In a Western-blot analysis of whole cell lysates, the antiserum reacted specifically with σ^{70}, σ^{32} and, additionaly, with at least three proteins with apparent molecular weights of 75, 27 and 23 kilodaltons. Major portions of σ^{70} and σ^{32} were recovered as associated forms with RNA polymerase, by glycerol gradient centrifugation of whole cell lysates while the other three cross-reacting proteins were not associated with RNA polymerase at least under the conditions empolyed. Purification and characterization of these proteins are in progress.

RNA Polymerase Sigma-Related Proteins in *Escherichia coli*, II. Heat-Shock Induction of Sigma-32 Synthesis

Nobuyuki FUJITA and Akira ISHIHAMA

The induction of heat-shock genes in *Escherichia coli* is under positive control of the *rpoH* (*htpR*) gene, which encodes σ^{32}, one of the sigma subunits of RNA polymerase. Purified holoenzyme containing σ^{32} ($E\sigma^{32}$) is capable of transcribing heat-shock genes *in vitro* while $E\sigma^{70}$ is virtually inactive (Fujita, N. *et al.* (1987) *J. Biol. Chem.*, 262, 1955–1859). Likewise, the genes transcribed by $E\sigma^{70}$ can not be transcribed by $E\sigma^{32}$.

A Western-blotting analysis using an antiserum raised against a tetradecameric peptide with the consensus sigma sequence revealed that the intracellular level of σ^{32} increases rapidly under heat shock (Fujita, N. *et al.* (1987),

Mol. Gen. Genet., in press). To reveal the induction mechanism of σ^{32} synthesis, we analyzed the transcription of the *rpoH* gene before and after heat-shock treatment.

S1 nuclease mapping and reverse transcriptase mapping indicated that the transcription of the *rpoH* gene is initiated, both *in vivo* and *in vitro*, from two major (P1 and P2) and one minor (P2*) promoters (Fujita, N. *et al.* (1987), *Mol. Gen. Genet.*, in press). The level of *rpoH* transcript from the downstream P2 promoter increases rapidly when *E. coli* cells are transferred from 30 to 42°C, while the transcript from the upstream P1 promoter remains at a constant level. Under these conditions, the metabolic stabilities of *rpoH* mRNAs are virtually unaffected. These observations indicate that heat-shock induction of σ^{32} synthesis is due to an increased transcription of the *rpoH* gene.

In vitro synthesis of *rpoH* mRNA was found to be absolutely dependent on the major species of RNA polymerase holoenzyme containing σ^{70} but not on the minor one ($E\sigma^{32}$). The mechanism of $E\sigma^{70}$-dependent transcription enhancement of the *rpoH* gene by heat shock is being currently analyzed.

Stringent Control Signals of *Escherichia coli*: Determination of Signal Strength in *in vitro* Transcription

Robert E. GLASS*, Akira ISHIHAMA, Teruaki NOMURA and Nobuyuki FUJITA

Several global control circuits responsible for regulating groups of genes are present in *Escherichia coli*. Stringent control is clearly part of an adaptive response to a short supply of substrates for translation. The presence of uncharged tRNAs in the ribosomal decoding site leads to the synthesis of two unusual nucleotides, ppGpp and pppGpp, which are the effectors of stringent control. Transcription *in vitro* of stringently controlled genes by purified RNA polymerase is subject to repression by ppGpp (Kajitani, K. and Ishihama, A. (1985) *J. Biol. Chem.*, 159, 1951–1957). Some mutations in the gene (*rpoB*) encoding the β subunit of RNA polymerase, render *E. coli* insensitive to amino acid starvation, *i.e.*, relaxed phenotype

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(Nene, V. and Glass, R. E. (1983), *FEBS Lett.*, 153, 307–310) and the mutants produce ppGpp-insensitive RNA polymerases (Glass, R. E. *et al.* (1986), *Mol. Gen. Genet.*, 203, 265–268). These observations indicate that the target for ppGpp is RNA polymerase. Among the stringently controlled genes, a GC-rich sequence known as 'discriminator' is conserved, spanning the region between the −10 signal and the transcription start site. It therefore appears that ppGpp-bound RNA polymerase recognizes this singnal and as a result is unable to initiate transcription.

This year, we measured the quantitative effect of ppGpp on various *E. coli* promoters with different discriminators, using both wild-type and relaxed RNA polymerases. This has allowed us to classify promoters based on their susceptibility to ppGpp. With the wild-type RNA polymerase, the hierarchy of susceptibility to ppGpp was: *rplJ*, *leuX*>*dnaQ*$_{P1}$, *dnaQ*$_{P2}$>*trp*, *alsS*>*lac*UV5; but, with the relaxed RNA polymerases, it was: *leuX*, *dnaQ*$_{P1}$ >*rplJ*, *dnaQ*$_{P1}$>*trp*, *alaS*>*lac*UV5 (Glass, R. E. *et al.* (1987) *Mol. Gen. Genet.*, in press). It was therefore concluded that the strength of the stringent control signal depends on the nature of the RNA polymerase as well as that of the promoter. It is interesting to note that the relaxed RNA polymerases are more sensitive to inhibition by ppGpp when measured with a promoter such as the *dnaQ*$_{P2}$ promoter which is not known to be susceptible to ppGpp.

Expression of the *leuX* Gene in *Escherichia coli*: Regulation at the Transcription and tRNA Processing Steps

Teruaki NOMURA and Akira ISHIHAMA

The *leuX* gene of *E. coli* confers the Su$^+$ phenotype and encodes a supressor, tRNA, which inserts leucine at the amber codon. By mapping with S1 nuclease and reverse transcriptase of the initiation sites for both *in vitro* and *in vivo* transcription, we found that this gene was organized into a single-gene operon carrying its own promoter and ρ-independent terminator (Nomura, T. *et al.* (1987), *J. Mol. Biol.*, in press). In spite of the accepted concept that in *E. coli* cells, primary transcripts of tRNA genes are rapidly processed into mature forms, we found that varying levels of the primary *leuX* transcript were accumulated in wild-type cells with respect to tRNA processing enzymes, but that the levels of mature tRNA stayed rather con-

stant under the culture conditions analyzed.

The quantitative measurement of both primary transcript and mature tRNA revealed that transcription of this gene was under both stringent and growth rate-dependent controls. These observations indicate that the expression of the *leuX* gene is regulated not only at the transcription step but also at the tRNA processing step.

Structure and Function of Genes for RNA Polymerase Binding Proteins. I. Regulation of Transcription for the SSP Gene

Hiroaki SERIZAWA and Ryuji FUKUDA

A set of transcription factors appear to participate in the control of transcription by interacting with RNA polymerase. Up to ten polypeptides which associate with RNA polymerase and may modulate the function of RNA polymerase during transcription have been identified in this laboratory. We isolated some of them, and have been studying their effects on transcription in the *in vitro* system. We have also been trying to isolate their respective genes to perform genetic analyses and study their physiological functions.

Stringent starvation protein (SSP) is one such polypeptide. In the previous issue, we reported on the structural analysis and genetic mapping of the SSP gene, as well as on the cloning of the gene (Fukuda *et al.*, *Mol. Gen. Genet*, **201**, 151, 1985). This year, we studied the regulation of the expression of the gene, and tried to identify its physiological functions.

As described previously, we have determined the 5′ end of the *in vivo* transcript of the SSP gene, which was identical for both transcripts prepared from cells grown under normal, or stringently starved conditions. However, the consensus promoter sequences could not be found at the −10 and −35 regions upstream from the transcription initiation site (Serizawa and Fukuda, *Nucleic Acids Research*, **15**, 1153, 1987). In order to identify promoter signals of the gene, deletion mapping of the 5′ flanking region was performed to test promoter activity. For this study, various fragments of that region were fused to *lacZ* genes cloned in a plasmid, and the activity of β-galactosidase was measured in the cells carrying the plasmids. As with ordinary promoters of *E. coli*, a fragment 40 bp upstream from the transcription initiation site was essentially required. In addition, the presence of an additional fragment 40 bp upstream from that essential region stimulated

the transcription seveal fold. This *cis*-acting region has AT clusters of 10 bp length, and the 3′ half of the region is enriched with pyrimidine residues. The DNA fragment containing this region exhibits abnormal mobility on polyacrylamide gel electrophoresis. This *cis*-acting region did not stimulate the *in vitro* transcription with purified RNA polymerase, suggesting a trans-acting factor(s) interacting with the region to enhance transcription.

Using purified RNA polymerase, we analysed the *in vitro* transcripts of the cloned SSP gene. We detected two kinds of transcripts, both of which were produced with RNA polymerase containing σ^{70}, but not with the enzyme containing σ^{32}. The 5′ ends of both *in vitro* products were extremely near to the site determined for the *in vivo* transcripts. The promoter activity *in vitro* of the gene is only 1/20 of that of the *lacUV5* promoter, and seemed to be very weak compared to the activity *in vivo*, also suggesting the presence of some transcription stimulation factor(s). We next investigated the effect of ppGpp on these *in vitro* promoters. One of them is inhibited by ppGpp, whereas the other is not. At present we are trying to determine which of the 5′ ends of the *in vitro* products is identical with that of the *in vivo* transcript.

Structure and Function of Genes for RNA Polymerase Binding Proteins. II. Examination of the Physiological Functions of SSP

Ryuji FUKUDA and Hiroaki SERIZAWA

To elucidate whether SSP is essential for cell growth or not, we tried to construct an *E. coli* strain in which SSP is not synthesized. As a preliminary step, we constructed an *E. coli* strain in which SSP is synthesized under the control of the *lac* promoter. In this strain, SSP is synthesized in the presence of IPTG, but not in the absence of the inducer, as shown by labeling the cellular proteins and detecting SSP, using the anti-SSP antibody. As such cells were able to grow normally in the absence of the inducer, it appears that SSP is dispensable for cell growth at least under normal culture conditions. We are now isolating another deletion mutant of the SSP gene, and studying the effect of SSP deletions on cell growth, when the cells are exposed to various stresses, such as amino acid strarvation or heat shock.

Molecular Mechanism of Transcription Initiation by Influenza Virus RNA Polymerase

Ayae HONDA, Kiyohisa MIZUMOTO*, Kyosuke NAGATA and Akira ISHIHAMA

The influenza virus genome consists of eight single-strand RNA segments of negative polarity. The RNA-dependent RNA polymerase associated with the RNP cores in virions is responsible for primary transcription of the viral genome into plus-strand mRNAs. The transcription initiation positions and the RNA polymerase-binding sites on viral RNAs were analyzed.

To determine the transcription initiation positions, we measured the priming activities of trinucleotide formation by dinucleotides of all possible base sequences (Honda, A. *et al.* (1986), *J. Biol. Chem.*, **261**, 5987–5991). Dinucleotide ApG, complementary to positions 1–2 from the 3′ termini of viral RNA segments, was found to be most active primer. Dinucleotide GpC, complementary over position 2–3, was also an active primer and directed the formation of either GpCpG or GpCpA. However, both dinucleotide CpG and CpU, complementary over position 3–4, were virtually inactive. These results indicate that transcription is initiated within the first four nucleotides at the 3′ termini of viral RNAs.

To determine the binding sites of RNA polymerase on the genome RNA segments, the ribonucleoprotein (RNP) cores were isolated from detergent-treated virions by glycerol gradient centrifugation. Subsequently, RNA polymerase-RNA (P-RNA) complexes were isolated from RNP by centrifugation on a double gradient of cesium trifluoroacetate (CsTFA) and glycerol. The RNP cores and the P-RNA complexes were subjected to 3′-terminal labeling with [^{32}P]pCp and T4 RNA ligase, and then to foot-printing analysis using double-strand-specific RNase V1 and single-strand-specific RNase T2. The results indicate that the 3′ termini of viral RNAs form intramolecular base-pairs with the respective 5′ termini and that the RNA polymerase is associated with a section approximately 18 bases from the 3′ termini of viral RNA segments (Honda, A. *et al.* (1987), *J. Biochem.*, in press).

In further experiments, RNA polymerase was dissociated from viral RNAs by centrifugation on CsTFA or CsCl gradients. The results of preliminary reconstitution experiments supported the model that RNA polymerase re-

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cognizes and associates at the termini of viral RNAs.

Assignment of the Physiological Role of Influenza Virus NP Protein

Kenji UÉDA, Kyosuke NAGATA and Akira ISHIHAMA

Genetic studies have indicated that the three P proteins (PB1, PB2 and PA) and the NP protein of influenza virus are absolutely required for viral RNA synthesis. In purifying the viral RNA polymerase, we obtained the RNP complex (a complex of all these proteins and viral RNAs) and the P-RNA complex devoid of the NP protein (Kato *et al.* (1986), *Virus Res.*, **3**, 115–127; Honda *et al.* (1987) *J. Biochem.*, in press). The RNP complex produces full-sized RNAs but the P-RNA complex only supports the synthesis of oligonucleotides. Reconstituted complexes from isolated P proteins and viral RNAs were found to catalyze only the limited elongation reaction (Honda, A., unpublished observations). These observations suggest that the NP protein is required for efficient elongation of RNA chains but not for initiation of RNA synthesis.

To confirm this hypothesis and to identify the physiological role of the NP protein, we established a transfection assay system of ribonucleoprotein complexes. For this purpose, mixtures of various RNA-protein complexes and DEAE-dextran were transfected into cultured cells and possible production of infectious viruses was examined. Transfection of purified viral RNAs did not produce viruses. On the other hand, the RNP complex could produce infectious virus (Uéda, K. *et al.*, submitted for publication). Infectivity of the P-RNA complex and the reconsituted RNA-protein complexes is presently being tested.

An *in vitro* System for Transcription and Replication of Influenza Virus RNA

Kyosuke NAGATA, Kaoru TAKEUCHI* and Akira ISHIHAMA

We developed an *in vitro* system for transcription and replication of influenza virus RNA using isolated nuclei from influenza virus-infected HeLa cells (Takeuchi, K. *et al.* (1987), *J. Biochem.*, **101**, 837–845). In this system,

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two species of positive-sense RNA transcripts, *i.e.*, mRNA and cRNA (complete copy of vRNA) and one negative-sense RNA transcript, vRNA, are synthesized. We carried out a detailed analysis of the molecular mechanism of the synthesis of positive-sense RNA transcripts.

While optimizing the isolated nuclear system, it was found that in the presence of low concentrations of salt, both mRNA and cRNA were synthesized, while in the presence of high concentrations of salt, mRNA was a predominant product. Thus, we prepared a salt-extract of nuclei (nuclear salt-extract=NE) and salt-treated nuclei (ΔN). The NE fraction mainly produced cRNA while mRNA was a major product synthesized by ΔN. The NE fraction was further fractionated by centrifugation. The precipitate (NEP) exhibited mRNA synthesis activity but with the addition of the supernatant fraction (NES) cRNA was the main product. The NES fraction alone exhibited a low level of cRNA synthesis activity. These observations strongly suggest that a regulatory factor present in the NES fraction is involved in the switch from mRNA to cRNA synthesis. Attempts are being made to isolate and characterize the putative regulatory factor(s).

Molecular Cloning of cDNAs of Influenza Virus RNAs and their Expression in Mouse Cells

Kunitoshi YAMANAKA, Kyosuke NAGATA, Yasuhiro HOSAKA*
and Akira ISHIHAMA

Recent studies have revealed that cellular immunity plays a crucial role in protection against influenza virus infection. In order to identify protein(s) and epitopes which are recognized by cytotoxic T lymphocytes, we are trying to establish a collection of cell lines, a single species of viral proteins by means of transfection of a specific cDNA to influenza virus (RNAs).

Ifluenza virus RNA were extracted from purified virions by a SDS–phenol method and full-length cDNAs were synthesized using synthetic primers and reverse transcriptase. Double-strand cDNAs were joined to either an *EcoR*I or *Sma*I linker, and then inserted into the respective cloning site of the pSP65 vector. cDNAs encoding the M2 or NS2 proteins were also constructed from full-sized M and NS cDNA, respectively.

For efficient expression of cDNA in mouse cells, a bovine papilloma virus DNA was used as an expression vector. It was equipped with an

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eukaryotic transcription promoter (either mouse metalothionein I promoter or SV40 early region promoter) including splicing signals as well as a signal for poly(A) addition. Recombinants containing influenza viral cDNAs were tranfected into mouse cells and their expression is presently being examined using immunological methods.

In vitro Splicing of Influenza Viral mRNA

Kyosuke NAGATA and Akira ISHIHAMA

Segment 7 RNA of the influenza virus genome codes for at least two polypeptides, M1 and M2 which are translated from separate mRNA species. Segment 8 also codes for two non-structural polypeptides, NS1 and NS2, translated from separate mRNA species. M1 and NS1 mRNAs are unspliced primary transcripts of viral RNA segment 7 and 8, respectively, whereas M2 and NS2 mRNAs are generated from M1 and NS1 mRNAs after splicing. In influenza virus-infected cells, the ratio of spliced to unspliced mRNAs changes depending on the time after infection. To elucidate the molecular mechanism of splicing and its control, we attempted to develop an *in vitro* splicing system using nuclear extracts prepared from either influenza virus-infected or unifected human cells.

To prepare the substrate RNA for splicing, a segment 8-specific cDNA was synthesized by AMV reverse transcriptase, cloned into a pSP vector and transcribed *in vitro* by SP6 RNA polymerase. Nuclear extracts prepared from infected cells exhibited a low level of splicing activity and yileded specific splicing intermediates only when ATP was supplemented. Nuclear extracts from uninfected cells, however, did not exhibit this activity. These observations suggest that a factor(s) exists in influenza virus-infected cells which affects the level of splicing. Studies to identify such a factor(s) are in progress.

Analysis of Temperature-sensitive Mutants of Influenza Virus. I. Establishment of a System for Quantitative Analysis of Viral RNAs

Eriko HATADA, Jun MUKAIGAWA* and Ryuji FUKUDA

To study the function of viral proteins participating in the transcription and replication of influenza virus, we have been analysing a collection of temperature-sensitive (ts) mutants of influenza A/Udorn/72, which have defects in viral RNA synthesis (Shimizu *et al.*, *Virology*, **117**, 38, 1982). In the previous issue, we reported the changes in protein synthesis exhibited by these mutants in MDCK cells. This year, we analysed viral RNA synthesis in MDCK cells infected with these mutant viruses.

The genome of influenza virus A comprises eight RNA segments of negative polarity. In the infected cell, three different types of viral RNA are synthesized, virion RNA (vRNA) and two types of transcripts of vRNA, mRNA and cRNA (the template for vRNA replication). mRNA contains a 3′ poly (A) tail and a 5′ capped end of 10 to 13 nucleotides derived from host cell RNA primers, and is an incomplete copy of vRNA in that it lacks a copy of the last 17 to 22 nucleotides at the 5′ end of vRNA. The other type of viral transcript, cRNA is a complete copy of vRNA, and is initiated without a primer. In addition, a portion of mRNA from segment 7 and 8 is processed to give two to three, and two species of spliced mRNA, respectively. Therefore 28 to 29 species of RNA in all are found in the infected cell and synthesis of each species is regulated temporally during the infectious process. To study the mechanism of viral RNA synthesis and its regulation, it is essential to develop a system for measuring the quantities of these RNA species individually. For this purpose, we established a quantitative hybridization system in which RNA probes intensely labeled with ^{32}P were used in a molar excess sufficient to overpower complementary RNAs present in the viral RNA samples. To get the RNA probes, 170–500 bp framgents individual viral ds-cDNAs were recloned in the SP-6 vectors, and the DNA fragments were transcribed with SP-6 RNA polymerase. The probes for mRNA and cRNA were obtained by transcribing the SP-6 recombinant DNAs, which gave the intact 5′ end sequence of vRNA. As mRNA deletes 16 nucleotides at the 5′ end of vRNA (see above), the RNA hybrid formed with mRNA is shorter than the cRNA hybrid. We can thus discriminate

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cRNA from mRNA on polyacrylamide gel electrophoresis for base sequencing. Transcripts of the opposite strand of cDNAs give the probes for vRNA.

To digest out the single-stranded region of the hybridized RNAs, we found nuclease T_1 is superior to nuclease S1, which is required in large amounts to digest RNA, and often cuts the double-stranded region of RNA. As the nucleotide lengths of hybrids formed with each probe differ from one another, the probes for eight segments can be combined into two groups. We can thus measure the quantities of 24 RNA species in four test tubes.

In this way, we elucidated the time course of the synthesis of individual RNA species in cells infected with wild-type virus with new results.

Analysis of Temperature-sensitive Mutants of Influenza Virus. II. Analysis of Influenza Virus RNA Synthesis in Cells Infected with ts Mutant Viruses Defective in RNA Segments 1 and 8

Eriko HATADA, Jun MUKAIGAWA*, Kazufumi SHIMIZU**
and Ryuji FUKUDA

RNA segment 1 of influenza virus encodes the PB2 protein which is one of the components of viral RNA polymerase and exhibits cap binding activity.

One of the ts mutants of this segment, ICRC27, exhibited severely reduced synthesis of M1 and HA, which are classified as late proteins at nonpermissive temperatures. In contrast, no decrease was seen in the synthesis of early proteins, NP and three P proteins.

RNA segment 8 encodes two proteins, NS1 and NS2 which are found only in infected cells but not in virions. NS2 mRNA is produced by splicing NS1 mRNA, but the functions of these proteins remains unknown. As previously reported, two NS1 mutants, ICR1629 and SPC45 (as determined by nucleotide sequencing) exhibited greatly reduced synthesis of the M1 protein as well as the HA protein at the nonpermissive temperature of 40°C. A reduced synthesis of NS1 protein was also observed in both mutants, and NS2 synthesis was greatly decreased in SPC45, whereas reductions in the synthesis of NP on the three P proteins were not obvious. Therefore, it

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appears that the two NS1 mutants have a defect in late viral protein synthesis as ICRC27 does.

No significant differences were seen in the viral protein synthesis for the NS2 mutant, ICR516.

Using the quantitative hybridization method as desribed above, we measured the quantities of mRNA, cRNAs and vRNAs for the eight genome segments which were synthesized in MDCK cells infected with these mutant viruses. At nonpermissive temperatures, the syntheses of all vRNAs were severely reduced in three ts mutants, ICRC27, ICR1629 and SPC45, which have defects in late protein synthesis. The quantities of cRNA as well as mRNA, except for those of NP and three P proteins, also decreased in these mutants. The results indicate that the primary block in these mutants is in the synthesis of cRNA or vRNA synthesis. Accordingly, the secondary transcription which depends on vRNA synthesis, does not occur, resulting in the defective synthesis of the late proteins. This suggests that NS1 as well as PB2 plays an essential role in the replicase complex. Strangely, the PB2 mutant exhibited an elevated synthesis of cRNA in segments 1 and 2 at permissive temperatures.

In the NS2 mutant, ICR516, the quantities of mRNA as well as cRNA were not reduced, but the synthesis of all vRNAs decreased at nonpermissive temperatures, suggesting the participation of the NS2 protein at some step of replication.

Assignment of the Function of Nuclear Factor I

Kyosuke NAGATA, Ken MATSUMOTO* and Fumio HANAOKA*

Nuclear factor I (NFI) has been purified as an essential factor involved in the initiation of adenovirus DNA relpication and shown to be a site-specific DNA binding protein (Hurwitz, J. *et al.* (1985) In: Genetics, Cell Differentiation and Cancer, pp. 15–14). Using an *in vitro* reconstituted chromatin, we found that DNAse-hypersensitive sites, which are often found in the transcriptionally active chromatin and the origins of some replicons, were generated in the neighbourhood of NFI-binding sites. To gain insight into the *in vivo* function(s) of NFI, we prepared a monoclonal antibody against NF-I from HeLa cells and performed an antibody-blotting analysis (Western blotting) of various cell lysates fractionated by gel electrophoresis.

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In parallel, we carried out a DNA-blotting analysis (South-Western blotting) of the same cell lysates using specifically labeled DNA probes.

The protein-blotting analysis revealed that NFI or NFI-like protein was present not only in HeLa cells (human) but also FM3A cells (mouse) and MDCK cells (canine), suggesting that NF-I is a ubiquitous and, therefore, essential protein. Although the molecular weight of purfied NF-I has been estimated to be 47 kdal, a cross-reactive protein with a molecular weight of about 100 kdal was also found in partially purified preparations of NF1. The interrelationship between the two proteins awaits further study.

Organization of Mitochondrial and Nuclear DNA in Cytoplasmic Male Sterile Rice

Saburo NAWA, Yoshio SANO and Taro FUJII

Two supercoiled circular DNAs, B-1 and B-2, found in rice mitochondria of male sterile cytoplasm, (*cms-boro*) rf_1rf_1, were cloned in the plasmid PUC. Using these as probes, the organization of mitochondrial and nuclear DNA in regard to sequences homologous to B-1 and B-2 was studied. It was found that the nuclear fraction from male sterile strains contained open circle forms of B-1 and B-2. These *oc* existed irrespective of the presence or absence of nuclear fertility-restoring genes. Although the supercoiled forms were not detected, it is not clear at present whether the circular molecules were not present naturally or changed to *oc* during preparation procedures of the nuclear fraction. No such DNAs were present in nuclear fractions from the strain having normal fertile cytoplasm, (*n-boro*)rf_1rf_1, or from cytoplasmic revertants.

EcoR 1 digestion of the high molecular weight DNA of mitochondria from *cms* strains gave four fragments homologous to B-1, while DNA from the normal strain and revertants lacked the most strongly labeled one of the four fragments. In the cae of B-2 homologus sequences, *EcoR* 1 digestion of mitochondrial DNA from *cms* strains produced five fragments, while DNA from the normal strain and revertants had only one of the five. Thus, the reversion to fertility in the male sterile cytoplasm resulted in the loss of some of the mitochondrial chromosomal sequences homologous to B-1 and B-2, as well as the loss of the free plasmids.

Sequences homologous to B-1 and B-2 were also present in the nuclear chromosomal DNA. *EcoR* 1 digestion of nuclear DNA from *cms* strains

produced at least six fragments which hybridized to B-1, while five B-1 homologous fragments were produced from the normal strain and revertants. Two of these fragments were conserved in both *cms* and the normal strain or revertants. Common six fragments homologous to B-2 were obtained in *EcoR* 1 digestion of nuclear chromosomal DNA from both *cms* and the normal strain or revertants, with additional three fragments being obtained from *cms* strains. These results show that a complex change in the organization of nuclear chromosomal sequences occurred in the cytoplasmic reversion. The presence of common DNA sequences in rice nuclear and mitochondrial genomes is also suggested.

DNA Supercoiling Enhances *In Vitro* Transcription of Fibroin Gene

Susumu HIROSE, Hisahiro TABUCHI and Yoshiaki SUZUKI*

In prokaryotes, DNA supercoiling is often an important requirement for proper gene expression. But little is known in eukaryotes. Using cell-free systems of transcription, we tested whether topological tension is also required for eukaryotic gene expression or not. A modified method for S1 nuclease assay was developed to quantitate amounts of radioactive transcripts in the presence of unlabeled homologus RNA. Using this method, we assayed *in vitro* transcription of the fibroin gene on circular and linear templates. In a posterior silk gland cell extract, covalently closed circular (ccc) DNA forms the superhelical state and supports three to 10 times more transcription than nicked circular or linear DNA does. (for details, see *J. Biol. Chem.* **260**: 10557–10562) A HeLa cell extract shows neither the supercoiling nor the preference of ccc DNA over the linear one. Upon fractionation of the posterior silk gland cell extract through phosphocellulose, both the flow-through and the 0.6 M KCl eluate fraction are required for supercoiling and efficient transcription on ccc template in the acceptor HeLa cell extract. While the 0.6 M KCl fraction has DNA topoisomerase II activity, the flow-through fraction contains a supercoiling factor which introduces the negative supercoiling into ccc DNA with the aid of topoisomerase II.

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Participation of the Upstream Region of the Fibroin Gene in the Formation of Transcription Complex *In Vitro*

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In posterior silk gland extracts, a region covering about 200 base pairs of DNA sequences upstream from the TATA box of the fibroin gene enhances transcription efficiency. In an attempt to examine the mechanism of this enhancement, the following studies were made. The addition of exogenous histones has an inhibitory effect on fibroin gene transcription in posterior silk gland extracts. The histones probably disturb a process in complex formation, because when transcription complexes were constructed with preincubation of the templates with the extracts, the inhibitory effect of histones was greatly reduced. Transcription of a fibroin gene construct, pFb5′Δ-238, with the upstream region beyond the TATA box was relatively less inhibited than that of pFb5′Δ-44 which lacks the upstream region. This tendency toward differential inhibition was observed in silk gland extracts but not in a HeLa cell extract and persisted even after complex formation in the silk gland extracts, suggesting a specific interaction of the upstream region with some factors in the extracts. The complexes formed on pFb5′Δ-44 are probably more susceptible to the inhibitory effect of histones. These results suggest a participation of the upstream region of the fibroin gene in the formation of stable transcription complexes in the promoter through an interaction with specific factors in the silk gland. Since the transcription-enhancing effect via the upstream region is augmented at a high histone/DNA ratio, it may mimic the *in vivo* situation in which the fibroin gene can be transcribed in the posterior silk gland even in the presence of excess suppressive materials. For details, see *Mol. Cell. Biol.*, **6**, 3928–3933.

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Tissue-Specific Transcription Enhancement of the Fibroin Gene Characterized by Cell-Free Systems

Yoshiaki SUZUKI*, Masaaki TSUDA*, Shigeharu TAKIYA*,
Susumu HIROSE, Etsuko SUZUKI*, Mayumi KAMEDA*
and Osamu NINAKI†

An upstream region of the *Bombyx mori* fibroin gene enhances transcription efficiency. Six cell-free extracts of transcription were used to characterize the nature of DNA signals and trans-acting factors responsible for the transcription enhencement of the fibroin gene. The upstream element of the fibroin gene involved in the enhancement can be divided into two regions. The proximal region, −72 to −32, is recognized as a common enhancing signal by all *B. mori* extracts from the posterior silk gland, middle silk gland, ovarian tissue, and an embryonic cell line. It is weakly recognized by an *Antheraea* silkworm cell line extract but not by a HeLa cell extract. The distal region, −238 to −73, appears to be a tissue-specific enhancing signal that is recognized more effectively by the posterior silk gland extract than by the middle silk gland extract. These observations suggest that the use of these cell-free systems can offer a means for the biochemical characterization of the trans-acting factors involved in the tissue-specific regulation of the fibroin gene. For details, see *Proc. Natl. Acad. Sci. USA*, **83**, 9522–9526.

Diversity in G+C Content at the Third Position of Codons in Vertebrate Genes and Chromosomal Banding Pattern

Toshimichi IKEMURA and Shin-ichi AOTA

In higher eukaryotes such as vertebrates, the codon-choice patterns of different genes in a single organism often differ significantly. Some genes are extremely G+C-rich at the codon 3rd position, whereas others are rather A+T-rich. We found that this G+C content of the 3rd letter was correlated to the G+C content of a large genome portion surrounding the gene; exons of genes with a high G+C% at the codon 3rd position are surrounded by G+C-rich introns and G+C-rich flanking sequences, and those with a

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low G+C% at the position by A+T-rich introns and flanking sequences. Analysis of G+C content distribution along DNA sequences using a DNA Sequence Data Bank supported the view that the vertebrate genome is a mosaic of regions with clear differences in their G+C content. The global variation in the G+C content throughout human genome thus found was attributed to the chromosomal G (Giemsa) banding or R (Reverse) banding, since the analysis on the GenBank sequence showed that the genes being mapped on R bands in the Human Genetic Map (the 8th edition) usually have the G+C content higher than 50% and those mapped on G bands have the content lower than 50%; on the basis of cytogenetic studies at the microscopic level, G bands have been believed to correspond to A+T-rich, late-replicating DNA segments, and R bands to G+C-rich early-replicating ones. For details, see *Nucl. Acids Res.*, **14** (1986): 6345–6355 and 8702.

Nucleotide Sequence and Molecular Evolution of Mouse IAP Elements

Shin-ichi AOTA, Takashi GOJOBORI, and Toshimichi IKEMURA

We determined the nucleotide sequences of cDNA and genomic clones for murine intracisternal type A particle (IAP) elements, which are retrovirus-like repetitive sequences in rodent genomes. The sequence of the cDNA resembled that of retrovirus RNA genomes in its lack of the U5 sequence within the 3′ long terminal repeat. By sequence comparison of our clones with reported rodent IAP elements, we located the probable *gag*, *pol*, and *env* gene regions. The sequences for the *pol*, *env*, and the 3′ two-thirds of the *gag* region were conserved among the IAP elements. In the regions, synonymous substitutions occurred more frequently than non-synonymous ones, which suggested that the regions in question were functionally constrained until fairly recently. The rate of nucleotide substitutions in the regions was estimated to be $6\text{–}10 \times 10^{-9}$ per site per year, and significantly higher than that of the cellular genes. These rates may exemplify a characteristic of the nucleotide substitutions for an endogenous retrovirus. *Gene* (1987), **56**, 1–17.

II. MICROBIAL GENETICS

On the Process of Cellular Division in *Escherichia coli*: Penicillin-Binding Protein 3 of *E. coli* is a Lipoprotein

Shigeru HAYASHI, Hiroshi HARA, Hideho SUZUKI
and Yukinori HIROTA

Penicillin-binding protein 3 (PBP-3) of *Escherichia coli* is a membrane protein that plays an indispensable role in cell division. It functions in the formation of a septum of the peptidoglycan sacculus and is one of the lethal tragets of β-lactam antibiotics. During the examination of the primary structure of PBP-3 deduced from the nucleotide sequence of the *ftsI* gene (Nakamura M. *et al.* 1983, *Molec. Gen. Genet.* **191**: 1), we noticed the existence of the amino acid sequence, Leu-Leu-Cys-Gly-Cys, in the NH_2-terminal region, which shows striking homology with the Leu-Leu-Ala-Gly-Cys consensus sequence for modification and processing of the precursor forms of Braun's lipoprotein and other bacterial lipoproteins. These lipoprotein precusors are modified with diglyceride in the SH group of cycteine at the last position in the consensus sequence, cleaved between glycine and cysteine residues by a specific processing enzyme, and acylated with fatty acid in the NH_2 group of the cysteine rsidue.

As expected from the existence of the consensus sequence, PBP-3 was covalently modified *in vivo* with [2-^{3}H]glycerol and [^{3}H]palmitic acid. Glycerylcysteine residue resulting from the modification of cysteine residue with thioester-linked diglyceride was detected in the acid hydrolysate of purified PBP-3, although such modification occurred only in a small fraction (about 8%) of total PBP-3 molecules. Fatty acids incroporated into PBP-3 were found in both mild alkali-labile and mild alkali-resistant linkages. Thus PBP-3 contains fatty acids ester-linked to glycerol and amide-linked to the NH_2-terminal cysteine residue. These results indicate that a portion of PBP-3 is a lipoportein which has the same NH_2-terminal modification as found in Braun's lipoprotein and other bacterial lipoproteins.

In Braun's lipoprotein, the fatty acid residues modifying the NH_2-terminal cysteine serve to anchor this protein to the outer membrane. It is probable that PBP-3 is also anchored to the outer membrane with its fatty acid-

modified NH_2-terminus. PBP-3 has several highly hydrophobic sequences in the COOH-terminal region and they are probably embedded in the cytoplasmic membrane. It seems likely that lipid-modified molecules of PBP-3 span the periplasmic space from the cytoplasmic membrane to the outer membrane. In this topography, the active site serine that occurs toward the middle of the molecule (Houba-Hérin *et al.* 1985, *Molec. Gen. Genet.* **201**: 499) would be located close to the peptidoglycan layer. PBP-3 is an enzyme acting on the peptidoglycan sacculus to change its structure for septum formation. The function of this enzyme might be regulated by modification with lipids. It is possible that only PBP-3 molecules arranged at the presumptive septal site are modified to become functional, and thus the modified molecules could be a small fraction.

Conversion of the α Component of Penicillin-Binding Protein 1b to the β Component in *Escherichia coli*

Hideho SUZUKI, Jun-ichi KATO, Youji SAKAGAMI,
Masaaki MORI, Akinori SUZUKI
and Yukinori HIROTA

Penicillin-binding protein (PBP) 1b of *Escherichia coli* is one of the essential enzymes involved in the process for extention of the murein sacculus. This protein is composed of three components as detected among proteins bound to [^{14}C]benzylpenicillin in membrane fractions and the component proteins are termed α, β and γ in order of decreasing apparent molecular weight. It has been suggested that the α and γ components are the primary products of the *ponB* (PBP 1b) gene and that their difference arises from the existence of different translation initiation codons within the same coding frame.

To confirm the two initiation codons and to elucidate the origin of the β component, the α component and the β component were purified as preparations uncontaminated by each other; the γ component was isolated; and the amino terminal sequences of the three components were determined. The N-terminal amino acid sequences for α and γ matched the reported nucleotide sequences following the 1st and the 2nd ATG in the *ponB* coding region, respectively, and also revealed that the methionine for translation initiation was removed. The N-terminal sequence of β indicated that β is derived with the removal of 24 amino acids from the N terminus of α.

The amount of β was found to increase with a simultaneous decrease in α during incubation of membrane fractions. Therefore, the origin of β was investigated to see if this component might be a product incidental to the preparation of membrane fractions. When the PBP 1b components were isolated from cells subjected to instantaneous protein denaturation by direct addition of trichloroacetic acid to the culture, essentially no β component was found. On the other hand, a substantial amount of β was detected accompanied by a decrease in α, in PBP 1b prepared from membrane fractions. The results were essentially the same for cells harvested at different growth phases, for those grown overnight, and for minicells; and led to the conclusion that β was formed from α primarily after cell distruption. (For details see *J. Bacteriol.* **169**: 891–893, 1987).

Molecular Cloning of *Bacillus subtilis* Gene Involved in Cell Division, Sporulation, and Exoenzyme Secretion

Yoshito SADAIE

The wild type *div-341* gene of *Bacillus subtiis* was cloned with a temperate phage rho-11 and recloned in a smaller temperate phage ϕ105. The resulting transducing phage ϕ105-*div-341*$^+$ carries the Cfrl3I chromosomal fragment containing the entire *div-341* gene which is involved in cell division, sporulation, exoenzyme secretion, competent cell formation, and autolysis (*Mol. Gen. Genet.*, **190**, 176–178; *J. Bacteriol.*, **163**, 648). The *div-341* strain carrying ϕ105-*div-341*$^+$ recovered wild type growth at restrictive temperatures suggesting that the wild type allele is dominant over the mutant allele. The *div-341* mutational site is very close to restriction sites produced by AluI, BcnI, HapII, HhaI, HinfI, MspI, EcoRI, EcoRV, HindIII, EcoT14I, MvaI, and TthHB8I as the transforming activity of the cloned fragment was completely lost with these restriction endonucleases.

III. IMMUNOGENETICS

Sexual Preference of Meiotic Recombination within the H-2 Complex

Toshihiko SHIROISHI, Tomoko SAGAI and Kazuo MORIWAKI

We previously reported that the B10. MOL-SGR congenic strain, H-2^{wm7} haplotype of which was derived from Japanese wild mouse, exhibits marked recombination enhancement within the H-2 complex (Shiroishi *et al. Nature* **300**: 370–372, 1982). In contrast to the standard recombination frequency of 0.3% (Klein, 1975), a tenfold higher value was calculated between the H-2K and H-2D marker loci, based on the screening of backcross progeny from females heterozygous for the wm7 haplotype. In that experiment, however, the recombination frequency was measured only in female mice. Here we present recently obtained data on recombination frequencies measured in males that are heterozygous for the H-2^{wm7} haplotype. The results are summarized in Table 1. In contrast to a higher recombination frequency in female mice, only one recombinant between C57BL/10J and B10. MOL-SGR was detected in the screening of 650 progeny generated from heterozygous males. No recombinants were found in crosses of B10. A×B10. MOL-SGR and A/Wy×B10. MOL-SGR, although heterozygous female with the same combination of H-2 haplotypes, gave rise to a number of intra-H-2 recombinants.

Table 1. Frequency of intra-H-2 recombination between H-2^{wm7} and common H-2 haplotypes of inbred mice in females and males.

Heterozygous partner with B10. MOL-SGR	Females		Males	
	No. recombinants/ Total no. mice	Frequency ±S.E. (%)	No. recombinants/ Total no. mice	Frequency ±S.E. (%)
B10. A (H-2^a)	16/539	3.0±0.7	0/301	0.0
B10. BR (H-2^k)	5/174	2.9±1.3	—	—
B10 (H-2^b)	8/204	3.9±1.4	1/176	0.5±0.5
A/wy (H-2^a)	3/117	2.6±1.5	0/173	0.0
Total	32/1,034	3.1±0.5	1/650	0.2±0.2

The present data thus indicates that male recombination in heterozygotes for the wm7 haplotype occurs at almost the same rate (0.2%) as was obsserved in heterozygotes between the common H-2 haplotypes of inbred mice. A genealogical study of recombination indicated that the post-meiotic stage is not involved in the generation of sexual preference of recombination enhancement, suggesting that the preference is a result of mexiotic-drive and a female specific mechanism is involved in the enhancement of meiotic recombination mediated by the H-2^{wm7} haplotype. For details, see *Immunogenetics* **25**: 258–262, 1987.

Genetic Polymorphism of a Serum Protein Found in Japanese Wild Mice

Yoshi-nobu HARADA, Kazuo MORIWAKI, and Takeshi TOMITA*

Genetic polymorphism of a serum protein in mice was studied using gel precipitation with alloantisera produced by reciprocal alloimmunization between BALB/c and MOM (a stranin derived from Japanese wild mice, *Mus musculus molossinus*). A protein migrating in the α region of serum proteins was identified as alphaprotein-2B (APH-2B). A gene locus controllong the serological variation of the APH-2 molecule was designated as *Aph*-2 (alphaprotein-2), in which two alleles were proposed, *Aph*-2^b allele for the positive reaction of APH-2B, and *Aph*-2^a for the negative reaction. The strain distribution of APH-2B is shown in Table 1. All common laboratory strains examined in this study had *Aph*-2^a, while three strains, MOM, Mol-Nis, and Mol-Tsm also had the *Aph*-2^b allele. In addition the BFM/2Ms strain established from European wild mice also possessed *Aph*-2^b. APH-2B was antigenically homologous to alphaprotein-1B (APH-1B), a serum protein reported previously (*Immunogenetics* **24**: 47–50, 1986).

Linkage analysis between the *Aph*-2 and *Aph*-1 loci controlling APH-1B was carried out using backcross progeny obtained from a cross of (BALB/c × MOM)F_1 mated to BALB/c. No recombination between the two loci was observed in 307 backcross progeny. These results suggest that the *Aph*-2 locus is closely linked to the *Aph*-1 locus.

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Table 1. Strain distribution of the serum component APH-2B in different strains of mice.

Phenotypes of APH-2B	Allele	Strains
Positive	*Aph*-2^b	MOM, Mol-Nis, Mol-Tsm, BFM/2Ms
Negative	*Aph*-2^a	A/HeN, AKR/J, AU/SsJ, BALB/cJ, BALA/cAn, CBA/J, CBA/StMs, CE/J, CS, C3H/HeJ, C3H. OL, C57BL/6J, C57BL/10J, C57BL/10Sn, C57L, C58/J, DBA/1J, DBA/2J, DD, DM, HRS/J, HTG, HTH, HTI, ITES, IITES, IXBL, KK, KR, KR/C, KSB, MOA, MOL-ANJ, MOL-MSM, MOL-NEM, MA/MyJ, NC, NZB, NZW/San, P/J, PL/J, RFM, RIIIs/J, SJL/J, SK/Cam, SM/J, SWR/J, WB/J, WN, YBR, 129/J, 129/Sv

Animal Model for Steroid 21-Hydroxylase Deficiency

Hideo GOTOH, Toshihiko SHIROISHI, Tomoko SAGAI and Kazuo MORIWAKI

So far, a number of H-2 recombinants have been produced from a cross of B10. A and wild-derived B10. MOL-SGR strains. One such recombinant, designated *aw*18, has a deletion of the complement component C4 and one of the steroid 21-hydroxylase genes, possibly due to unequal recombination. The mouse homozygous for the *aw*18 haplotype is lethal in the neonatal period (Shiroishi *et al. Proc. Natl. Acad. Sci. USA*, 84: 2819–2823, 1987).

To determine 21-hydroxylase activity of animals homozygous for the *aw*18 haplotype, we tested the level of serum 17α-hydroxyprogesterone (17-OHP) which is a cortisol precursor for 21-hydroxylase. A radioimmunoassay system using the 17α-hydroxy-progesterone radioimmunoassay-kit (CSI-Sorin) was utilized.

Animals to be tested were generated from an intercross of *aw*18/*b* heterozygotes. We collected serum samples by heart puncture from 0.5-day-newborn mice, and determined the H-2 haplotype of these individuals both by the cytotoxicity test for thymocytes and by the Southern blotting analysis using a class II gene probe. To measure the level of 17-OHP, 5 μl of serum was assayed for each individual.

The serum 17-OHP concentration of *aw*18/*b* and *b*/*b* haplotypes ranged from 2.0 ng/d*l* to 5.7 ng/d*l*, and was considered normal. No differences

were observed in 17-OHP levels between *aw*18/*b* and *b*/*b* haplotypes. On the other hand, *aw*18/*aw*18 indicated markedly high levels of serum 17-OHP, ranging from 5.1 ng/d*l* to 46.7 ng/d*l*. These results clearly demonstrated that the *aw*18/*aw*18 mouse is deficient for 21-hydroxylase, and the 21-OHase genes which still remained in the *aw*18 haplotype was biologically inactive.

In human, deficiency of steroid 21-hydroxylase is one of most common inborn error of metabolism. The *aw*18 recombinant haplotype is expected to provide useful and sole animal model for this disease.

Clinical characterization for C4 deficiency of *aw*18/*aw*18 homozygous mouse is still unknown.

The Low Polymorphic Nature of the H-2 Class I Genes in Japanese Wild Mouse

Tomoko SAGAI, Toshihiko SHIROISHI and Kazuo MORIWAKI

In spite of extremely high polymorphism in H-2 class I genes, relatively low polymorphism or oligomorphic features of class I genes has been observed in some geographically isolated populations of European and North American wild mice (Klein, J., 1981). We made a serological survey of Japanese wild mouse populations with respect to genetic polymorphism of the H-2K antigen. This study revealed that two private alloantisera reacted predominantly with Japanese wild mice. One of them, anti-H-2K^{f}, reacted with 34% of the mice tested. While another, anti-H-2K^{u}, reacted with 19% of the mice. To more precisely characterize H-2K^{f} antigenicty of Japanese wild mice, we established monoclonal antibodies against the H-2K^{f} antigen using the B10. MOL-TEN1 congenic strain as an immunization donor. The H-2 complex of this strain was derived from the Japanese wild mouse having a previously determined H-2K locus of f haplotype. Of 11 monoclonal antibodies obtained, six appeared to be H-2^{f} specific using the panel test on standard H-2 haplotypes of indbred mice. These 6 antibodies, however, showed different reactivities to wild mice. The other 5 antibodies exhibited crosseractivities with some inbred strains (Table 1). With these antibodies, most Japanese wild mice testing positive to an anti-H-2K^{f} private alloantiserum, H-2K.26, showed identical reaction patterns to those of the B10. MOL-TEN1 and B10. M strain. An RFLP analysis using two DNA probes which hybridize to the 3′ and 5′ ends of the H-2K gene respectively, did not indicate any differences in autoradiogram patterns

Table 1. Similar reactivities with anti B10. MOL-TEN1 H-2 class I monoclonal antibodies in Japanese wild mice

Mice	Monocloal antibodies and Specificities											Reac-tivities to anti H-2K. 26
	MS 51 f	MS 52 f	MS 53 f	MS 55 f	MS 56 f	MS 68 f	MS 54 f,p	MS 59 f,p	MS 60 f,K^b	MS 61 f,s	MS 69 f,p,s	
B10. M and A. CA	+	+	+	+	+	+	+	+	+	+	+	+
B10. MOL-TEN1	+	+	+	+	+	+	+	+	+	+	+	+
M. m. mol. Aziro	+	+	+	+	+	+	+	+	+	+	+	+
Niigata	+	+	+	+	+	+	+	+	+	+	+	+
Iiyama	+	+	+	+	+	+	+	+	+	+	+	+
Shizuoka	+	+	+	+	+	+	+	+	+	+	+	+
Kyoto	+	+	+	+	+	+	+	+	+	+	+	+
Omiya	+	+	+	+	±	±	+	+	−	+	+	+
Mito-1	−	±	±	±	−	+	+	+	−	+	±	+
Mito-2	+	+	+	+	−	±	+	+	n.t.	+	+	±

among these wild mice. This wider distribution of stable H-2K^f antigenicity suggests that the Japanese wild mouse population has a relatively low polymorphic nature in regards to the H-2K gene.

H-2 Controlled Genetic Susceptibility to Pulmonary Adenomas Induced by Urethane and 4-nitroquinoline 1-oxide in A/Wy Congenic Strains

Nobumoto MIYASHITA and Kazuo MORIWAKI

The genetic effect of H-2 complex on the development of chemically induced pulmonary adenomas was clearly demonstrated in H-2 congenic strains with an A/Wy background having a high susceptibility to pulmonary adenomas. A single subcutaneous injection of either urethane or 4-nitroquinoline 1-oxide (4NQO) was given to nine strains of mice. Among them, the number of adenoma foci per mouse was significantly higher in A/Wy (haplotype H-2^a), A/J (H-2^a), A.TL (H-2^{t1}) and A.AL (H-2^{a1}), than in A.BY (H-2^b), A.SW (H-2^s), A.CA (H-2^f) and A.TH (H-2^{t2}) strains. In addition,

Table 1. Effect of H-2 complex on urethane- or 4NQO-induced pulmonary adenoma in H-2 congenic strains with an A/Wy background

Strain	H-2 haplotype	Urethane treatment[a]		4NQO treatment[a]	
		Tumor No., mean±S.E.M.	Incidence: No. of mice with tumors/ total no. of surviving mice	Tumor No., mean±S.E.M.	Incidence: No. of mice with tumors/ total no. of surviving mice
A/WySnJ	a	26.8±1.3	35/35	9.5±2.1	11/13
A/J	a	30.6±1.4	24/24	9.0±2.2	10/13
A. SW	s	12.5±0.8*[b]	50/50	5.0±0.5*	24/25
A. TH	t2	18.0±1.0*	39/39	5.4±1.1*	20/26
A. AL	a1	26.3±1.3	36/36	11.0±2.9	11/11
A. TL	t1	29.0±1.5	20/20	9.0±1.7	14/14
A. CA	f	16.8±0.6*	58/58	4.8±0.9*	30/37
A. BY	b	13.8±0.6*	58/58	5.3±0.9*	12/13
B10. A	a	1.2±0.2*	20/26	NT[c]	NT

[a] Mice received an injection of 1.5 mg urethane/g of body weight or 12.5 μg 4NQO/g of body weight.

[b] Student's t-test was applied. *$P<0.001$, in comparison with A/Wy strain.

[c] Not tested.

the average number of adenoma foci in A/Wy (H-2^a) was more than 20-times that in the B10. A (H-2^a) strain (Table 1). Thus, multiplicity of adenoma foci appeared to be regulated by at least two genes, one located in the I or S region in the H-2 complex and the other in the non-H-2 genetic background. The genes in the H-2 complex were distinct from that for pulmonary adenoma susceptibility (*Pas*)-1. For details, see *Jpn. J. Cancer Res.* **78**: 494–498 (1987).

IV. DEVELOPMENTAL AND SOMATIC CELL GENETICS

Comparative Studies on the Antimutagenic Actions of Vitamin C and its derivatives in Cultured Chinese Hamster Cells

Yukiaki KURODA

In a previous study, it was reported that vitamin C had strong inhibitory effects on the cytotoxic action and 6-thioguanine (6TG) resistant mutations induced by ethyl methanesulfonate (EMS) in cultured Chinese hamster V79 cells. In the present study, the effects of vitamin C and its derivatives on cytotoxicity and mutagenicity of EMS were compared in Chinese hamster V79 cells.

EMS had a cytotoxic effect on the cells, showing an LD_{50} of 541 μg/m*l*. In the presence of vitamin C or its derivatives at concentrations of 50 or 100 μg/m*l*, the cytotoxicity of EMS expressed at LD_{50} was reduced to more than 1,000 μg/m*l* for vitamin C, and 639 μg/m*l* for iso-vitamin C. Dehydro-vitamin C enhanced the cytotoxicity of EMS, giving an LD_{50} of 234 μg/m*l*. EMS showed a strong activity for inducing 6TG-resistant mutations in V79 cells. At a concentration of 1,000 μg/m*l*, EMS induced 6TG-resistant mutations at a frequency of 88×10^{-5}. Vitamin C decreased EMS-induced mutations by 2/3–3/4. Dehydro-vitamin C and iso-vitamin C also decreased the EMS-induced mutations by 1/2 to 2/3.

When cells were treated with vitamin C after treatment with EMS, the induced mutation frequency was not affected, while pretreatment with vitamin C was effective in reducing EMS-induced mutations. Vitamin C mixed with EMS and incubated for 2 hours in the absence of cells, also had a strong effect in reducing mutations. This suggests that vitamin C was desmutagenic rather than antimutagenic against induction of mutations by EMS.

Combined Effects of MMS and EMS after Time Lag Treatment on Mutations in Cultured Chinese Hamster Cells

Yukiaki KURODA, Hajime KOJIMA* and Hiroaki KONISHI*

It was reported in the previous study that the combined effects of simultaneous treatments with methyl methanesulfonate (MMS) and ethyl methanesulfonate (EMS) on 6-thioguanine (6TG)-resistant mutations in Chinese hamster V79 cells were additional or synergistic, depending on the sequence of treatments with both chemicals.

In the present work, to elucidate more details concerning the mechanisms of the combined effects of both chemicals, cells were treated with the two chemicals successively at different times for 3 hours, or incubated in normal medium for 3 hours between two treatments of both chemicals.

The sequence of treatments with two chemicals as well as incubation in normal medium markedly affected cell survival. Especially, in the case of MMS treatment, cell survival after posttreatment with EMS showed a greater decrease than after pretreatment with EMS. In most cases, cell survival after successive treatments was lower than that after simultaneous treatments, and increased by incubation in normal medium for 3 hours between two treatments with EMS and MMS.

On the other hand, the induced mutation frequency after successive treatments was higher than that after simultaneous treatments, while the sequence of treatments with two chemicals showed no detectable effect. Incubation in normal medium between two treatments with EMS and MMS decreased induced mutation frequency, suggesting that a repair mechanism for mutational damages caused by the initial treatment may be operating during the incubation period.

Age-dependent Accumulation of Spontaneous Mutations in Human Embryonic Organs

Yukiaki KURODA

Many theories have been proposed on the mechanism of aging. In most of these theories, it is suggested that some changers or modifications in the

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genetic information of cells may be related to the mechanism of aging. In the present work, changes in the frequency of spontaneous mutations in cells of organs in human embryos at various gestation stages were examined. With the cooperation of several sanitary hospitals in the Mishima area, various organs such as lungs, hearts, livers, kidneys and skins were dissected from artificially aborted human embryos at various stages ranging from 6 weeks to 24 weeks. These organs were dissociated into single cells by treatment with trypsin. Inocula of 10^5 cells in petri dishes were incubated in 8-azaguanine (8AG)-containing medium. The number of colonies of 8AG-resistant mutant cells were counted.

Among cells obtained from various organs in 6 week embryos, only lung cells formed colonies in normal medium, whereas cells obtained from other organs did not form colonies. Cells obtained from other organs in embryos at more advanced stages did form colonies. Lung cells showed the highest colony-forming activity among cells obtained from various organs. The colony-forming activity of lung cells obtained from various ages was compared. Such activity was low in cells obtained from embryos at early gestation stages, increased gradually with the development of embryos, reaching its height in 13 week embryos and went down again to a low activity in cells obtained from 24 week embryos.

On the other hand, the frequency of 8AG-resistant mutant cells was also low in lung cells obtained from early embryos, reaching a peak in 13 week embryos, and again went down in more advanced stages.

The ratios of the number of colonies of 8AG-resistant mutant cells to those of normal cells were compared among cells obtained from embryos at various stages. Ratios increased lineally with the progress of the embryonic development, indicating that mutations may be accumulated in normal organs with the progress of embryonic development. Results also suggest that the ratios obtained in a certain district may be an indicator of the all over strength of mutagenic factors in that district.

The Fine Structure of Embryonic Cells of *Drosophila melanogaster* Cultured in the Presence of Ecdysterone

Yutaka SHIMADA and Yukiaki KURODA

In a series of tissue culture studies of embryonic cells for *Drosophila mela-*

nogaster, we examined the fine structure of these cells cultured with ecdysterone.

Undifferentiated cells dissociated from post-gastrula embryos of the wild-type strain (Oregon-R of *Drosophila melanogaster*) were cultured in medium K-17 supplemented with 15% fetal calf serum, 0.1 μg/m*l* fetuin, and 10 μg/m*l* ecdysterone, fixed in glutaraldehyde and osmium, and thin sections were examined with an electron microscope.

In addition to cell types previously reported (muscle cells, nerve cells, and electron-dark and light cells of unidentified nature), we observed the formation of cuticule and trachea in the cultured cells.

The cuticle was a homogeneous structure with a dense line lining multilayered lamellar structures. Each lamella was composed of simple epithelial-like cells. Between lamellae, obliquely running fibrillar structures were found. Around the lamellae, dense cells with many vacuoles were seen.

The trachea was a hellow-like structure found within parenchymal cells. It appeared to be formed initially by a similar mechanism as that in the development of nerve myelin. The center of onion-like running membranes disappeared to canalize the cell and, thus, the lumen of trachea was formed.

These results suggest that the fine structure of insect cells cultured with ecdysterone my be useful in analyzing the more detailed characteristic actions of specific genes during development.

Embryonic Development of a Maternally Affected Embryonic-Lethal Mutant in *Drosophila melanogaster*

Kiyoshi MINATO and Masa-Aki YAMADA

Embryonic development of a maternally affected embryonic-lethal mutant, fs(1) MAY-263 (Yamada, M. A., 1978), in *Drosophila melanogaster*, was observed by time-lapse photographs taken through an optical microscope. Eggs laid by female flies homozygous for the above mutant gene developed normally to the end of the cellular blastoderm stage, but thereafter the formation of the ventral furrow was very weak compared to a normal embryo and, hence, a little dorsal extension of germ band was oberved. Instead, many inward invaginations of the external layer were found dorsally and ventrally in these embryos. Consequently, at the last stage of embryonic

developemt, they had some compartments formed by epidermal layers and had less developed internal organs of the gut, trachea, and Malpighian tubule. Because the embryos moved actively within the vitelline membrane at that time, they appeared to have some muscle development. Although the embryos never hatched, they had nearly consumed the yolks. These observations suggest that the embryos die not from metabolic defects but from some teratogenic anomalies.

Whole Embryonic Culture of Spontaneous Parthenogenetic Diapausing-Eggs in *Bombyx mori*

A. MURAKAMI, Y. OHTSUKI* and T. KITAZAWA*

It is comparatively easy to artificially initiate the development of eggs without fertilization in the *Bombyx* silkworm. So it is assumed that spontaneous activation commonly occurs in nature. In fact, we observed that virgin female moths frequently produced spontaneous parthenogenetic eggs in almost all races of the silkworm. We reported that the frequency of naturally-occurring parthenogenesis is markedly different among several strains, ranging from 3.6% to 32.1%, with rates flucutating from batch to batch. Only a small proportion of the well developed eggs survive beyond the diapause period. It is well known that when a normally-fertilized diapausing egg is taken from its chorion and explanted in Grace's medium, the embryo instantly begins to develop to the stage immediately preceding hatching. In such a case, spontaneous parthenotes could be rescued by releasing the diapause state and allowing development to the last stage.

In the present experiment, eggs laid from F_1 female moths in a cross J 106♀ × Cambodge ♂ were used, because females in this cross showed the highest rate (32.1%) of natural parthenogenesis in the silkworm. When virgin females were placed on the egg-card, they began their oviposition a few days after emergence and it lasted to about the 10th day. The eggs, obtained in such a manner were kept at 25°C to make diapause proceed for about 4–12 weeks after oviposition until the time when they were used for culturing. For the culture the whole embryo and yolk, enclosed by the serosal membrane, was taken from the egg-chorion in a 0.85% NaCl solution and then cultured in hanging-drops of Grace's medium at 25°C tor 2 weeks according to Takami *et al.* (1966) without renewing the medium. After

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cultivation for 2 weeks some eggs were dissected in 0.85% NaCl solution to examine the development of embryos.

Results showed that the growth of spontaneous parthenogenetic embryos *in vitro* varied among different ages of embryos after oviposition. Most four to five week-old parthenogenetic diapausing-eggs grew to the stage of bristle formation (or the late stage of morphogeneisis) as partial embryos, while the majority of twelve week-old eggs developed completely at least up to the stage of bristle formation as normally-fertilized diapausing eggs did. A few others grew to the stage of larvae ready to hatch. It is of interest to note that the post-diapause embryonic development was observed even in some poorly pigmented parthenogenetic diapausing-eggs to an extent similar to well pigmented eggs.

The present finding and others clearly indicate that in whole embryo culture, spontaneous parthenogenetic diapausing eggs were able to be partly rescured from unavoidable genetic death. It is also suggestive that natural parthenogenesis is common in the *Bombyx* silkworm as it is in the order *Hymenoptera.*

Studies on Voltinism in Bombyx with Special Reference to Extra-Embryonic Membranes

Akio MURAKAMI

Diapausing eggs of the silkworm are easily reactivated by various means, electric shock, brushing, immersion in warm HCl solution, and so on, Umeya 1937, 1939) pointed out that prickling of diapausing eggs served as a stimulus for termination of diapause and some eggs immediately resumed their post-diapause embryonic growth. Also, deprivation of chorions from normally-fertilized diapausing eggs leads to the termination of diapause as reported by many researchers. In this insect the diapause phenomenon is generally dependent upon maternal traits. The eggs of silkworms are surrounded by three main extra-embryonic membranes, chorion, vitelline membrane and serosal memberane. The former two membranes are maternal products and the latter one is a zygotic product. On the other hand, all three membranes in parthenogenetic eggs are maternal in origin differing from fertilized-eggs. In spite of such differences, when spontaneous parthenogenetic whole embryos are explanted in Grace's medium after they deprivation of chorions they instantly resume their embryonic growth as fertiliz-

ed diapausing-eggs do. This finding clearly suggests that the deprivation of chorions may stimulate the termination of diapause, while the serosal membrane may not be concerned in the event. Various artificial treatments to disrupt diapause may change the property of chorions by increasing the permeability of oxygen into the embryo and yolk complex covered by serosal membranes. It seems that mechanical wounding may modify physiological conditions of the embryo-yolk complex.

In the previous report it was found that most spontaneous parthenogenetic diapausing-eggs in nature could not survive beyond the diapause period in contrast to fertilized diapausing-eggs. When parthenogenetic eggs were provided with shelter prior to the termination of diapause, most of them were able to develop to a fairly advanced stage as seen in the fertilized eggs. In general, diapausing eggs of the silkworm are remarkably resistant to unfavorable environments because they are highly inactive physiologically. Although parthenogenetic eggs, which may have a certain genetic defect in serosal membranes, could not overcome an early step in the termination of diapause *in vivo*, they could do so under *in vitro* culture conditions. Among various liquid culture media, Grace's medium appeared the best sited for whole embryonic development, probably owing to its pertinent osmotic pressure on the embryo-yolk complex rather than as a nutritional source. In brief, a high mortality of spontaneous parthenogenetic diapausing-eggs in nature may be caused by genetic defects in functions of a serosal membrane and/or its secondary product, a serosal cuticle.

Genetic Studies on Voltinism of a Tropical Race in *Bombyx*

Akio MURAKAMI

A tropical polyvoltine race, Cambodge, produces generally nondiapausing eggs in the Tropics throughout the year. In Japan, however, this polyvoltine race is giving rise to moths which lay nondiapausing eggs during the mild climate season, from spring to early autumn, under natural conditions, while some individuals are often giving rise to moths which lay diapausing eggs. This latter phenomenon particularly appears in the early part of autumn, suggesting that this egg diapause is a physiological and developmental adaptation for increasing the probability of survival during periods of low temperatures or a shortage of feed in the Temperate Zone. In the climate of Japan,

it was once difficult to preserve such a tropical silkworm race, because of the shortage of mulberry leaves as feed. At present it is possible to keep various tropical races throughout the year due to the development of an artificial diet. Accordingly, the tropical race has been preserved with diapasuring eggs having a low hibernating ability. An attempt to selecet and/or establish a highly stable polyvoltine line from the race Cambodge has been made, taking advantage of the artificial diet. The experimental temperature throughout the present study was constantly kept at 25°C and light conditions in the laboratory were controlled by natural sun light through two small windows in addition to fluorescent lighting.

A highly stable polyvoltine line was obtained in the course of only three generations. At the same time, a low line was also selected. However, the the high line rarely produced nondiapause batches regardless of season. The unstable line both diapause and nondiapause batches, J106, Aojuku, and C108 are typical divoltine races giving rise to moths which lay diapausing eggs under the natural conditions in Japan. To further clarify the genetic constitution of the tropical polyvoltine race, the F_1 eggs of a cross between Cambodge females and J106 males, were analyzed for the extent of hibernation. Results showed that most F_1 eggs were nondiapause. Similar tendencies were observed in crosses between Cambodge females and males of various divoltine lines, C108, Aojuku, and Daizo.

When females of various lines were crossed with a fixed Cambodge male, the F_1 eggs were diapause without exception. These observations clearly confirmed that a genetic behavior of voltinism in Cambodge is maternal inheritance as observed by Nagatomo (1926) and by Umeya (1925). The nondiapausing eggs laid by Cambodge females were colorless or non-pigmented and consequently they were designated as a non-pigmented and nondiapause (*npnd*), while the color of diapausing eggs was yellow ocher, peculiar various tropical races. The F_1 moths from a cross between Cambodge females and J106 males laid only diapausing eggs, while those of J106 females and Cambodge males laid mostly nondiapausing eggs. The situation was also confirmed for several different crosses using males of various strains, such as C108, Aojuku, and Daizo. These findings indicate that the nondiapause trait depends on the male parent, suggesting that the genetic factor responsible for the polyvoltine and non-pigmented egg traits in Cambodge is located on the sex-chromosome of the Cambodge race as suggested by Nagatomo (1926) and Umeya (1925).

Studies on the Voltinism Gene, *pnd*, in a Tropical Race of *Bombyx*

Akio MURAKAMI

In a previous study, two different lines, a highly stable polyvoltine line and an unstable polyvoltine line were developed from a tropical polyvoltine race, Cambodge. The unstable line includes a line with a low polyvoltine tendency. In *Bombyx*, it has been shown that the Cambodge race is a stable polyvoltine mutant, which lays pigmented but non-diapause (*pnd*) eggs. It originated from an Indonesian polyvoltine race (Katsumata, 1968). Eggs, homozygous for *pnd*, are continuously nondiapause throughout the year regardless of environmental conditions, while heterozygous eggs (*pnd*/+) are diapause. The gene having such voltinism is located on the 11th (*pnd*) and 12th (*pnd*-2) linkage groups. Accordingly, it is of interest to analyze the relationships between the highly stable polyvoltine line in Cambodge and the *pnd* gene.

When *pnd* females were crossed with highly stable polyvoltine line males of Cambodge, most female moths laid diapausing eggs, but some laid nondiapausing eggs. As a matter of course, F_1 eggs of a reciprocal cross, Cambodge females $\times$ *pnd* males, showed non-hibernation depending on physiological conditions already experienced by females. Thus, it is clear that some Cambodge lines preserved in our laboratory may have a *pnd* gene in heterozygous as well as homozygous states. Accordingly, it can be presumed that genetic constitutions concerning nondiapause traits in Cambodge are expressed as follows: Y/*npnd*: +/+, Y/*npnd*: *pnd*/+ and Y/*npnd*: *pnd*. The first two lines may correspond to unstable lines, while the last one to the highly stable polyvoltine line. The stable line would have been selected out under either natural or laboratory conditions in Japan. Such being the case, the stock of Cambodge seems to have been maintained as Y/*npnd*: *pnd*/+ and Y/*npnd*: +/+. A similar situation would be observed for all stocks of Cambodge in Japan, because this tropical race was originally introduced by Dr. Y. Tanaka from Taiwan to the University of Kyushu in 1935 and subsequently transferred to the National Sericultural Experiment Station, from which the stock has generally been distributed to several research institutions.

Rescue of the SRO-mediated Male-specific Lethal Effect by Cytoplasmic Transplantation in *Drosophila melanogaster*

Masa-Aki YAMADA and Saburo NAWA

Sex-ratio organisms (*Spiroplasma* SRO) are transovarially transmitted into progeny of Drosophila and specifically kill male zygotes. It has been shown that in crosses of females carrying SROs and ring X-males, fewer gynandromorphs were obtained than with controls and those obtained had only small patches of XO-tissues (Tsuchiyama *et al.* 1978, *Genetics* **89**, 711). SROs microinjection experiments suggested that SROs affected male zygotes (with one X-chromosome) at early developmental stages (Omura-Tsuchiyama *et al.* 1980, *Jpn, J. Genet.* **55**, 484; ————, 1981, *ibid.* **56**, 628).

In order to study the male killing action of SROs, We tested whether or not SRO-mediated lethal male zygotes were rescued by microinjection of normal egg cytoplasms. About 2 *nl* (1–2% of egg volume) of egg cytoplasm was injected into SRO-infected eggs at the cleavage stage. Eggs carrying four recessive genes, *y cv v f*, on the X chromosome were used to discriminate males rescued by co-transplanted female nuclei (with two X chromosomes).

In the injections with normal egg cytoplasm at the cleavage stage, 11 of 77 adults obtained (14%) were males carrying SROs in hemolymphes. They had no mosaics exhibiting normal characteristics, indicating that the males had no cells with nuclei from the normal donor. In the case where the cytoplasm was injected into blastodermal embryos, no males were obtained. On the other hand, in injection experiments either with cytoplasms of normal non-cleavage eggs or with SRO-infected cleavage eggs, all adults obtained (131 and 126, respectively) were females. Injections of the cytoplasm of SRO-infected cleavage eggs into normal eggs at the same stage was used as a control and females and males were obtained at a 1: 1 ratio.

These results suggest that the cytoplasm of normal eggs at the cleavage stage contain a substance essential to the normal development of male zygotes, but which is inactivated by SROs.

The Mechanism of Elimination Of Interstitial Cell Lineage in a Mutant Hydra Strain Containing Temperature-Sensitive Interstitial Cell Lineage

Hiroyuki TERADA and Tsutomu SUGIYAMA

The interstitial cell lineage in the mutant strain sf-1 hydra is temperature sensitive. When these hydra are grown at a restrictive temperature (23°C or higher) they lose nearly all interstitial cells and some of their differentiation products (nematoblasts, namatocytes, nerve cells and gland cells) within 48 hours. The mechanism responsible for this cell elimination process was investigated.

Hydra were macerated, the resultant dissociated cells stained with Feulgen, counterstained with Fast green, and then examined for evidence of phagocytosis of interstitial cells by epithelial cells. In some epithelial cells maintained at the elevated temperature, the cytoplasm had large phagocytic vacuoles that contained partially degraded cells, whose nuclei had highly-condensed and intensely Feulgen-positive chromatin granules. This indicates that, as in colchicine-treated (Campbell, 1976) or starved (Bosch and David, 1984) wild-type hydra, epithelial cells in the mutant strain sf-1 eliminate interstitial cells by phagocytosis.

The incidence of phagocytosis was higher in sf-1 tissue maintained at the elevated temperature than that at the normal temperature. However, the observed incidence was relatively low (maximally 0.14 phagocytosed cell per epithelial cell) and appeared to be insufficient to account for the very rapid loss of the interstitial cell lineage at the elevated temperature in this strain. We concluded that elimination of the interstitial cell lineage in strain sf-1 takes place in part by phagocytosis and in part by other yet-unidentified mechanisms (*cf.*, Marcum *et al.*, 1980).

Minimal Critical Size for Hydra Regeneration

Hiroshi SHIMIZU and Tsutomu SUGIYAMA

In hydra, a piece of tissue with approximately 1/30 of the total number of cells of a normal polys can regenerate an entire organism while a smaller piece cannot. Although hydra has a great capacity for regeneration, factors which determine minimal tissue size necessary for regeneration are presently

unknown. In the present study we investigated in detail the process of regeneration from a small picece of hydra tissue, in order to identify the factor(s) determining minimal regeneration size and to gain instight into the underlying principle of hydra pattern formation.

A small square tissue was excised from the body column of a wild strain of *H. magnipapillata* (105) and allowed to regenerate at 18°C. The tissue excised was initially a two layered cell sheet, consisting of an ectodermal and an endodermal layer. Some tissues disintegrated shortly after excision. Within a day, others developed into spheres having a continuous ectodermal layer outside, a continuous endodermal layer inside, and an empty cavity at the center. The entire surface of these spheres was covered by mucous material. Regeneration occurred from these spheres in the next several days.

Twenty-four hours after excision, the diameter of the sphere was measured by a micrometer under a dissecting microscope. It was found that the diameter of the smallest spheres produced was 0.2 mm. These spheres contained an average of about 250 epithelial cells. Various attempts to produce spheres less than 0.2 mm in diameter all failed. This suggests that 0.2 mm represents the minimal tissue mass necessary to form a stable spherical structure. Any tissue too small to form this structure apparently died and disintegrat within 24 hr after excision, presumably due to ion leakage from the tissue.

Spheres ranging in size form 0.20 to 0.45 mm were all found to be capable of regenerating normal hydra. The rate of regeneration, however, varied greatly, depending on the tissue size. It was about 5% for the smallest size (0.2 mm), gradually increasing as size increased, and reaching a value of 100% at 0.45 mm. In addition, time required for regeneration also varied depending on tissue size, ranging from about 8 days for 0.2 mm to about 3.5 days for 4.5 mm.

These features of regeneration were compared to similar features in computer-simulated pattern formation processes based on the reaction-diffusion mechanism.

In the reaction-diffusion system, consisting of a line of cells with homogeneous morphogen distribution, the gradients of the morphogens are not formed if the system size is below a finite critical limit. On passing through this critical limit, gradients of the morphogens appear with the following two features. First, as the system size increases, the gradient level increases

in proportion to ($L-L_c$), where L is the system size and L_c is the critical limit size. Secondly, the time steps required to reach the final steady state decreases in proportion to ($L-L_c$). These two features are qualitatively similar to the two experimentally obtained features of the regeneration described above, suggesting that a chemical reaction-diffusion mechanism plays an important role in hydra regeneration, and that 0.2 mm is close to the minimal critiacal size for pattern formation.

It is probable that two independent factors are involved in determining the minimal size of hydra regeneration. One is the survival factor. Excised tissue too small to form a stable spherical structure can not survive. The other is the space factor for prepattern formation. Prepattern for regeneration (presumably by the reaction-diffusion mechanism) can not be established if the system size is too small. The spherical tissue mass 0.2 mm in diameter and containing about 250 total epithelial cells corresponds, within the accuracy of the analyses, to the minimal limit for both factors in the standard wild type strain.

V. CYTOGENETICS

Theoretical Bases for Karyotype Evolution
1. The Minimum-Interaction Hypothesis

Hirotami T. IMAI, Takeo MARUYAMA, Takashi GOJOBORI,
Yutaka INOUE, and Ross H. CROZIER

Recent literature has shown that the relative probabilities of occurrence for various categories of spontaneous chromsomal mutations do not match those predicted by either the random-contact-and-exchange model or the random-breakage-and -reunion model. These models do not take into account recent findings that, during the meiotic prophase and especially at pachytene, eukaryote chromsomes are attached by each end to the nuclear membrane, leading to a configuration we term the "suspension-arch structure".

We recalculated the relative probabilities of the occurrence of spontaneous chromosomal mutations given the suspension-arch structure and assuming that these rearrangments arise from errors in the resolution of interlockings between bivalents and a special type of crossover that we call the "hetero-site" crossover. From these calculations we found that the relative probability of the occurrence of reciprocal translocations (the most fitness-damaging rearrangement) declines with increases in chromosome number and in nuclear volume. We also found that paracentric inversions occur increasingly more oftern than pericentric ones as the centromere position becomes more terminal and the distance between attachments to the nuclear membrane becomes greater (this distance increases as nuclear volume increases). These results are in accord with cytogenetic data from *Drosophila*, humans, and ants.

A puzzling phenomenon is that the relative rates of radiation-induced chromosomal mutations differ greatly from those calculated under the suspension-arch structure model and from rates of spontaneous chromsomal mutations. We propose the testable hypothesis that (1) most spontaneous chromosomal mutations occur in synaptonemal complexes and involve crossovers and errors in the resolution of interlockings, and (2) radiation and chemical mutagens allow rearrangements when chromsomes intersect at any stage.

These considerations lead to the "minimum-interaction hypothesis", which states that karyotype evolution has been in large part shaped by selection to reduce the occurrence of such fitness-reducting spontaneous chromosomal mutations as reciprocal translocations. Some of the response to this selection is the result of an improvement in DNA-repair mechanisms, an increase in contraction of chromosomes caused by higher-order helix formation, and the development of sex-chromosome heteropycnosis. We concentrate on examining two further interacting responses in the light of this hypothesis. One of these is increase in nuclear volume, but if the ratio of genome size to nuclear volume is high, then an increase in chromosome number, caused by such factors as centric fission, is adaptive because it recduces the occurrence of reciprocal translocation. Although chromsome number can be reduced by centric fusion, such instance seem to be "back eddies" in the main stream of karyotype evolution. For details see *Am. Nat.* **128**: 900–920 (1986).

Polymorphism of a Y Chromosome Repeated Sequence among Asian *Mus musculus* and Related European Subspecies

Pierre BOURSOT* and Kazuo MORIWAKI

pY353/B, a sequence specific to the mouse Y chromosome and representing part of a small multisequence family (Bishop *et al.* 1985, Nature 315: 70–72), was used to proble Southern blots of DNA in males from a variety of Asian mice. Sixteen wild derived strains (maintained at the National Institute of Genetics) of various geographical origins (China, Korea, Taiwan, The Philippines and Indonesia) were tested. They represent a sample of the two parapatric Asian subspecies, *M. m. musculus* (northern type) and *M. m. castaneus* (southern type). Among the 13 restriction enzymes used, three (Eco RI, Hae III and Hind II) did not show any interstrain differences. The other ten (Hind III, Bgl II, Kpn I, Bam HI, Taq I, Hinf I, Alu I, Hpa I, Pvu II and Pst I) produced two patterns, one found in a strain from Bogor (Indonesia), the other found in all other strains. The unique pattern for the first 3 enzymes and the pattern common to the 10 others were found identical to those shown by a European *M. m. musculus* strain (MBT, from Bulgaria). Five strains of Japanese mice of various origins ranging from Hokkaido to Kyushu also displayed the same patterns.

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Using enzymes that best discriminate Bogor from the other Asian strains (Pst I, Hap I, Alu I and Pvu II), we compared the Bogor strain to two other *M. musculus* subspecies, *M. m. bactrianus* (one strain from Iran) and *M. m. domesticus* (4 wild derived strains from England, Canada, France and Bulgaria). The patterns obtained were very similar, but small differences with Hpa I, Pvu II and Pst I showed Bogor and *bactrianus* to be identical but different from the 4 *domesticus*, which were indistinguishable among themselves.

The main conclusions are as follows:

(1) Among the four subspecies of the complex species *M. musculus*, pY353/B identifies two subsets which are well differentiated from each other: *M. m. domesticus* and *M. m. bactrianus* on the one side, *M. m. musculus* and *M. m. castaneus* on the other. Slight differences were detected between the two first subspecies, but the two last are indistinguishable with this probe.

(2) The Bogor strain, believed to belong to *M m. castaneus*, carries a *M. m. bactrianus* Y chromosome. This indicates that genetic introgression has occurred between these two subspecies in Indonesia.

Acknowledgments: We thank C. E. Bishop for providing the probe pY353/B.

Are NORs (Nucleolar Organizer Regions) Necessary for the Formation of Robertsonian Translocations?

Yasuyuki KURIHARA, Heinz WINKING*, François BONHOMME** and Kazuo MORIWAKI

The standard karyotype of *Mus musculus*, including the laboratory mouse, consists of 20 pairs of acrocentric chromosomes, but some European wild mice have a reduced chromosome number (2n=22–39) due to fusions of acrocentric chromosomes at their centromeres (Robertsonian translocations). The mechanism which caused these centric fusions in European wild mice is controversial. In some cases, NORs, cytologically defined ribosomal RNA genes, have been shown to play an essential role in Rb translocations in man and mouse. From the accumulated data already reported, we examined all chromosome combinations so far involved in Rb fusions to de-

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termine whether NORs are prerequisite for the formations of metacentric chromosomes in European wild mice. Statistical examination suggested that NOR-bearing chromosomes are not necessarily required for the Rb exchanges, but probably also inhibit them when two NOR-bearing chromosomes are concerned. This is inconsistent with the above hypothesis.

Previously we reported that some Rb-mice are probably an intersubspecies hybrid between *M. m. domesticus* and *M. m. bactrianus* based on the restriction fragment length polymorphisms (RFLPs) of ribosomal RNA genes. Further analysis of RFLPs in genomic DNAs of European wild mice carrying either the normal karyotypes or Rb-exchanges confirmed the unique genetic character of Rb-mice as such.

Chromosomal Locations of NORs (Nucleolar Organizer Regions) in Standard Strains of the Laboratory Mouse

Yasuyuki KURIHARA, Dong Sang SUH* and Kazuo MORIWAKI

Genes coding for ribosomal RNA are cytogenetically demonstrated by silver staining. On the average, a mouse has about 200 copies of ribosomal RNA genes, which are repeated tandemly and located on several different chromosomes. Their chromosomal locations have so far been reported only in four laboratory mouse strains among hundreds. We attempted to survey widely the distribution of NOR in various mouse strains.

Spleen cell cultures stimulated by concanavalin-A and lipopolysaccharide were employed for the chromosome preparations. After chromosome identifications by G-banding, a silver staining procedure modified from Howell and Black was applied to the samples. At least 35 well spread karyotypes were analyzed in each strain. As summarized in Table 1, chromsomal locations of NORs in the 10 inbred strains were restricted to five pairs of chromosomes, #12, #15, #16, #18 and #19, but the frequencies of the appearance varied. For example, the 129/J strain had NORs on chromosomes #12, #16, #18 and #19. Most cells (>80%) had NORs on #12, #18 and #19. Only 20% of the cells had NOR on #16, in addition to #12, #18 and #19. Similar results were reported previously on chromosome #16 in the BALB/c strain. This phenomenon was explained by the reduced number of ribosome RNA genes on #16. Alternatively, we can also assume that the regulated expression of

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Table 1. NORs distributions and frequencies of appearance in inbred strains of mice

Strain	No. of karyotypes analyzed	Chromosome number and appearance of NORs (%)									
		10	11	12	13	14	15	16	17	18	19
SM/J	40							96		94	
CBA/J	40						95			98	41
CE/J	45			67			86			42	
C3H/J	38						97	68		67	
129/J	35			90				20		83	87
RIII/J	41			93			83			70	96
SJL/J	41			31						96	91
I/J	39			85			73			77	89
WB/ReJ-W	38			82				67		37	89
NZB/B1NJ	38			89				38		93	66

ribosome RNA genes might cause this phenomenon.

Effect of Partial Deletion of Y Chromosome on Morphology and Protein Composition of Mature Mouse Spermatozoa

Józefa STYRNA*, Dong Sang SUH** and Kazuo MORIWAKI

Spermatozoa of males from the inbred mouse strains differ widely in the percentage of abnormal heads and in efficiency of fertilization. The differences in sperm abnormality were found to be polygenically determined by a small number of autosomal genes and the role of Y chromosome was detected (Krzanowska H., 1976 *Genet. Res.* **28**: 189). To get more insight into the function of Y chromosomal genes, B10BR/SgSn and B10BR-Y^{del}/Ms congenic males originated from B10BR/SgSn by partial deletion of Y chromo some were used for comparative morphological and biochemical analysis of mature sperm.

The two congenic strains differ considerably with respect to the proportion of abnormal spermatozoa which amounts to 21.9% in B10BR/SgSn and as much as 59.6% in B10BR-Y^{del}/Ms strain. In the F_1 generation a heterosis effect was observed if B10BR/SgSn males were crossed to females from

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SJL/J, AKR/J. C3H/HeJ, CBA/J and BALB/cAnN strain (0.6–3.6% of abnormalities) but not if B10BR-Y^{del}/Ms males were used for matings (19.9–62.7% of abnormalities respectively). It appeared that not only the total percentage of abnormal sperm, but also the frequency distribution of abnormality classes were characteristic for a given strain. In B10BR-Y^{Pel}/Ms males the most frequent is class with flat acrosomal part (49.4% of all abnormalities), but in B10BR/SgSn males only 1.2% abnormal heads belong to this category.

Three distinct sperm fractions were recovered following density gradient centrifugation in 1.6 M sucrose: SDS-soluble proteins mostly derived from plasma membrane and acrosome, SDS-insoluble tail components and SDS-insoluble head components. A significant reduction of a major-39.000 molecular weight component in SDS-soluble fraction of B10BR-Y^{del}/Ms sperm was detected by polyacrylamide gel electrophoresis. Triple staining procedure used for evaluation of site of acrosomal enzymes activity showed a failure of acrosome staining for about 30% of B10BR-Y^{del}/Ms sperm. The silver staining for proteinase activity was performed for sperm separated by sedimentation in Percoll density gradient. Upper layers containing mostly Y-bearing sperm exhibit 87.3% negatively stained cells.

These results suggest that deleted fragment of Y chromosome contains gene(s) exerts a regulatory function on the synthesis of acrosomal proteinase. Low production of the proteinase can cause inability of Y-bearing sperm for fertilization. This conclusion is also supported by evidence that progeny derived from matings with B10BR-Y^{del}/Ms males shows significantly distorted sex ratio with lower proportion of males ($x^2=31.3$ $p>0.02$).

High sperm abnormality in mutant males and absence of heterosis effect indicate that a factor affecting head shaping of spermatozoa is also located on the Y chromosome and can be separated from the other polygenes influencing the same character.

Establishment of Mouse Strains Congenic for Mitochondrial DNA for Transplantation Experiments

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Transplantation of cells and tissues is a useful technique in biomedical reseach, especially in developmental biology, immunology and oncology. It

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is often important to be able to easily distinguish donor from host cells of any organ with high sensitivity and reliability in quantitative analysis. For this purpose, we have developed unique congenic strains that carry mitochondrial DNA (mtDNA) of a restriction enzyme phenotype rarely found among laboratory mice. The mtDNA-congentic strains have at least two advantages: The restriction enzyme phenotype of mtDNA can easily be detected with high sensitivity from any tissue or cell samples, and its amount can be precisely determined by Southern blot hybridization (P. Bursot, H. Yonekawa and F. Bonhomme, *Mol. Biol. Evol.*, in press).

We have bred and established four mtDNA-congenic mouse strains by successive backcrosses, using ddY as maternal progenitor: C57BL/6J-mtJ, C57BL/10Sn-mtJ, DBA/2Yok-mtJ and DDY/Yok-mtJ. The closed colony of the ddY stock contains two types of mtDNA: the common type of standard laboratory mice and the mtJ (for Japan) type that differs by its restriction enzyme phenotype. Comparison of ddY mice with wild subspecies of the house mouse, *Mus musculus*, suggests that the mtJ type of mtDNA comes from an Asian race of *M. m. musculus* (previously termed *M. m. molossinus*) (H. Yonekawa *et al.*, *Curr. Top. Microbiol. Immunol.* **127**, 62, 1986). The mtJ type has so far been found only in the ddY and RR laboratory strains established in Japan, and not in any wild mice (H. Yonekawa *et al.*, *Differentiation* **22**, 222, 1984).

It is known that mtDNA can affect cell surface antigenicity throught Mta, the maternally transmitted antigen of mice, which can cause rejection of transplanted cells by a host of different mtDNA type (K. Fischer Lindahl, Trends Genet. **1**, 135, 1985). Fortunately, the mtJ type mtDNA shares the common α phenotype with more than 95% of all laboratory strains and wild mice, and thus we avoid the complications that would follow an immune response against cells congenic for other types of mtDNA (K. Fischer Lindahl, H. Yonekawa and K. Moriwaki, to be published).

At generations N9 to N12, we examined enzyme markers from 12 chromosomes and found, as expected, that they had all become fixed for the allele of the paternal backcross parent. We therefore concluded that the nuclear genes of all four mtDNA-congenic strains were now identical to those of their respective inbred partner strain.

To investigate whether mtDNA-congenic mice are useful for transplantation experiments, we made two chimeric mice by aggretation of embryos from strains C57BL/6J-mtJ and DBA/2Yok. At 8 weeks of age, these spot-

ted mice were killed and 15 organs (brain, kidney, testis, heart, muscle, thymus, spleen, peripheral blood, liver, pancreas, salivary gland, lung, stomach, small intestine, and large intestine) were removed for mtDNA typing with restriction enzymes. From brain, liver, kidney, testis, heart and muscle, mtDNA was isolated by the cleared lysate method and the restriction fragments patterns detected by ethidium bromide staining and by Southern blot analysis. The restriction phenotype of the mtDNA of the other organs was examined by Southern blot analysis only, using total DNA from these organs and purified mtDNA, nick-translated with ^{32}P-α-CTP, as a probe. All organs showed both mtDNA restriction phenotypes, but the composition varied from 5 to 50% of the mtJ type between organs. These results show that mtDNA can be typed in any tissue and the mtDNA-congenic strains can be usefully applied in transplantation experiments.

VI. MUTAGENESIS AND RADIATION GENETICS

Des-mutagenic Action of Refined Corn Bran

Tsuneo KADA,† Tadashi INOUE, Masako HARA
and Masayasu TAKEUCHI*

Many kinds of desmutagens, which are defined as agents that inactivate mutagens by reacting directly with them, have been identified in various natural sources and the possible role in environmental mutagenesis has been suggested by many investigators.

We report here that refined corn bran (RCB), a dietary fiber derived from the mechanical refining of corn hulls, irreversibly adsorbed and inactivated various environmental mutagens. When RCB was added at a concentration of 10 mg/m*l* to an aqueous solution of dinitropyrene, one of the most abundant environmental carcinogen, 91.6% of the mutagenicity towards Salmonella tester strain TA-98 disappeared. Under similar conditions decreases in mutagenicity of dinitropyrene using wheat bran and cellulose powder were 58.4% and 43.0%, respectively. The adsorption of dinitropyrene to the fibers appeared irreversible since little mutagenicity was recovered by washing the treated fibers with aqueous buffer solutions of various pH's. Even with an organic solvent (methanol: ammonium hydroxide; 50: 1), only two thirds of the mutagenicity of dinitropyrene was recovered. RCB was also able to inactivate mutagenic heterocyclic amines such as IQ, Trp-P-1, Trp-P-2, Glu-P-1, and Glu-P-2.

Considering that RCB is not digested in the human body, it can adsorb and inactivate mutagens/carcinogens and cause them to be rapidly excreted. RCB may therefore have a protective effect against dangerous carcinogens in the human body.

† Deceased on November 14, 1986.
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Cobaltous Chloride, an Antimutagen, Enhances Genetic Recombination

Tadashi INOUE and Tsuneo KADA

In earlier studies, we demonstrated that cobaltous chloride, a potent antimutagenic metal compound, stimulated the RecA protein *in vitro* enhancing the formation of D-loop which is thought to be an intermediate in genetic recombination. The metal compound also elevated the ATPase activity of the RecA protein, which is expressed during the unwinding of double-stranded DNA. These data suggest that this metal compound exhibits its antimutagenicity elevating recombinational activity in the cells. In order to confirm this concept, we examined whether the metal compound stimulates genetic recombination *in vivo* using a newly developed assay system.

Plasmid pMW334 (Am^RTc^S) and pTH4 (Cm^RTc^S) were derived from pBR322 (Am^RTc^R) and pACYC184 (Cm^RTc^R), respectively. Plasmids pMW334 and pTH4 were made Tc^S through *in vitro* mutagenesis at different sites in the identical Tc genes of parental plasmids. In cells harboring both pMW334 and pTH4, genetic recombination between the Tc gene of pMW334 and pTH4 yields a $Tc^RAm^RCm^R$ phenotype which can easily be detected.

In this system, cobaltous chloride increased recombination frequency by several-fold suggesting that the antimutagenicity of the metal compound can be ascribed to the stimulation of error-free recombination repair activity catalyzed by the RecA protein.

Bio-antimutagenic Effect of L-ethionine on the Spontaneous Mutagenesis in *Salmonella* and *Bacillus subtilis*

Tadashi INOUE and Tsuneo KADA

Ethionine, the ethyl analogue of the essential amino acid methionine, is one of the well-known hepatocarcinogen. Recent results reported from this and other laboratories suggest that ethionine interferes with DNA metabloism resulting in a alteration of mutation frequency in treated cells. In order to gain more insight into the genetic action of ethionine, we examined the genetic effect of ethionine using bacterial systems which exhibit high spontaneous mutation frequencies due to known molecular mechanisms.

Salmonella TA98 and TA100 are known to exhibit relatively high spon-

taneous mutation rates due to the presence of plasmid pKM101 which encodes genes for error-prone repair enzymes. When applied to these strains, L-ethionine diminished spontaneous mutation frequencies in a dose-dependent manner, and at 5 μg/m*l* of L-ethionine every few mutant colonies deeveloped whereas D-ethionine did not exhibit such an antimutagenic effect at all. L-ethionine also abolished spontaneous mutations in the parental strains. TA1538 and TA1535, which do not harbor the plasmids, indicating that plasmid-mediated mutagenesis is not specifically involved in the expression of the bio-antimutagenic activity of L-ethionine.

Another system we examined was the spontaneous mutagenesis in *Bacillus subtilis* NIG1125 in which an error-prone DNA replicating enzyme causes the induction of a high spontaneous mutation rate. Also in this system, L-ethionine was very effective in reducing spontaneous mutation induction, indicating a possible involvement of the error-prone DNA replicating enzyme in the expression of bio-antimutagenic activity of this amino acid.

One of the most probable targets for the action of L-ethionine is the methionine in cells. Ethionine may be incrorporated into protein in place of methionine or these two amino acids may compete with each other during methylation of biomolecules including proteins and nucleic acids. This idea is supported by the fact that the bio-antimutagenic effect of L-ethionine was no longer observed when L-methionine, but not D-methionine, was present in the medium. From these results, we suggest that modification by L-ethionine of an enzyme functioning in repair/replication is a causal factor for the bioantimutagenic action of this amino acid.

Adenosine Deaminase Activity in Wasted Mouse, a Radiation Sensitive Mutant

Tadashi INOUE, Hideo TEZUKA and Tsuneo KADA

The mouse mutant "wasted" (*wst/wst*) is a putative animal model for ataxia-telangiectasia (A-T), a human genetic disease characterized by chromosomal instability, radiation-hypersensitivity, immune-deficiency and increased predisposition to cancer. Although the wasted mouse shares many characteristics with A-T, it also resembles another human genetic disease SCID (severe combined immunodeficiency syndrome) whose causal factor is known to be anomaly of adensoine deaminase (ADA). Recently Abotte *et al.* reported the decreased level of ADA in erythrocytes of the

wasted mouse indicating that *wst* is the structural gene for ADA. According to our previous observations, however, target organs of wasted mutation are restricted to lymphoid organs such as spleen or thymus, and the wasted phenotype appears age-dependently. We therefore examined ADA activity in the extracts from spleens of wasted and control animals of various ages in order to confirm the observation of Abotte *et al.* However, differences in the ADA activity between wasted and control animals could not be detected throughout the developmental stages examined. Simple interpretation that reduced ADA activity in erythrocytes in wasted mouse is a result of mutation in the structural gene for ADA should therefore be re-examined. We are now comparing the ADA gene from wasted mouse with that of normal mouse at sequence level.

Ultraviolet Induction of Chromosome Aberrations and Separation Inhibition in Chromosomes of the *rad-2* Strain of *Caenorhabditis elegans*

Yoshito SADAIE and Tamiko SADAIE

The nematode *Caenorhabditis elegans* is transparent and has less than 1000 somatic cells with established cell lineage. Transparency permits studies on the mechanisms of DNA repair and mutation induction using ultraviolet irradiation. In preceding reports (No. 35, No. 36) we described the gamma-ray induced chromosome aberrations in early embryonic cells and a repair of damages responsible for the aberrations, depending on *rad-2* gene activity. In this study we examined the induction of chromsome aberrations by ultraviolet irradiation. Results showed that ultraviolet irradiation induced aberrations in the *rad-2* strain but not in the wild type strain. Furthermore, ultraviolet strongly inhibited separation of metaphase chromosomes only in the *rad-2* strain. This might suggest that ultraviolet suppresses DNA synthesis in the *rad-2* strain followed by a defective separation of the centromeric regions of the chromosomes. Therefore the *rad-2* gene might be directly involed in DNA repair, and the aberrations induced by ultraviolet might result from a defective DNA repair process in the *rad-2* strain.

A Cytogenetic Study on the Radiosensitivity of Bone Marrow Cells of Wasted Mice

Hideo TEZUKA, Koichi TAMAI, Tadashi INOUE
and Tsuneo KADA

The induction kinetics of chromsomal aberrations and of micronuclei in bone marrow cells of wasted mice were compared with those in controls. Dose-response studies 24 hours after gamma-ray irradiation revealed the presence of numerous aberrations in metaphases of bone marrow cells in wasted mice but only a few micronuclei in polychromatic erythrocytes, in contrast to what was observed in controls. Time-course studies conducted at a constant gamma-ray dosage of 1 Gy revealed delayed recovery from radiation-induced chromosomal aberrations and a low and delayed appearance of micronuclei in wasted mouse bone marrow.

These results suggest either the presence of a sensitive stage to radiation-induced damage in the wasted mouse cell cycle or a severe delay during the condensation and the expulsion of nuclei from erythroblasts.

The Mode of Action of Vanillin, an Food Additive, on Chemically Induced Cytotoxicity and Mutations in Cultured Chinese Hamster Cells

Koichi TAMAI, Hideo TEZUKA, Yukiaki KURODA
and Tsuneo KADA

Some chemicals in our environment have significant effects on the biological properties of living cells. Vanillin is frequently used as a flavoring in food, and this chemical has been found to have a potential of suppressing chemically induced mutations by enhancing a function of rec A-dependent error-free DNA repair in *E. coli* (Ohta *et al.*, 1986, *Food Chem. Toxic.* **24**: 51–54).

We examined possible modifying effects of the chemical, vanillin, on cytotoxicity and genetic alterations in cultured Chinese hamster V79 cells treated with two different types of mutagens, EMS (ethylmethanesulfonate) and H_2O_2 (hydrogen peroxide). In the case of EMS treatment, a significant decrease in cell numbers survived and a significant increase in induced 6-thioguanine-resistant mutants have been found in cells by co-treatment with vanillin and EMS when compared with cells treated with EMS alone. These effects were observed as a function of vanillin dosage. In contrast, we found

a different kind of action of H_2O_2 from that of vanillin on V79 cells. Vanillin shows a pronounced effect on recovery from H_2O_2-caused cytotoxicity. A preliminary result showed that vanillin treatment decreased the frequency of chromosomal aberrations induced by H_2O_2.

These results suggest that biological effects of vanillin are dependent on the types of mutagens used and that some different mechanisms may operate in cellular repair of chemically induced DNA lesions in cultured mammalian cells.

VII. POPULATION GENETICS

Diffusion Model of Population Genetics Incorporating Group Selection, with Special Reference to an Altruistic Trait

Motoo KIMURA

In order to investigate under what conditions an altruistic trait evolves through group selection, the following diffusion model was formulated. Consider a species consisting of an infinite number of competing groups (demes), each having a constant number of reproducing members and in which mating is at random. Then consider a gene locus and assume a pair of alleles A and A', where A' is the "altruistic allele." Let x be the relative frequency of A' within a deme, and let $\phi=\phi(x;t)$ be the density function of x at time t such that $\phi(x;t)\Delta x$ represents the fraction of demes whose frequency of A' lies in the range $(x, x+\Delta x)$. Then, we have

$$\frac{\partial\phi}{\partial t}=\frac{1}{2}\frac{\partial^2}{\partial x^2}\{V_{\delta x}\phi\}-\frac{\partial}{\partial x}\{M_{\delta x}\phi\}+c(x-\bar{x})\phi\ ,$$

where $M_{\delta x}$ and $V_{\delta x}$ stand for the mean and variance of change in x per generation (due to mutation, migration, individual selection and random sampling of gametes) within demes, and c is a positive constant (called the coefficient of interdeme competition) and $\bar{x}$ is the mean of x over the species, i.e. $\bar{x}=\int_0^1 x\phi dx$. By studying the above diffusion equation at a steady state ($\partial\phi/\partial t=0$), a condition is obtained for group selection to prevail over individual selection in the evolution of an altruistic trait. For details, see *Stochastic Processes and Their Applications* (Ed. K. Itô and T. Hida) pp. 101–118. Lecture Notes in Mathematics, Springer-Verlag, Berlin.

DNA and the Neutral Theory

Motoo KIMURA

The neutral theory claims that the great majority of evolutionary changes at the molecular (DNA) level are caused not by Darwinian selection but by

random fixation of selectively neutral or nearly neutral mutants. This theory also asserts that the majority of protein and DNA polymorphisms are selectively neutral and that they are maintained in the species by mutational input balanced by random extinction. In conjunction with diffusion models (the stochastic theory), of gene frequencies in finite populations, it treats these phenomena in quantitative terms based on actual observations.

Although the theory has been strongly criticized by the 'selectionists', supporting evidence has accumulated over the years. Particularly, the recent outburst of DNA sequence data lends strong support to the theory, both with respect to evolutionary base substitutions and DNA polymorphism, including rapid evolutionary base substitutions in pseudogenes. In addition, the observed pattern of synonymous codon choice can now be readily explained in the framework of this theory. I reviewed these recent findings in the light of the neutral theory. For details, see *Phil. Trans. R. Soc. London B* **312**: 343–354.

Actual Number of Alleles Contained in a Multigene Family

Tomoko OHTA

By using a simple model of gene conversion, the actual number of alleles contained in a multigene family was theoretically studied. It was shown that the Ewens' sampling theory is applicable in predicting the actual number of alleles in a gene family of a genome. However, the actual number of gene families forming the total population becomes larger or smaller than the predicted value by the sampling theory, depending upon the relative magnitude of the rates of two homogenization processes; intra-genome and within the population. For details, see *Genet. Res., Camb.* **48**: 119–123.

Population Genetics Theory of Multigene Families with Emphasis on Genetic Variation Contained in the Family

Tomoko OHTA

The significance of repetitive DNA families in evolution has three aspects; to increase genetic information, to regulate expression of structural genes, and to increase themselves as selfish DNA. Various existing multigene families (or gene clusters, supergenes and gene complexes) are real examples

of the first aspect. Their evolution is characterized by concerted change due to a continued occurrence of unequal crossing-over, gene conversion and transposition. A population genetics theory concerning evolution was developed based on identity coefficients. Transitional equations of allelism, an allelic identity coefficient, and two nonallelic coefficients are presented for a model of gene conversion and duplicative transposition under the assumption of constant copy number per genome. Results of numerical studies show that by adjusting parameter values such as copy number, conversion rate and transposition rate, any desired gene family may be attained with respect to genetic variability contained in the family. Natural selection on these parameters is termed "indirect selection." Actual gene families range from those with highly uniform members to those made of quite diverse gene copies, and they are likely to be attained by indirect selection. For details, see *Evolutionary Processes and Theory*, (Eds. S. Karlin and E. Nevo) 239–253, Academic Press, New York.

Population Genetics of an Expanding Family of Mobile Genetic Elements

Tomoko OHTA

A model of an expanding family of dispersed repetitive DNA was studied. Based on previous results using the model of duplicative transposition, an approximate solution for presenting allelisms and identity coefficients as functions of time was obtained, and theoretical predictions were verified by Monte Carlo experiments. Results showed that even if the copy number per genome increases very rapidly, allelism and identity coefficients may take a long time to reach equilibrium. Changes in allelism and allelic identity are similar to those of homozygosity at an ordinary single locus, whereas those of nonallelic identity can be much slower, particularly when the copy number per genome is large. Thus, many existing families of highly repetitive sequences may represent nonequilibrium states for nonallelic identity. The present model may be extended to include other evolutionary forces such as gene conversion or the recurrent insertion from normal gene copies. For details, see *Genetics* **113**: 145–159.

An Attempt to Estimate the Effective Size of an Ancestral Species Common to Two Extant Species from which Homologous Genes are Sequenced

Naoyuki TAKAHATA

When DNA sequence data on various kinds of homologous genes sampled from two related species are available, the effective size of their ancestral species can be inferred from a simple consequence of gene genealogical considerations. This method, when applied to the common ancestral species of human and rat, human and mouse, human and bovine, or rodents and bovine estimates their effective sizes all to be of the order of 10^7, supporting the view that these species indeed shared, around 75 million years ago, a common ancestral species from which they are descended. The effective size thus estimated would imply that the ancestral species was abundant enough to have ample opportunity for adaptive radiation. The extent of silent polymorphism in that species might have been very large, possibly comparable to the number of silent substitutions accumulated in a gene after mammalian divergence. Some causes that may alter these results and require a more elaborated statistical analysis were discussed. For details, see *Genet. Res., Camb.* **48**: 187–190.

Private Alleles in a Partially Isolated Population II. Distribution of Persistence Time and Probability of Emigration

Naoyuki TAKAHATA and Montgomery SLATKIN

To study statistically useful properties of "private" alleles in partially isolated populations, we developed and analyzed a general Wright-Fisher model of migration, mutation, and selection with an arbitrary degree of dominance. Instantaneous killing of the process due to emigration of a mutant leads to one of two diffusion processes with a killing term. One (*weak gene flow*) is the boundary case of the other (*strong gene flow*), which can cover a wide range of gene flow. The diffusion process subject to strong gene flow is similar to that studied by S. Karlin and S. Tavaré (1983, *SIAM J. Appl. Math.* **43**: 31–41). The spectral decompostition of the transition probability density of mutant allele frequencies is presented in the case of strong gene flow. The fate of mutants in a deme is governed largely by

random sampling drift and emigration. When the emigration rate is high, the quantitative features of "private" alleles are due mostly to the distribution of alleles after their initial spread to other populations and may have fairly long histories since their first appearance. For details, see *Theor. Pop. Biol.* **30**: 180–193.

A Stochastic Model of Gene-Culture Coevolution Suggested by the "Culture-Historical Hypothesis" for the Evolution of Adult Lactose Absorption in Humans

Kenichi AOKI

A stochastic model of gene-culture coevolution, suggested by the "culture historical hypothesis" of Simoons and McCracken, was presented. According to this hypothesis, adult lactose absorption, believed to be an autosomal dominant trait, attained a high frequency in some human populations due to positive selection pressure induced by culturally determined milk use in those populations. Two-dimensional Kolmogorov backward equations with appropriate boundary conditions were derived for the ultimate fixation probability of milk users, of the gene for adult lactose absorption, and of both jointly, and for the average time until fixation of the gene. These boundary value problems were solved numerically by the Gauss-Seidel method. I defined a theoretical measure of the correlation between gene and culture in terms of the three ultimate fixation probabilities. Monte Carlo simulations were conducted to check and extend the numerical results and also to obtain the first arrival time at gene frequency 0.70, which is approximately the highest observed frequency in any population. Two results that pertain to the culture historical hypothesis were obtained. First, the incomplete correlation observed between adult lactose absorption and milk use does not necessarily constitute evidence against the hypothesis. Secondly, for the postulated genetic change to have occurred within the 6000-year period since the advent of dairying, either the effective population size was of the order of 100, or, if it was of a larger order, the selection coefficient probably had to exceed 5%. For details, see *Proc. Natl. Acad. Sci. USA* **83**: 2929–2933.

Stable Polymorphic Equilibria in a Toy Model of Group Selection

Kenichi AOKI

A basic model of group selection was formulated by Eshel (1972) and corrected for an "unnatural" assumption by Roughgarden (1979). The model assumes two counteracting selective forces, group selection and individual selection, in a deme-structured population; and was proposed in relation to the evolution of altruistic behavior. In this study, I derived further properties of this model, concentrating on the special case of two haploid individuals per deme. Specifically, I demonstrated the existence and stability of polymorphic equilibria in the extreme case of migration rate 1. The existence and stability of other equilibria were studied systematically by defining four regions in the (m, k)-parameter space for fixed s. Here, k is a measure of group selection, s is the selection coefficient against the individually deleterious type, and m is the migration rate. One region appears to correspond to stable polymorphic equilibria. Results were discussed in relation to the critical equality $k=2Nms$ where N is the deme size (Aoki, 1982), and also in relation to polymorphic equilibria in the diffusion approximation when mutation is ignored (Kimura, 1983, 1984; Ogura and Shimakura, submitted). For details, see *Jpn. J. Genet.* **61**: 481–490.

Gene Flow in Natural Populations of *Drosophila melanogaster*

Tsuneyuki YAMAZAKI, Jong-Kil CHOO, Takao K. WATANABE and Naoyuki TAKAHATA

Neutral or nearly neutral genes may not always be appropriate for estimating the level of gene flow in a geographically structured population unless the level is rather low, or the population size is small. In this experiment, we dealt with natural and cage populations of *D. melanogaster* and studied lethal allelism rates and protein variation simulataneously. A survey of 14 protein loci, together with frequencies and within- and between-population allelism rates of lethal second chromosomes, was carried out in five (four Japanese and one Korean) natural populations and one cage population. It was found that lethal allelism rates decreased rapidly as geographical distance between two populations increased, while variation at protein

loci showed a remarkable similarity over all populations examined. These findings suggest that there are very high levels of gene flow in these natural populations and that selection at protein loci which can maintain substatial geographic variation, if present, is overshadowed by gene flow. There is no indication that invasion of *D. melanogaster* to the Far East occurred so recently that the frequencies of lethal chromsomes are still in nonequiribrium. For details, see *Genetics* **113**, 73–89.

The Relationship between Viability and Number of P Elements in the Second Chromosome of *Drosophila melanogaster*

Terumi MUKAI, Ko HARADA and Shin-ichi KUSAKABE

The copy number of one kind of transposable element is limited to a certain range in one individual. There must be some factor by which the maximum number per individual is determined. The P element, one of the transposable elements in *D. melanogaster*, induces deleterious mutations when this active element moves on the chromosome. However, it appears that activity of transposition gradully disappears when the P type changes to the Q and from Q to M′. We examined the relationship between the copy number of all types of P elements and the viability of their carriers.

Two populations were chosen, the Raleigh, N. C. population and the Nagasaki population. Results of the gonad sterility test for the P-M hybrid dysgenesis are shown below as the percentage of each type: In this table, *n* stands for the number of tested lines. These results indicate that P elements in vaded the Nagasaki population earlier than the Raleigh population and in the former no active P element can be seen.

Type	P	Q	M or M′	Others
Nagasaki (n=81)	0.0%	60.5%	37.0%	2.5%
Raleigh (n=68)	50.0%	26.5%	10.3%	13.2%

The average number of P elements in the second chromosomes were estimated in homozygotes by an *in situ* hybridization method: The Nagasaki population: 13.20±4.26 (1984); The Raleigh population: 15.22±4.76 (1984), 17.50±4.36 (1970). The figures in parentheses indicate the years when the

samples were taken. It is likely that as the number of active P elements descreases, the copy number of the element slightly decreases but not significantly so.

Finally, the relative viabilities of second chromosomes were estimated by the *Cy* method, and a correlation was calculated between viability and the copy number of P elements. This test has not been completed yet, but, thus far, the following results have been obtained:

The Nagasaki $\hat{r}=-0.12$ ($n=23$)

The Raleigh $\hat{r}=-0.24$ ($n=15$)

Both estimates are not significantly different from 0 but they are negative. Studies are continuing.

VIII. EVOLUTIONARY GENETICS

Conservation of Alleles and of Genic Heterozygosity in Small Managed Populations

Takeo MARUYAMA

Data has been presented on the loss of alleles from populations of small fixed size. Emphasis has been placed on the effect which the allele frequency distribution and the interlocus distribution of heterozygosity will have on the pattern of allelic loss. Rare alleles are rapidly lost during the initial sampling period, and continue to be lost for several generations following the establishment of small population size. The nonequilibrium nature of the process of loss of genetic variability is stressed. The rapid loss of rare alleles and the preservation of intermediate and high frequency alleles will result in (1) heterozygosity declining much more slowly than allele number, and (2) the establishment of genetically similar populations when sampled from the same base population. For details, see *Zoo Biology* **5**: 171–179.

Relative Contributions of Germline Gene Variation and Somatic Mutation to Immunoglobulin Diversity

Takashi GOJOBORI and Masatoshi NEI

The relative contributions of germline gene variation and somatic mutation to immunoglobulin diversity were studied by comparing gene sequences with their rearranged counterparts for mouse V_H, V_κ, and V_λ genes. The mutation rate at the amino acid level was estimated to be 7.0% in the first and second complementarity-determining regions (CDRs) and 2.0% in the framework regions (FRs). Differences in mutation rates at the nucleotide level between the CDRs and FRs were of the same order of magnitude as those for the amino acid level. Analysis of amino acid diversity or nucleotide diversity indicated that the contribution of somatic mutation to immunoglobulin diversity is about 5%. However, the contribution of somatic mutation to the number of different amino acid sequences of immunoglobulins is much larger than that estimated by the analysis of amino acid diversity, and more than 90% of the different immunoglobulins seem to be generated

by somatic mutation. Examination of the pattern of nucleotide substitution has suggested that clonal selection after somatic mutation may not be as strong as generally believed. For details, see *Mol. Biol. Evol.* **3**: 156–167 (1986).

A Sequence Homology between the pX Genes of HTLV-I/II and the Murine IL-3 Gene

Takashi GOJOBORI, Shin-ichi AOTA, Tadashi INOUE and Kunitada SHIMOTOHNO

Searching the protein sequence database for amino acid sequences homologous to the *x-lor* sequence in the pX region of human T-cell leukemia virus types I and II (HTLV-I/II), we found that there is a region of 38 amino acids where the murine interleukin 3 (IL-3) sequence has a 40% homology with the *x-lor* sequence. A statistical analysis shows that this homology is highly significant with a probability of 1.57×10^{-10}. Since various T-cell lines established by HTLV-induced transformation have been demonstrated to produce several lymphokines including IL-3, it is possible that the activation of the IL-3 gene may have been an important step in the production of this leukemia. Thus, biological functions of the pX gene involved in the mechanism of HTLV-induced leukemogenesis may be related to those of IL-3. For details, see *FEBS letters* **208**: 231–235 (1986).

Simple Methods for Estimating the Number of Synonymous and Nonsynonymous Nucleotide Substitutions

Masatoshi NEI and Takashi GOJOBORI

Two simple methods for estimating the number of synonymous and nonsynonymous nucleotide substitutions were presented. Although they give no weights to different types of codon substitutions, these methods give essentially the same results as those obtained by Miyata and Yasunaga (1980) and by Li *et al.* (1985). Computer simulation indicated that estimates of synonymous substitutions obtained by the two methods are quite accurate unless the number of nucleotide substitutions per site is very large. It was shown that all available methods tend to give an underestimate of the number of nonsynonymous substitutions when the number is large. For details, see *Mol. Biol. Evol.* **3**: 418–426 (1986).

Evolution of Nested Genes with Special Reference to Cuticle Proteins in *Drosophila melanogaster*

Etsuko N. MORIYAMA and Takashi GOJOBORI

One of the pupal cuticle protein (PCP) genes has been found within an intron of a Drosophila house keeping gene, Gart locus, which encodes three enzymes in the purine pathway. This intronic gene has been described as a gene within a gene, and the gene is now called a "nested" gene. This "nested" gene structure has been taken as an unambiguous exception to the classical linear model of gene organization, particularly in the nuclear genomes of eukaryotes. Since the intronic PCP gene has a sequence homology with larval cuticle protein (LCP) genes, it should have been derived from one of the LCP genes or their ancestral gene. Therefore, we attempted to elucidate the evolutionary origin of the "nested" gene structure using these genes. Comparing nucleotide sequences of four LCP genes with those of the PCP genes, we studied possible phylogenetic relationships among these five genes. The results obtained suggest that the PCP gene may have originated from its ancestral gene before duplication of the LCP genes occurred. Using the number of synonymous (silent) substitutions, we than estimated the divergence time between the PCP gene and LCP genes to be about 70 million years (Myrs). The estimated divergence time between these genes is much larger than that of *D. melanogaster*'s sibling species (about 2.5 Myrs). Thus, it is probable that the "nested" gene structure can be seen not only in *D. melanogaster*, but also in other Dorsophila species.

Higher Rates of Nucleotide Substitution in Drosophila than in Mammals

Etsuko N. MORIYAMA and Takashi GOJOBORI

To examine whether the rate of nucleotide substitution is affected by the generation time of organisms, we attempted to estimate an accurate rate of synonymous (silent) substitution in Drosophila lineages. We used the nucleotide sequences of alcohol dehydrogenase (Adh) and heat shock protein 82 (hsp 82) genes for a total of eight species of Drosophila and estimated the number of synonymous substitutions. From paleontological, paleobiogeographical and electrophoretic data, divergence times between *D. melangaster* and its sibling species, between *D. melanogaster* and *D. pseudoobscura* and

between *D. melanogaster* and *D. virilis* have been estimated to be about 2.3, 30, and 40 Myrs, respectively. Using these values, we estimated the rate of synonymous substitution in Drosophila lineages to be 10.5×10^{-9}/site/year. This rate is approximately two times higher than that of rodents and ten times greater than higher primates. The higher rate in Drosophila may be explained by the shorter generation times of the Drosophila species, though the possibility that the mutation mechanism in Drosophila may differ from that in mammals cannot be excluded. For details, see *Jpn. J. Genet.* **62**: 139–147.

Expansion of *Drosophila simulans* in Japan

Masayoshi WATADA, Yutaka INOUE
and Takao K. WATANABE

Drosophila simulans is distributed throughout the world in association with human habitation. This species, however, had never been collected in Japan before 1972 except on Ogasawara Island. In 1974 and 1975, many *D. simulans* individuals were collected in Kitakyushu and the Tokai district. In 1976 the species was found to be distributed in two separated areas; Kyushu and the Kanto-Tokai districts. The intervening area comprising the Kinki and Chugoku districts, was almost free of *D. simulans*.

Domestic and semi-domestic species of *Drosophila* were collected in Japan from 1977 to 1985 in order to study the expansion of *D. simulans* and its effect on other species. Newly established populations of *D. simulans* were recorded in the western mainland (Kinki and Chugoku). In addition, some isolated populations of *D. simulans* were found in Sapporo, Akita and Miyakojima. The frequency of *D. simulans* temporarily changed in the newly invading localities before its successful colonization. Comparisons of the proportions of collected flies suggested that the expansion of *D. simulans* affected *D. melanogaster* severely in domestic sites and *D. lutescens* slightly in semi-domestic sites. For details, see *Zool. Sci.* **3**, 873–883.

Computer Study of Molecular Evolution

Takashi MIYATA

The proto-oncogne c-*fms*/macrophage colony-stimulating factor receptor and platelet-derived growth factor (PDGF) receptor are closely related in

sequence. Both have a structual property of cell-surface receptors consisting of an extracellular domain of putative five repeat units, a transmembrane region and a cytoplasmic tyrosine kinase domain homologous to the *src* family (Coussens *et al.*, 1986; Yarden *et al.*, 1986). We showed that the extracellular domains of these growth factor receptors exhibit significant homologies in sequence with myelin-associated glycoprotein (MAG) and the neuronal cell adhesion molecule (N-CAM), known to be members of the immungoglobulin superfamily. The degrees of homologies are in the range of 34% for the c-*fms*/MAG pair to 19% for the PDGF receptor/N-CAM pair, and the probabilities that these homologies are realized by chance are less than 6.3×10^{-5}. Furthermore the three repeat units of c-*fms* and the two repeat units of the PDGF receptor show statistically significant homologies with 33 known immunoglobulin-related sequences for regions around invariant cysteine residues involved in disulfide bonding. Thus these growth factor receptor genes are a new subgroup of the immunoglobulin gene superfamily encoding an immunoglobulin-related extracellular domain and an *src*-related cytoplasmic protein kinase domain, and possibly evolving through the shuffling of primordial genes from distinct gene families. An additional homology of a *Drosophila* neuron-specific glycoprotein with Thy-1 was also shown.

IX. HUMAN GENETICS

Incidence of Genetic Disease in Japan

Ei MATSUNAGA

In 1934, T. Komai, who compiled 741 pedigrees of some 80 kinds of hereditary diseases and abnormalities published in Japanese medical literature, was struck by the finding that, when compared with what was known for Europeans, there was very little difference either in the kinds of disease or in the mode of transmission. As instances of rare exception, he mentioned that Oguchi's disease and Leber's optic-nerve atrophy were much commoner in Japan than in Europe while there was no authentic case of Huntington disease known in this country. Since then, numerous hereditary disorders have been documented in Japan, and as far as phenotypic similarity of the morbid genes is concerned, the general principle first pointed out by Komai, that there is very little ethnic difference, was substantiated, even for mental disorders such as schizophrenia and Down's syndrome. However, information about the frequency of individual disorders in the general population is still limited. The following are a summary based on available data collected from literature.

The incidence at birth of various types of chromosomal abnormalities is virtually the same in both Japanese and European populations. Most Mendelian disorders so far investigated, including monogenic hypercholesterolemia and neurofibromatosis, are as common in Japan as in Europe and North America. As instances of rare exception, tuberous sclerosis, congenital cataract (dominant and recessive), congenital muscular dystrophy (Fukuyama type), Oguchi's disease, acatalasemia, corneal amyloidosis and xeroderma pigmentosum, seem more frequent in Japan than in Europe and North America, while Huntington disease, phenylketonuria and cystic fibrosis are much less frequent in Japan. The overall frequency of chromosomal and some Mendelian disorders is estimated at about 1 in 100 newborn infants. There are two foci of familial amyloid neuropathy, one in Kyushu and the other, the second largest concentration of this disorder, in central Japan. With declining frequencies of consanguineous marriages and the breaking down of geographical and social isolates in this country,

it is expected that the incidence of all kinds of recessive disorders will decrease gradually to some extent. However, the prevalence rate of patients with hereditary diseases and disabilities in the general population is generally increasing owing to an improvement in medical care and therapy. Details are in press in *Human Genetics*, Proceedings of the 7th International Congress, Berlin, 1986.

Paternity Determination in Two Cases with Hereditary Disease

Ei MATSUNAGA

If, in paternity determination, a child is affected with a rare dominant disorder and the same condition is also found in a putative father, it will constitute strong evidence that he is the biological father of the child. However, if the genetic disease in the child is of a kind which arises usually as a result of fresh mutation, then the determination of paternity will be very difficult.

We recently experienced a case of paternity determination in which the plaintiff, a 25-year-old woman, and the defendant, a 50-year-old man, were both affected with congenital aniridia, whereas the mother of the plaintiff, a classmate of the defendant in primary school, was mentally retarded but had normal irises. It was ascertained that aniridia ran in the family of the defendant; this condition was inherited from his father, and two sisters and one niece were also affected, showing an autosomal dominant pattern of inheritance. Examination of 13 genetic markers in the three persons gave results consistent with the paternity of the defendant. Since the frequency of this condition in Japan seems to be of the order of 10^{-5}, it was concluded that the defendant is the father of the plaintiff.

In the second case, the plaintiff, a 19-year-old boy, was affected with typical Down's syndrome. Because the boy showed distinct features peculiar to Down's syndrome, it was difficult to detect phenotypic resemblances between the plaintiff and the defendant, a 50-year-old man. However, examination of genetic markers revealed that there was no inconsistency with the paternity of the defendant. In particular, both the plaintiff and the defendant possessed a rare Gc variant (plaintiff 2-1C1, mother 2-1S and defendant 1A1-1C1). It was concluded that the defendant was in all probability the father of the plaintiff. Details were reported in the *Jpn. J. Legal Med.* **40**: 520,

1986.

A T→C Transition in Codon 110 of the Human β-Globin Gene is a Novel Cause of β-Thalassemia Phenotypes

Takashi IMAMURA, Hitoshi NAKASHIMA and Yuji NARITOMI*

Thalassemia results from inherited defects in the rate of synthesis of one or more of the globin chains, which leads to clinical manifestations of anemia, hemolysis and ineffective erythropoiesis. The similar but genetically distinct hemolytic syndrome, unstable hemoglobin-hemoglobinopathies, result from an inherited structural alteration in one of the globin chains, through which physical stability of the molecule is affected. The presence of one of these particular abnormal hemoglobins in a patient is associated with chronic hemolysis and anemica, due to the denatured hemoglobin precipitates formed *in vivo*.

We now report evidence that a single nucleotide substitution in the third exon of the β-globin locus of an affected Japanese patient produced the β-thalassemia phenotype through a post-translational mechanism. This novel human globin gene mutation resulted in the substitution of proline for leucine at position 110 in the G-helix of the β-globin chain. Both molecular stability of the β-globin subunit and $\alpha_1\beta_1$ dimer formation, the first step in hemoglobin tetramer formation, were impeded resulting in the β-thalassemia phenotype.

Although the common molecular mechanisms for thalasemia are produced by abnormal mRNA processing and by early or late termination, the molecular lesion which is defined in this study was not due to gross mRNA deficiency. The structural variant was so unstable that it could not be detected in cells by any means. Thus, the globin chain deficiency, apparently similar to that in thalassemia, resulted from synthesis of the variant hemoglobin subunits which were extremely unstable in amounts equal to their gene dose (or slightly less), having a greatly accelerated rate of denaturation and removal. The mutation could be identified at the DNA level upon digestion with a restriction enzyme *Msp I*. This detection of the mutation on the gene level is of significant advantage for differential diagnostic purposes.

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Distinct Clustering of Mitochondrial DNA Types among Japanese, Caucasians and Negroes

Satoshi HORAI, Takashi GOJOBORI and Ei MATSUNAGA

A phylogenetic tree constructed by genetic distances among the 62 mitochondrial DNA types indicated that at least two distinct lineages exist in the Japanese population. In order to evaluate the evolutionary significance of this finding, a phylogenetic tree of mtDNAs among three major racial groups was constructed by combining our data with those for Caucasians and Negroes analyzed by Cann (1982) using 11 restriction enzymes. A total of 117 restriction types were observed among the three major racial groups. It is to be noted that any restriction type in one racial group was unique to that group. The average number (d) of nucleotide substitutions for Japanese was almost the same as that for Caucasians, whereas the value for Negroes was about two times larger than those for the other two races. This implies that Negroes are much more diverse with respect to mtDNA than Caucasians or Japanese.

We also estimated the number of nucleotide substitutions for all pairs of individuals in the three races and constructed a phylogenetic tree for the 117 types by the unweighted pair group method (Sokal and Sneath 1963; Nei 1975). We tentatively classified all lineages into 8 clusters designated as C1 to C8. While three of them (C3, C7 and C8) represent interminglings of individuals from different racial groups, the remaining five show distinct clustering of the same racial group: C1 (consisting of 9 types) may be called a Negroid cluster, C2 (13 types), C4 (6 types) and C6 (24 types) Japanese, and C5 Caucasian, although one out of 19 types of the C5 was derived from Negroes.

In the tree, the C1 and C2 clusters diverged first from the rest of the clusters. As indicated in a previous paper (Horai and Matsunaga, 1986), C2 (group I), which represented 18% of the Japanese, was quite different from the rest (group II) of the Japanese in two ways; individuals of C2 showed a higher level of polymorphism as revealed by several restriction enzymes, and they all showed a HaeIII variant (morph 2), due to a length polymorphism characterized by a 7 bp deletion in a 141 bp restriction fragment. None of the other Japanese clusters exhibited this length polymorphism. In the data analyzed by Cann (1982) the Hae III morph 2 was found in six Asians and one black individual, but none was detected among the Caucasians. In the

case of this length polymorphism, an independent occurrence of variation seems unlikely, because exactly the same amount of electrophoretic mobility shift was identified in the variant individuals. Some Mongoloids and a part of Negroids exhibiting the HeaIII morph2 may share a common ancestor.

In our analysis, the branching point of C1 and C2 clusters goes back to about 170,000 years ago, if we assume that the rate of nucleotide substitution in mtDNA is 2×10^{-8}/site/year. On the other hand, Nei and Roychoudhury (1974), using gene frequency data for 28 protein loci, estimated that the divergence time between the Caucasoid and the Mongoloid (Japanese) races is 50,000 years ago, whereas that between the Negroid and the Caucasoid-Mongoloid group is about 120,000 years ago. The standard errors involved in the estimation of divergence times must be large in both cases. Moreover, our analysis referred to gene phylogeny, whereas Nei and Roychoudhury's was concerned with the time of population splitting. However, our finding may suggest that the time of gene divergence was much earlier than the time of population splitting. In other words, prior to the racial divergence there were two ancestral types of mitochondrial genes, one for C1 and the other for C2. An alternative hypothesis is that the time of divergence between some parts of the Negroid and Japanese was still earlier than that estimated from the protein data.

With respect to C3, C7 and C8, the intermingling of different racial groups may be due to the ancient polymorphism of human mtDNAs. This intermingling may also be explained by gene migration. In particular, the intermingling between American whites and blacks could be due to recent admixtures (Reed, 1969). However, such gene admixtures between Japanese and the other two races are unlikely because the Japanese population seems to have been isolated for a long time.

Althogh there are several possible explanations for the interpretation of the overall clustering patterns in the tree, the most pertinent explanation is that the ancestral human population was already polymorphic in the mitochondrial genome before the divergence of the three major races. For details, see *Jpn. J. Genet.* **61**: 271–275.

An Apparent Discrepancy between Chain Length and Electrophoretic Mobility of Restriction Fragments: a Case of Human Mitochondrial DNA

Satoshi HORAI, Tadashi INOUE and Ei MATSUNAGA

Restriction enzyme analysis has been used as a powerful tool for detecting genetic variations in human mitochondrial (mt) DNA. We previously analyzed mtDNA from 116 Japanese with restriction enzymes of four or five base pair recognition and postulated that there was an insertion of about 60 bp in the region form URF2 to $tRNA^{Asn}$ as compared to the published sequence (Anderson *et al.* 1981, *Nature* **290**: 457). The insertion was common to all individuals screened, and was clearly demonstrated by three different enzyme digestions. The apparent presence of the insertion was also confirmed by double digestion experiments with enzymes of six base pair recognition (Horai and Matsunaga 1986, *Hum. Genet.* **72**: 105).

In order to localize the insertion in the fragment, we sequenced the fragment in question. Contrary to expectations, there was no insertion in the fragment and the actual length of the fragment was found to be exactly the same as that derived from the sequence data reported by Anderson *et al.* (1981). To clarify this discrepancy between the sequence data and the gel electrophoretic analysis, we compared the mobility of the fragments in question in native gel with those in a denaturing gel. Results showed that the mobility of the restriction fragments in native gel does not necessarily reflect real molecular weight. These findings suggest that certain fragments had peculiarly higher structures resulting in slower mobility in the native gel electrophoresis. However, a computer analysis of the sequence data of the fragment in question failed to show any specific secondary structures or biases of base compositions.

In conclusion, we should be sufficiently careful when assessing molecular weight of restriction fragments by native gel electrophoresis, assuming a linear relationship between mobility and the logarithm of the molecular weight. These data were published in *Hum. Genet.* **75**: 73–74.

Mitochondrial DNA Polymorphisms in Japanese Monkeys, *Macaca fuscata*, Derived from Four Local Population

Kenji HAYASAKA, Satoshi HORAI, Takayoshi SHOTAKE,
Ken NOZAWA and Ei MATSUNAGA

We analyzed mitochondrial DNA polymorphisms of 10 Japanese monkeys, *Macaca fuscata*, derived from four local populations using 18 restriction enzymes. Of the 18 enzymes used, polymorphisms of cleavage patterns were observed in 12 enzyme digestions. By combining the cleavage patterns for each enzyme, the 10 samples were classified into four restriction types. Each of the four types was found exclusively in the respective population. The four types of mtDNAs had 43 to 49 restriction sites for the 18 enzymes. The number of nucleotide substitutions per site among the 10 samples was estimated to range from 0 to 0.0236 and the average was 0.0132. This average value is several times larger than that observed in human populations but within the range of those observed in great apes and rodents.

The phylogenetic tree constructed using the number of nucleotide substitutions revealed that the four types were divided into two clusters. If we assume the rate of nucleotide substitution to be 2×10^{-8} per year per site, these two clusters diverged approximately 1×10^{6} years ago. From the data of blood protein polymorphisms, the divergence time between Japanese monkeys and rhesus monkeys, which are considered to be the closest relatives of the former, was estimated at 5×10^{5} years. This discrepancy between divergence times may result from either a higher rate of nucleotide substitutions or the divergence of mtDNAs before the divergence of species.

Our observation that each local population has its own unique restriction type of mtDNA is in agreement with both the maternal inheritance of mtDNA and the life history of this species whose females stay in their native populations for their entire lives. This observation is also in agreement with the study of blood protein polymorphisms which revealed a low genetic variability within local populations and restricted distribution of variant alleles. For details, see *Jpn. J. Genet.* **61**: 345–359.

X. APPLIED GENETICS

Genic Analysis of Rice Isozymes and Their Expression in Calli

Ryuji ISHIKAWA*, Toshiro KINOSHITA* and Hiroko MORISHIMA

About 40 isozyme loci have so far been identified in rice. To assign those isozyme loci to the respective chromsomes, a series of trisomics derived form a Japanese variety were crossed with some Indica varieties and wild strains. Segregations in F_2 populations so far examined revealed that *Cat*-1 belongs to linkage group I (chromsome 6), *Amp*-2 to linkagegroup *su* (chromosome 12), *Pgd*-1 to linkage group VIII (chromsome 9), *Pox*-2, *Acp*-1 and *Sdh*-1 to linkage group d_{33} (chromosome 4), respectively. Together with the loci already known, 12 isozyme loci were assigned to six chromsomes.

To verify the expression of isozymes in calli, allelic expression of 14 loci were examined in the embryo-derived calli. Calli cultured for two to three months and those cultured for more than one year were compared to evaluate the effect of age. Six loci (*Pgi*-1, *Pgi*-2, *Cat*-1, *Pgd*-1, *Sdh*-1, *Est*-2) showed the same phenotypes as in the original plants and their expression was quite stable, so that they could be used as useful genetic markers selection *in vitro*. *Pox*-1 and *Pox*-2 never appeared in the calli. Phenotypic expression of the other six loci was altered or modified during the culture, and it seemed difficult to distinguish allelic differences. Tissue specificity observed in the original plants tended to disappear in calli.

Latent Differentiation towards the Indica and Japonica Types in the Asian Common Wild Rice

Hiroko MORISHIMA and Lilian GADRINAB**

In our earlier studies we accepted evidence that the wild ancestors of the Asian cultivated rice did not differentiate into the Indica and Japonica types, which represent the two major phylogenetic groups in cultivars, and that this differentiation occurred along with the process of domestication. This

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problem was reexamined using a wider range of materials and characteristics (both phenotypic traits and isozymes).

Analysis of variation and covariation of 23 characteristics and 12 isozyme loci revealed that distinct differentiation into the Indica and Japanica types as found in the cultivars is not fully developed among wild strains. Yet, a trace of non-random character association characterizing the Indica and Japonica types was detected. Moreover, this trend towards differentiation found in phenotypic characteristics and that found in isozymes were significantly related to one other. Most wild strains formed a continuous array between typical Indica and Japonica types. Chinese wild rices were closer to the Japonica type than the strains from other regions. It was also found that among the Asian wild rices the perennial type (supposedly primitive type) was more Japonica-like than the annual type (advanced type). Indica vs. Japonica differentiation must reflect a complex combination of historical incidence and consequences of selection during evolutionary divergence.

Gametic Selection under Different Conditions in Rice

Y. I. SATO, R. ISHIKAWA and H. MORISHIMA

Mendelian segregation assumes that all pollens or sperms are given equal chance of fertilization. Is this assumption always correct? We sometimes observe significant segregation distortions and fluctuations in F_2 populations of rice, though no particular genes causing segregation distortion are involved. This suggests that a relative proportion of fertilization by pollen grains with different genotypes is influenced by the environment. To confirm this, allelic frequencies at nine isozyme loci were compared among four samples of F_2 seeds born on F_1 plants obtained from the same cross but grown at different places (Sapporo, Mishima, Kyoto and Taichung). The data indicated that segregation ratios of three loci were significantly different among F_2s derived from F_1s grown at different places. In addition, F_1 plants of another cross were grown in the summer (outdoor) and the winer (greenhouse) seasons in Mishima. In this cross, a marker gene (*gl*) controlling hairiness of hull segregated. Frequencies of *gl gl* hommozygous plants were 0.169 and 0.112 in the F_2 populations which were obtained from F_1 plants grown in the summer and winter seasons, respectively. Since these experiments were only preliminary, we were unable to detect a general tendency. However,

some important questions were raised.

1. Selection in gametic phase must be more a general phenomenon than previously expected. Population genetic studies taking the genetic expression of the gametes into consideration is needed to elucidate its consequences in the sporophyte generation.
2. Breeding materials in early generations are often grown in greenhouses during winter for generation acceleration. Is it truly good for the efficiency of breeding?

Ecological Genetic Studies in Wild Rice Populations

P. BARBIER* and H. MORISHIMA

The Asian common wild rice *Oryza perennis* shows an intraspecific differentiation into annual and perennial ecotypes. Both types differ by several life-history traits, particularly in the use of sexual vs asexual reproduction and the mating system. Annuals are predominantly selfers that reproduce by seeds, and perennials are predominantly outbreeders but reproduce largely by asexual means.

Seven natural populations located in Thailand were studied regarding various phenotypic charactes and isozymic loci, in order to examine the relationships between life-history traits (sensu lato) and the distribution of genetic variability between and within populations.

Outcrossing rates were estimated from progeny of single mother plants sampled from three populations, using nine isozyme loci. Maximum likelihood estimates were about 10% for annuals and 50-60% in perennials. In outbreeders, inbreeding coefficients were generally higher than expected equilibrium F values. This could be explained by the existence of consanguineous matings between genotypes that are spatially distributed in a patchy manner.

The selfing annual populations exhibited low intra-population genic diversities, and the outbreeding perennial populations high diversities, in most cases both in the seeds and juvenile plants sampled. However, significantly lower diversities in juveniles as compared with those in seeds were found in one annual and one perennial population. We tried to interpret these results by looking at the demographical records available for these populations. In the annual, the decrease in genic diversity was related to a decrease

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in the number of individuals (due to mortality of juvenile plants). Such annual populations are likely to be subject to drift when exposed, for instance, to unexpected drought after recruitment of seedlings. In the perennial population, the variability observed in seeds, which is generated by pollen flow, possibly including pollen from outside of the population and sexual recombination, is probably seldom used, since newly recruited plants are mainly asexual propagules of the founders.

On the other hand, in another perennial weedy population which propagates both by seeds and asexually, a high genic diversity was found both in seeds and in juvenile plants. This indicates that the level of variability in natural populations is strongly connected to the mating system (opportunities for gene flow), but also depends on other life-history traits such as the mode of recruitment.

Search for Genes Controlling Photoperiodic Sensitivity in Rice Using Linkage with Isozyme Genes

R. ISHIKAWA, Y. I. SATO and H. MORISHIMA

Rice is a shortday plant in which flowering time is accelerated under shortday conditions. The photoperiodic sensitivity (PS) of the rice plant has not been fully studied from the viewpoint of genetics. In order to understand the genetic variablility of this trait in various cultivars, three F_2 populations derived from three cultivars with differing degrees of PS and a non PS tester strain were each divided into two groups and grown under shortday and longday conditions respectively. Some of the segregants grown under longday condition did not head due to their strong PS. Therefore, among obtained progeny, the frequencies of alleles derived from PS cultivars at loci linked with PS genes were expected to be lower under longday condition than those observed under shortday condition. In this experiment, eight isozyme loci were analyzed using the progeny seeds of headed F_2 plants. Results obtained suggest the existence of four PS loci. Strong PS seemed to be controlled by combinations of PS alleles at two or more loci. Allelic frequencies of three isozyme loci belonging to linkage group I (chromosome 6), were consistently different between shortday and longday conditions in three populations. A PS locus (*Se*-1) already reported belongs to this linkage group. The PS alleles detected here may be on the *Se*-1 locus. It was assumed that another PS locus was located on the same chromosome

being linked with *Cat*-1. An additional one was revealed to be on chromosome 5.

Phylogenetic Differentiation of the Japanica Types of Asian Cultivated Rice

Y. I. SATO and H. MORISHIMA

Asian cultivated rice (*Oryza sativa* L.) is classified into two major varietal groups, the Indica and Japonica types. Analysis of genetic structure within the Japonica type was attempted by principal component analysis using 13 morphological and physiological characters. The first principal component showed a latent phase represented by the variation axis of 'panicle length vs. panicle number' type. Scores of this component (Z_1 score) showed a bimodal distribution. The Japonica type is apparently differentiated into two types. Judging from the obtained larg loadings of the certain characters used by Oka (1958) for discrimination of his tropical and temperate Japonicas, the two types extracted by PCA seemed to correspond with his tropical and temperate types. Tropical Japonicas were panicle length type, while temperate Japonicas were panicle number types. These two types were fairly different in geographical distribution pattern; as their names indicate troipcal and temperate Japonica type tend to distribute in the tropical and temperate zones, respectively. They also showed differences in the frequency of the *Hwc*-2 gene for F_1 weakness. The Japonica types have been differentiated through various selections and adapted to different environments.

Wx protein of Induced Mutants in Rice

Yoshio SANO, Mitsuko KATSUMATA and Etsuo AMANO

As previously reported, a *cis*-acting regulatory mutation produced Wx^b from Wx^a. The frequency of Wx^b which drastically reduces the *Wx* protein level as well as amylose content in rice endosperm seems to have increased during domestication. Thus, naturally occurring variants were useful for recognizing the importance of regulatory functions at the *waxy* locus of rice. Unfortunately, naturally occurring variants are difficult to use for interpreting regulatory functions without the use of near isogenic lines, since their genetic backgrounds may lead to misinterpretation and their evolutionay origins are often obscure. A number of induced variants including *waxy*

and the low amylose mutatuion may give us a great opportunity for a better understanding of the *Wx* gene expression. When the induced mutants share a clear parent-progeny relationship, any biochemical changes detected are likely to be the cause of the mutant gene(s).

The *waxy* and low amylose mutants obtained were readily distinguishable from their endosperm appearances when compared with the original line of Norin 8. All mutants gave a 3 normal: 1 mutant ratio when crossed with T65 (nonwaxy), indicating that their mutant endosperms are each controlled by a single recessive gene. When the 11 *waxy* mutants were crossed with a *waxy* strain (T65*wx*), no normal endosperm segregated in F_2, showing that the mutant genes involved are allelic to *wx*. On the other hand, the 5 low amylose mutants gave normal, intermediate and waxy segregants in a ratio of 9: 3: 4, 3: 1 or 9: 7 in crosses with T65*wx*. The endosperm appearance of a low amylose mutant (74LA5) was close to its normal counterpart, so intermediate segregants were not distinguishable from normal segregants in F_2. Another mutant (76LA3), showed an endosperm appearance close to *waxy* and gave a segregation ratio of 9: 7 in F_2. These results indicate that the genes involved in low amylose mutants are inherited indepndently of *wx* and they have different effects on endosperm appearances although the allelism test among the mutants remains to be studied.

Amylose content and *Wx* protein level were examined in these mutants to look into regulatory changes at the *wx* locus. Out of 11 *waxy* mutants, only 3 almost completely lacked amylose and the *Wx* protein. It should be noted that naturally occurring *waxy* variants all belong to this type. The other 8 waxy mutants produced the *Wx* protein more or less and 3 of them produced traces of amylose, suggesting that some mutants produce an inactive form of the *Wx* protein. A mutant gene of 74*wx*2 seemed to affect only the level of the *Wx* protein, since the degree of reduction was similar in both amylose and the *Wx* protein when compared with to those of N8. Further evidence regarding the inactivation of the *Wx* protein was gained from the 75*wx*1 mutant. The mutant had a *Wx* protein about 1,500 daltons larger than that of the Norin 8 as determined by comparisons of migration rates. An inactive form of the *Wx* protein larger than normal size was also detected in maize, which resulted from an insertion of a controlling element into *wx* locus. Low amylose mutants affected the quantitative level of *Wx* protein as well as amylose. Endosperm appearance seemed to be closely associated with amylose content. The mutant genes obtained were regulatory in nature.

Since they show different genic action and phenotypic expression, different types of regulatory genes might be involved.

Some Comments on Seed Sampling form Natural Plant Populations

K. YONEZAWA and H. ICHIHASHI*

A seed sampling method for field collection was theoretically investigated with respect to the number of plants per site (population), m, and seeds per plant, n. Populations which are at genetic equilibrium with mixed selfing and outcrossing were assumed as the target populations to be collected, and the probability that all (two or three) of the alleles existing in the target populations are taken up was calculated for different rates of selfing and different combinations of ma and n. It was shown that in predominantly selfing populations, the results of sampling primarily depend on the plant number m, but a defect due to a shortage in plant number can well be compensated for by an increase in the seed number per plant, unless the selfing rate is as large as or larger than 0.95. In predominantly outcrossing populations, on the other hand, no substancial difference is found regardless of whether the plant number or the seed number per plant is increased. The optimum size of sampling is not much affected by the number of (indepenent) loci and alleles per locus to be collected, but is largely determined by the frequency of the rareest allele at any locus. Sampling about ten plants, with 10 to 20 seeds being collected from each plant, is estimated to be sufficient unless very infrequent (0.05 or fewer) alleles are to be collected from highly selfing populations. (Proceedings of the fifth International Symposium on Crop Exploration and Utilization of Genetic Resources, Taichung, 1986).

Variations in Protein Fractions from Rice Endosperm

Toru ENDO

Many aspects of the differences between Indica and Japonica types of rice cultivars have been studied, including seed protein contents. For sequentially extracted protein fractions from rice endosperm, the content

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ratios of albumin, globulin, prolamin and glutelin of some cultivars have also been described (Padhy and Salunkhe 1979, *Cereal Chem.* **56**: 389). Here I report on the content ratios of five Indica and five Japonica cultivars (see table).

Varieties	Albumin %	Globulin %	Prolamin %	Glutelin %
Indica group				
Peiku	8.6	11.6	1.9	77.9
130	10.9	4.3	11.4	73.4
419	19.4	2.3	17.9	60.0
713	21.3	3.3	2.6	72.8
717	23.9	3.8	4.1	68.1
Japonica group				
Taichung	23.0	9.8	3.3	63.9
Kosihikari	17.6	8.5	6.7	67.2
Harebare	37.0	7.8	1.5	53.7
Murasaki	12.2	4.4	8.9	74.5
Kochi-aka	14.3	2.4	12.6	70.7

Albumin, globulin and prolamin fractions were extracted from 10 g of 100-mesh endosperm powder with specific extractants, 0.01 M Tris-HCl, pH 8.5, for albumin; 0.5 M NaCl, pH 6.5, containing 0.2 M Tris and 0.2 M ascorbic acid for globulin; and 80% t-butanol for prolamin. In all cases the extraction was carried out overnight at 4°C in the presence of 0.1 M 2-mercaptoethanol. Glutelin was extracted with 0.1 M NaOH for 15 min and precipitated with ascorbic acid at about pH 6.

It can be noted from the table that there are no significant difference in content ratios between Indica and Japonica groups and that there are considerable differences among the cultivars of each group. For instance, albumin content is much higher in Harebare and 717 than the others, and globulin content is appreciable higher in Peiku and Taichung. Prolamin content is variable within both groups. Although the endosperm protein contents depend on agrotechnical, climatic and storage conditions, the above-mentioned differences must be partially due to differential genetic systems among the cultivars empolyed.

It shoud be noted, however, that isoelectrofocusing polyacrylamide gel-electrophoresis (IEF-PAGE), covering the pH 2–11 range, gave blurred banding patterns for albumin, globulin and prolamin samples, but gave clear patterns for glutelin. On the other hand, immobilized pH gradient polyacryl-

amide gel-electrophoresis (IPG-PAGE), covering the pH 4–10 range, gave sharp bandmorphs for globulin and glutelin samples, and gave clear but weak bands, small in number, for the albumin and prolamin samples. These results suggest that the overnight-extraction method caused a denaturation of most protein fractions resulting in blurred bands by IEF-PAGE and leading to a few sharp bandmorphs by IPG-PAGE. Although the overnight-extraction method is not necessary for qualitative experiments, it is desirable for a quantitative examination of the protein content ratio. Comparative analysis of the bandmorphs among the cultivars is now under investigation.

XI. DATABASE

Publications from Genetic Resources Section, Genetic Stocks Reseach Center

Shin-ya IYAMA

The following publications were released from Genetic Resources Section this year.

1. "Catalogue of *Escherichia coli* Genetic Stocks in National Institute of Genetics". (Compiled by A. Nishimura and S. Iyama) 181 pages.

This was completed in cooperation with the Microbial Stocks Section. The catalogue listed 2,177 main genetic stocks of *E. coli* out of about 10,000 those maintained in the Institute. Each stock was described with its accession number, strain name, sex, containing genetic markers together with prophage and plasmid, if any. A list of 1353 marker genes and chromosomal deficiencies was added. Under each item, the accession numbers of stocks possessing the marker were indicated so as to be used as an index for finding the stocks interested.

2. "Drosophila Stock List in Japan 1986". 56 pages.

Previous list published in 1985 was updated. This issue included 1250 Drosophila stocks kept in various places in Japan. Each stock was indicated with the place of maintenance. Wild and mutant stocks of *D. melanogaster*, *D. simulans*, *D. ananassae*, *D. hydei*, *D. virilis* and other 115 species were included.

3. "Rice Genetics Newsletter Vol. 3". 128 pages. (in English).

This volume contained the followings:

(1) Rules for gene symbolization in rice proposed by the Committee on Gene Symbolization, Nomenclature and Linkage Groups of Rice Genetics Cooperative.

(2) Lists of recommended gene symbols for chlorophyll deficiencies and leaf spots, heading behavior, anthocyanin coloration, grain size and shape, and isozymes.

(3) Lists of gene symbols and gene markers supplemented to the previous ones.

(4) List of about 200 recent publicaitons on rice genetics.

(5) 62 research notes on rice genetics.

Data Base Design of DNA Base Sequences

Sanzo MIYAZAWA

DNA base sequencing have become so easy that DNA base sequences analyzed are rapidly growing. This recent trend prompts biologists to use computer to analyze DNA base sequences. Typical examples of common analyses are those such as homology search, restriction enzyme site map, and open reading frame search. Search and retrieval of specific base sequences from DNA sequence data are prerequisite to such analyses. Thus, a DNA data base is demanded to efficiently manipulate sequence data. The unix operating system has been chosen as a target system to develope a data base, because of a portable operating system. By taking advantage of superior tools for interprocess communication in unix such as shared memory and pipeline, basic tools for data manupulation are being implemented as filters at the shell level.

Basic tools are provided such as

- to output specified entries,
- to output specified types of records in specified entries,
- to output the name of entries with strings specified in regular expression,
- to output base sequences specified in regular expression with their entry names by searching a whole data base, and also
- to convert formats.

These tools are used with unix tools such as "sort", "uniq" and so on to search and retrieve entries by keywords such as author names, the titles of papers, species names, locus names, and others. In addtion, a wide variety of application programs for sequence analyses will be incorporated into the data base system.

This type of simple search and retrieval system has an advantage to be portable among unix systems whether computer is a large, mini or personal computer. Although portability is lost, an alternative method to build data bases is to use one of data base management systems that are commercially available. Relational data base is advantageous, because it is easy to build and also it may allow detailed search and retrieval. We are planning to build a relational data base to manage data entry in the DNA Data Bank of Japan as well as for its use as a research tool.

PUBLICATIONS

AKETAGAWA, J., MIYATA, T., OHTSUBO, S., NAKAMURA, T., MORITA, T., HAYASHIDA, H., MIYATA, TAKASHI, and IWANAGA, S.: Primary structure of limulus anticoagulant anti-lipopolysaccharide factor. J. Biol. Chem., **261**: 7357–7365, 1986.

AKETAGAWA, J., MIYATA, T., OHTSUBO, S., NAKAMURA, T., MORITA, T., HAYASHIDA, H., MIYATA, TAKASHI, IWANAGA, S., TAKAO, T. and SHIMONISHI, Y.: Primary structure of anticoagulant, anti-lipopolysaccharide factor, from the limulus *Tachypleus tridentatus*. J. Biol. Chem. (in press).

AOKI, K.: A stochastic model of gene-culture coevolution suggested by the "culture historical hypothesis" for the evolution of adult lactose absorption in humans. Proc. Natl. Acad. Sci. USA, **83**: 2929–2933, 1986.

AOKI, K.: Stable polymorphic equilibria in a toy model of group selection. Jpn. J. Genet. **61**: 481–490, 1986.

Aota, S. and Ikemura, T.: Diversity in G+C content at the third position of codons in vertebrate genes and its cause. Nucl. Acids Res. **16**: 6345–6355 & 8702, 1986.

BEHRENS, P. Q., NAKASHIMA, H., YOKOTA, E. and RIGGS, A. F.: The structure of hemocyanin II from the horseshoe crab, *Limulus polyphemus*. J. Biol. Chem. **261** (23): 10520–10525, 1986.

CARSTEN, A. L., BENZ, R. D., COMMERFORD, S. L., HUGHES, W., ICHIMASA, Y., IKUSHIMA, T., and TEZUKA, H.: Further studies on the genetic damage to bone marrow and other somatic effects following exposure to low level tritium. NIRS-M-52 (Proc. 2nd Workshop on Tritium Radiobiology and Health Physics), 258–275, 1985.

FUERST, P. A. and MARUYAMA, T.: Fate of genetic variation in captive 300 populations. Zoo Biology **5**: 171–179, 1986.

FUJII, T. and TANO S.: Mutagenic activities of EMS on somatic (M_1) and recessive (M_2) mutations in the soybean test system. Environ. Exp. Botany **26**: 191–195, 1986.

FUKUDA, R, and HATADA, E.: RNA synthesis of influenza A viruses: Temperature-sensitive mutants defective in RNA synthesis. (in Japanese) Uirus (Virus) **36**: 203–219, 1986.

FURUSATO, T., TAKANO, J., JIGAMI, Y., TANAKA, H., and YAMANE, K.: Two tandemly located promoters, artificially constructed, are active in a *Bacillus subtilis* α-amylase secretion vector. J. Biochem., **99**: 1181–1190, 1986.

GLASS, R. E., JONES, S. T. and ISHIHAMA, A.: Genetic studies on the β subunit of *Escherichia coli* RNA polymerase, VII. RNA polymerase is a target for ppGpp. Mol. Gen. Genet. **203**: 265–268, 1986.

GLASS, R. E., JONES, S. T., NENE, V., NOMURA, T., FUJITA, N. and ISHIHAMA, A.: Genetic studies on the β subunit of *Escherichia coli* RNA polymerase, VIII. Localisation of a region involved in promoter selectivity. Mol. Gen. Genet. **203**: 487–491, 1986.

GLASS, R. E., HONDA, A. and ISHIHAMA, A.: Genetic studies on the β subunit of *Escherichia coli* RNA polymerase, IX. The role of the carboxyterminus in enzyme assembly. Mol. Gen. Genet. **203**: 492–495, 1986.

GOJOBORI, T., and NEI, M.: Relative contributions of germline gene variation and somatic mutation to immunoglobulin diversity. Mol. Biol. Evol. **3**: 156–167, 1986.

GOJOBORI, T., AOTA, S., INOUE, T. and SHIMOTOHNO, K.: A sequence homology between the pX genes of HTLV-I/II and the murine IL-3 gene. FEBS Let. **208**: 231–235, 1986.

HATTORI, M., BUSE, J. B., JACKSON, R. A., GLIMCHER, L., FORF, M. E., MINAMI, M., MAKINO, S., MORIWAKI, K., KAZUYA, H., IMURA, H., STRAUS, W. M., SEIDMAN, J. G. and EISENBARTH, G. S.: The NOD mouse: Recessive diabetogenic gene in the major histocompatibility complex. Science **231**: 733–735, 1986.

HAYASAKA, K., HORAI, S., SHOTAKE, T., NOZAWA, K. and MATSUNAGA, E.: Mitochondrial DNA polymorphism in Japanese monkeys, Macaca fuscata. Jpn. J. Genet. **61**: 345–359, 1986.

HAYASHIDA, H., KUMA, K. and MIYATA, T.: Sequence homology between *mas* oncogene product and rhodopsin family. Nature **323**: 116, 1986.

HIRAO, I., ISHIKAWA, M., and MIURA, K.: Partial synthesis of leader sequence of phage f1 coat protein mRNA. Chemistry Letters. (1986): 1929–1932.

HONDA, A., MIZUMOTO, K. and ISHIHAMA, A.: RNA polymerase of influenza virus: Dinucleotide-primed initiation of transcription at specific positions on viral RNA. J. Biol. Chem., **261**: 5987–5991, 1986.

HORAI, S. and MATSUNAGA, E.: Mitochondrial DNA polymorphism in Japanese II. Analysis with restriction enzymes of four or five base pair recognition. Human Genetics **72**: 105–117, 1986.

HORAI, S., GOJOBORI, T. and MATSUNAGA, E.: Distinct clustering of mitochondrial DNA types among Japanese, Caucasians and Negroes. Jpn. J. Genet. **61**: 271–275, 1986.

HOUBA-HÉRIN, N., HARA, H., INOUYE, M. and HIROTA, Y.: Binding of penicillin to thiol-penicillin-binding protein 3 of *Escherichia coli*. Identification of its active site. Molec. Gen. Genet. **201**: 499–504, 1985.

IHARA, M., GADRINAB, L. U., SIREGAR, U. J., and IYAMA, S.: Genetic control of alcohol dehydrogenase and estimation of some population parameters in *Hopea odorata* Roxb. (Dipterocarpaceae). Jpn. J. Genet. **61**: 127–136, 1986.

IKEGAMI, M., MORINAGA, T., and MIURA, K.: Infectivity of virus-specific double-stranded DNA from tissue infected by bean golden mosaic virus, a geminivirus. Ann. Phytopathol. Soc. Jap. **52**: 735–739, 1986.

IMAI, H. T.: Modes of species differentiation and karyotype alteration in ants and mammals. In "*Modern Aspects of Species*" (ed. by K. Iwatsuki, P. H. Ravan, and W. J. Bock), 87–105, Univ. of Tokyo Press, Tokyo, 1986.

IMAI, H. T., MARUYAMA, T., GOJOBORI, T., INOUE, Y. and CROZIER, R. H.: Theoretical bases for karyotype evolution. I. The minimum interaction hypothesis. Amer. Nat. **128**: 900–920, 1986.

IMAMURA, T.: The thalassemia syndromes. (in Japanese) Handbook of Internal Medicine, Vol. 86-A (ed. by Y. Yoshida, S. Oguma and H. Uchino), 99–113, Nakayama-Shoten, Tokyo, 1986.

IMAMURA, T.: Thalassemia syndromes. (in Japanese) Clinical Investigations **30**: 381–388, 1986.

INOUE, T., TEZUKA, H., KADA, T., AIKAWA, K. & SHULTZ, L. D.: The mouse mutant "wasted": An animal model for ataxia-telangiectasia. In "*Antimutagenesis and Anticarcinogenesis: Mechanisms*" (ed. by D. M. Shankel & others), 323–335, Plenum Press, New York, 1986.

INOUE, T., MURAKAMI, K. and FUJII, T.: Mutagenic potential of cordycepin (3′-deoxyadenosine) in Salmonella and soybean tester strains. Mutation Res. **174**: 179–182, 1986.

INOUE, T., AIKAWA, K., TEZUKA, H., KADA, T. and SHULTZ, L. D.: Effect of DNA damaging agents on isolated spleen cells and lung fibroblasts from the mouse mutant "wasted", a putative animal model for ataxia-telangiectasia. Cancer Res. **46**: 3979–3982, 1986.

INOUE, T., SUZUKI, O., KADA, T. and FUJII, T.: Bio-antimutagenic effect of L-ethionine on the spontaneous mutagenesis in *Salmonella* and *Bacillus subtilis*. Jpn. J. Genet. **61**: 461–467, 1986.

ISHIHAMA, A.: Transcription signals and factors in *Escherichia coli*. In "*Adv. Biophy.*", **21**, 163–173, 1986.

ISHIHAMA, A., FUJITA, N. and NOMURA, T.: Promoter selectivity of *Escherichia coli* RNA polymerase. In "*RNA Polymerase and the Regulation of Transcription*", Elsevier Sci. Publ. Comp., New York, 1986.

ISHIHAMA, A., MIZUMOTO, K., KAWAKAMI, K., KATO, A. and HONDA, A.: Proofreading function associated with the RNA-dependent RNA polymerase from influenza virus. J. Biol. Chem. **261**: 10417–10421, 1986.

ISHIHAMA, A.: A proposal for promotion of virus research. (in Japanese) Uirus (Virus) **36**: 181–183, 1986.

ISOBE, Y. and SHIMADA, Y.: Organization of filaments underneath the plasma membrane of developing chicken skeletal muscle cells *in vitro* revealed by the freeze-dry and rotary replica method. Cell Tissue Res. **244**: 47–56, 1986.

ISOBE, Y. and SHIMADA, Y.: Cytoskeleton in embryonic skeletal muscle cells. Bio Essays **4**: 167–171, 1986.

KADA, T., INOUE, T., OHTA, T. and SHIRASU, Y.: Antimutagens and their modes of action. In "*Antimutagenesis and Anticarcinogenesis: Mechanisms*"(ed. by D. M. Shankel & others), 181–196, Plenum Press, New York, 1986.

KADA, T., INOUE, T., MORITA, K. and NAMIKI, M.: Dietary desmutagens. In "*Genetic Toxicology of the Diet*" (ed. by I. Knudsen), 245–253, Alan R. Liss, Inc., New York, 1986.

KATOH, Y., HASEGAWA, T., SUZUKI, T. and FUJII, T.: Plant regeneration from the callus derived from mature embryo of Hiproly barley, *Hordeum distichum* L. Agric. Biol. Chem. **50**: 761–762, 1986.

KIJIMA, S. and KIJIMA, H.: Statistical analysis of channel current from a membrane patch. I. Some stochastic properties of ion channels or molecular systems in equilibrium. J. Theor. Biol. (in press)

KIJIMA, S. and KIJIMA, H.: Statistical analysis of channel current from a membrane patch. II. A stochastic theory of a multi-channel system in the steady-state. J. Theor. Biol. (in press)

KIKUNO, R. and MIYATA, T.: Slowly evolving *Drosophila* mitochondrial genes. Mol. Biol. Evol. (in press)

KIMURA, M.: Diffusion model of population genetics incorporating group selection, with special reference to an altruistic trait. "*Stochastic Processes and Their Applications*", (ed. by K. Itô and T. Hida), 101–118, *Lecture Notes in Mathematics*, Springer-Verlag, Berlin, 1986.

KIMURA, M.: Diffusion models of population genetics in the age of molecular biology. "*The Craft of Probabilistic Modelling*", (ed. by J. Gani and C. C. Heyde), 150–165, Springer-Verlag, Berlin, 1986.

KIMURA, M.: DNA and the neutral theory. Phil. Trans. Roy. Soc. London, Series B, **312**: 343–354, 1986.

KITAGAWA, H., KITAMURA, N., HAYASHIDA, H., MIYATA, T. and NAKASHIMA, S.: Structural relationship of the three rat kininogen genes and their evolution. J. Biol. Chem. (in press)

KOJIMA, H., KONISHI, H. and KURODA, Y.: Combined effects of chemicals on mammalian cells in culture. I. Effects of methyl methanesulfonate (MMS) and ethyl methanesulfonate (EMS) on the mutation induction. Mutation Res. **164**: 272, 1986.

KURODA, Y.: Genetic and chemical factors affecting chemical mutagenesis in cultured mammalian cells. In "*Antimutagenesis and Anticarcinogenesis: Mechanisms*" (ed. by D. M. Shankel & others), 359–375, Plenum Press, New York, 1986.

KURODA, Y.: *In vitro* studies on the spermatogenesis of *Drosophila melanogaster*. In "*Techniques in the Life Sciences, Techniques in Vitro Invertebrate Hormones and Genes*" (ed. by E. Kurstak & H. Oberlander), C124, 1–6, Elsevier Biomedical, 1986.

KURODA, Y.: Differentiation of adult structures in cultures of embryonic tissues from *Drosophila melanogaster*. In "*Techniques in the Life Sciences, Techniques in Vitro Invertebrate Hormones and Genes*" (ed. by E. Kurstak & H. Oberlander), C215, 1–5, Elsevier Biomedical, 1986.

KURODA, Y.: Antimutagenic activity of vitamin C in cultured mammalian cells. Mutation Res. **164**: 273, 1986.

KUSUDA, J., TAZIMA, Y., ONIMARU, K., NINAKI, O. and SUZUKI, Y.: The sequence around the 5′ end of the fibroin gene from the wild silkworm, *Bombyx mandarina*, and comparison with that of the domesticated species, *B. mori*. Mol. Gen. Gent. **203**: 359–364, 1986.

MARUYAMA, T., GOJOBORI, T., AOTA, S. and IKEMURA, T.: Codon usage tabulated from the GenBank genetic sequence data. Nucl. Acids Res. **14**: Suppl. r 151–197, 1986.

MATSUNAGA, E.: Paternity examination of two cases with hereditary disease. (in Japanese) Jpn. J. Legal Med. **40**: 520, 1986.

MIYASHITA, N. and MORIWAKI, K.: Genetic mechanism governing development of tumors in mammalian forms. (in Japanese) In "Progress in Cancer Research '86" (ed. by Y. Yamamura & others), Vol. 23, 183–190, Nakayama-shoten, Tokyo, 1986.

MIYASHITA, N.: Effect of major histocompatibility complex on the development of carcinogen-induced pulmonary adenomas in mice. (Abstract in English) J. Juzen Med. Soc. **95**: 161–169, 1986.

MIYATA, T., TOH, H. and ONO, M.: Homology between IgE-binding factor and re-

trovirus genes. Nature **322**: 484, 1986.

MORISHIMA, H.: Wild progenitors of cultivated rice and their population dynamics. In "*Rice Genetics*", 3–14, IRRI, Manila, 1986.

MORIWAKI, K.: Molecular and cytogenetical differentiation of *Mus musculus* species. In "*Modern Aspects of Species*" (ed. by K. Iwatsuki, P. Raven and W. J. Bock), 221–225, Univ. of Tokyo Press, Tokyo, 1986.

MORIWAKI, K., MIYASHITA, N., SUZUKI, H., KURIHARA, Y. and YONEKAWA, H.: Genetic features of major geographical isolates of *Mus musculus*. In "*The Wild Mouse in Immunology*" (ed. by M. Potter, J. H. Nadeau and M. P. Cancro), *Current Topics in Microbiology and Immnuology* 127, 55–61, Springer-Verlag, Berlin. 1986.

NAKASHIMA, H., BEHRENS, P. Q., Moore, M. D., Yokota, E. and RIGGS, A. F.: Structure of hemocyanin II from the horseshoe crab, *Limulus polyphemus*. Sequences of the overlapping peptides, ordering the CNBr fragments, and the complete amino acid sequence. J. Biol. Chem. **261** (23): 10526–10533, 1986.

NAKASHIMA, H., TOKUNAGA, S., KONISHI, H., NISHIDA, H., TSURU, A., NAKAMURA, S. and MATSUNAGA, A.: A case of small bowel ileus strangulated by falciform ligament. (in Japanese) Med. J. Mutual Assoc. **35**: 449–451, 1986.

NEI, M., and GOJOBORI, T.: A simple method for estimating the number of synonymous and nonsynonymous nucleotide substitutions. Mol. Biol. Evol. **3**: 418–426, 1986.

NOMIYAMA, H., JINNO, Y., MATSUDA, I., SHIMADA, K. and MIYATA, T.: Amplification of human argininosuccinate synthetase pseudogenes. J. Mol. Biol. **192**: 221–233, 1986.

NOMURA, T., FUJITA, N. and ISHIHAMA, A.: Promoter selectivity of *Escherichia coli* RNA polymerase: Alteration by fMet-tRNA$_f^{Met}$. Nucleic Acids Res. **14**: 6857–6870, 1986.

OHTA, T.: Population genetics theory of multigene families with emphasis on genetic variation contained in the family. "*Evolutionary Processes and Theory*", (ed. by S. Karlin & E. Nevo), 239–253, Academic Press, New York, 1986.

OHTA, T.: Population genetics of an expanding family of mobile genetic elements. Genetics **113**: 145–159, 1986.

OHTA, T.: Actual number of alleles contained in a multigene family. Genet. Res. **48**: 119–123, 1986.

OKAMURA, H., YANAGI, Y., IMARI, Y., KUDO, J., ISHIBASHI, N. and IMAMURA, T.: Werner's syndrome associated with cholangiocarcinoma. Jap. J. Med. **25** (2): 179–183, 1986.

ONO, M., YASUNAGA, T., MIYATA, T. and USHIKUBO, H.: Nucleotide sequence of the human endogenous retrovirus gene related to the mouse mammary tumor virus gene. J. Virol. **60**: 589–598, 1986.

SAGAI, T., SHIROISHI, T., MORIWAKI, K., BONHOMME, F., PETRAS, M. L., THOHARI, M., YU, Z. C., LU, D. Y. and CHO, W. S.: Characterization of newly isolated monoclonal antibodies against MHC of a Japanese wild mouse. Immunogenetics **24**: 361–367, 1986.

SAKOYAMA, Y., HONG, K-J., BYUN, S. M., HISAJIMA, H., UEDA, S., YAOITA, Y., HAYASHIDA, H., MIYATA, T. and HONJO, T.: Nucleotide sequences of immunogloblin epsilon genes of chimpanzee and orang utan: DNA molecular clock and hominoid evolution. Proc. Natl. Acad. Sci. USA **84**: 1080–1084, 1986.

SANO, Y.: Sterility barriers between *Oryza sativa* and *O. glaberrima*. In "*Rice Genetics*", IRRI, Manila, 1986.

SANO, Y., KATSUMATA, M. and OKUNO, K.: Genetic studies of speciation in cultivated rice. 5. Inter- and intraspecific differentiation in the *waxy* gene expression of rice. Euphytica **35**: 1-9, 1986.

SASAKI, Y. F., IMANISHI, H., WATANABE, M., SEKIGUCHI, A., MORIYA, M., SHIRASU, Y. and Tutikawa, K.: Mutagenicity of 1,2-dibromo-3-chloropropane (DBCP) in the mouse spot test. Mutation Res. **174**: 145–147, 1986.

SATO, Y. I., CHITRAKORN, S. and MORISHIMA, H.: The Indica-Japonica differentiation of rice cultivars in Thailand and its neighboring countries. In "*New Frontiers in Breeding Researches*" (ed. by B. Napompeth & S. Subhadrabandhu), 185–194, Kasetsart Univ., Bangkok, 1986.

SHIMADA, Y. and ISOBE, Y.: Cytoskeletal organization in embryonic chick skeletal muscle cells *in vitro* revealed by detergent-extraction, freeze-dry method. In "*Molecular Biology of Muscle Development*" (ed. by C. Emerson, D. A., Fischman, B., Nadal-Ginard and M. A. O. Sidiqui), pp. 725–739. Alan R. Liss, Inc., New York, 1986.

SHINKAWA-TACHI, K., MORIMOTO, K., KOIZUMI, A. and Kuroda, Y.: Combined effects of chemicals on mammalian cells in culture. II. Effects of ethyl methanesulfonate (EMS) and arabinosyl cytosine (Ara-C) on mutation induction. Mutation Res. **164**: 281, 1986.

SHIRASU, Y., MORIYA, M., TEZUKA, H., TERAMOTO, S., OHTA, T. and INOUE, T.: Mutagenicity of pesticides. In "*Mutation, Cancer and Malformation*" (ed. by E. H. CHU and W. M. GENEROSO), 617–624, Plenum Press, New York, 1984.

SHIROISHI, T., SAGAI, T., SAKAI, S. and MORIWAKI, K.: Lethal deletion of the C4 and 21-hydroxylase genes in the mouse H-2 class III regions, caused by meiotic recombination. Proc. Natl. Acad. Sci. USA **84**: 2819–2823, 1987.

SHIROISHI, T., SAGAI, T. and MORIWAKI, K.: Sexual preference of meiotic recombination within the H-2 complex. Immunogenet. (in press)

SUZUKI, H., MIYASHITA, N., MORIWAKI, K., KOMINAMI, R. MURAMATSU, M., KANEHISA, T., BONHOMME, F., PETRAS, M. L., YU, Z. C. and LU, D. Y.: Evolutionary implication of heterogeneity of the nontranscribed spacer region of ribosomal DNA repeating units in various subspecies of *Mus musculus*. Mol. Biol. Evol. **3**: 126–137, 1986.

SUZUKI, H.: Penicillin-binding proteins. (in Japanese) Biseibutsu **2**: 81–89, 1986.

SUZUKI, Y., TSUDA, M., TAKIYA, S., HIROSE, S., SUZUKI, E. KAMEDA, M. and NINAKI, O.: Tissue-specific transcription enhancement of the fibroin gene chraracterized by cell-free systems. Proc. Natl. Acad. Sci., USA, **83**: 9522–9526, 1986.

TACHIBANA, H. and ISHIHAMA, A.: Correlation between the rate of productive transcription initiation and the strand-melting property of *Escherichia coli* promoters. Nucleic Acids Res. **13**: 9031–9042, 1986.

TAKAHATA, N.: An attempt to estimate the effective size of the ancestral species common to two extant species from which homologous genes are sequenced. Genet. Res. **48**: 187–190, 1986.

TAKAHATA, N. and SLATKIN, M.: Private alleles in a partially isolated population. II. Distribution of persistence time and probability of emigration. Theor. Pop. Biol.

30: 180–193, 1986.

TANABE, N., KIJIMA, H., and MARUHASHI, J.: Trinitrobenzen sulfonic acid (TNBS) increases transmitter release at the frog neuromuscular junction. Proc. Japan Acad. 63B (in press)

TAYA, C. and MORIWAKI, K.: Effect of H-2 complex on the growth of embryo-derived teratomas in mice. Am. J. Reprod. Immunol. **10**: 35–38, 1986.

TEZUKA, H., INOUE, T., NOGUTI, T., KADA, T. and SHULTZ, L. D.: Evaluation of the mouse mutant "wasted" as an animal model for ataxia telangiectasia. I. Age-dependent and tissue-specific effects. Mutation Res. **161**: 83–90, 1986.

TOMITA, I., NAKAMURA, Y., YAGI, Y. and TUTIKAWA, K.: Fetotoxic effects of mono-2-ethylhexyl phthalate (MEHP) in mice. Environ. Health Perspect. **65**: 249–254, 1986.

TSUDA, M., HIROSE, S. and SUZUKI, Y.: Participation of the upstream region of the fibroin gene in the formation of transcription complex *in vitro*. Mol. Cell. Biol. **6**: 3928–3933, 1986.

UEDA, S., WATANABE, Y., HAYASHIDA, H., MIYATA, T., MATSUDA, F. and HONJO, T.: Hominoid evolution based on the structures of immunoglobulin epsilon and alpha genes. Cold Spring Harbor Symp. **51**: 429–432, 1986.

WATADA, M., INOUE, Y. and WATANABE, T. K.: Expansion of *Drosophila simulans* in Japan. Zool. Sci. **3**: 873–883, 1986.

YAMANE, K., NAKAZAWA, K., NAKAMURA, K., MINEKURA, M., TAKANO, J., SHIROZA, T., SOHME, A. and FUJITA, T.: Secretion activity of the *Bacillus subtilis* α-amylase signal peptides with different lengths in *Bacillus subtilis* and *Escherichia coli* cells. *In "Bacillus Molecular Genetics and Biotechnology Application"* (ed. by A. T. Ganesan and J. A. Hoch), p. 411, Academic Press, New York, 1986.

YAMAZAKI, T., CHOO, J-K., WATANABE, T. K. and TAKAHATA, N.: Gene flow in natural populations of *Drosophila melanogaster* with special reference to lethal allelism rates and protein variation. Genetics **113**: 73–89, 1986.

YONEKAWA, H., GOTOH, O., TAGASHIRA, Y., MATSUSHIMA, Y., SHI, L.-I., CHO, W. S., MIYASHITA, N. and MORIWAKI, K.: A hybrid origin of Japanese mice "*Mus musculus molossinus*". In "*The Wild Mouse in Immunology*" (ed. by M. Potter, J. H. Nadear and M. P. Cancro), *Current Topics in Microbiology and Immunology* 127, 62–67, Springer-Verlag, Berlin, 1986.

YONEZAWA, K. and OKA, H. I.: Conservation methods of crop populations with mixed selfing and outcrossing. Proc. of 5th SABRAO Int. Cong.: 575–584, 1986.

YONEZAWA, K., TATEMATSU, N., SPETSOV, P. and TSUNEWAKI, K.: Increasing genetic variability in common wheat by utilizing alien cytoplasms——Effects of four *Aegilops* cytoplasms on the cross, Norin 26×Norin 61. Jpn. J. Breed. **36**: 262–273, 1986.

YOO, I. D., FUJII, T., SANO, Y., KOMAGATA, K., YONEYAMA, T., IYAMA, S. and HIROTA, Y.: Dinitrogen fixation of rice-*Klebsiella* associations. Crop Sci. **26**: 297–301, 1986.

YUKUHIRO, K. and MUKAI, T.: Increased determental load possibly caused by a transposon in a local population of *Drosophila melanogaster*. Jpn. J. Genet. **61**: 25–43, 1986.

ABSTRACTS OF DIARY FOR 1986

Biological Symposium

247th	meeting	Jan. 23	The mechanism of RNA polymerase: Evidence in support of rotationary translocation model for transcription (D. Dennis)
248th	—	Apr. 3	Ecosystem assembly (G. Sugihara)
249th	—	Apr. 30	Immortal genes (S. Ohno)
250th	—	June 11	Structure and regulation of *Bacillus subtilis* RNA polymerase sigma-43 operon (R. H. Doi)
251st	—	July 7	Mitochondrial DNA studies on Australian resella parrots (R. H. Crozier)
252nd	—	July 29	Genetic-epidemiologic studies in Hungary (A. Czeizel)
253rd	—	July 30	Sex and sexual selection: a role for parasites? (W. D. Hamilton)
254th	—	Sept. 1	Genetics of susceptibility to mammary cancer in mice (J. Hilgers)
255th	—	Nov. 10	The Neutralism/panselectionism controversy and some geckos (G. Pasteur)
256th	—	Nov. 27	*In vitro* transcription of eukaryotic genes (J. L. Manley)

Mishima Geneticists' Club

310th	meeting	Jan. 23	Protein evolution and the partitioned structure of genes (M. Go)
311th	—	Feb. 14	Invertebrate taste reception (H. Kijima)
312th	—	Mar. 4	The molecular structure, function and evolution of hemocyanins (H. Nakashima)
313th	—	Mar. 13	Structure and function of *Heliozoan* microtubule (Y. Shigenaka)
314th	—	Mar. 28	Analysis of human rotavirus genome (Y. Furuichi)
315th	—	Mar. 31	Construction of transforming retrovirus

		carrying *ras* induced cellular gene, TGF-α (S. Watanabe)
316th meeting	Apr. 11	Role of DNA conformation in the transcription (S. Hirose)
317th —	May 29	Genetics of mouse complement system (S. Sakai)
Special program	Oct. 17	New methods of homology search for non-conservative proteins: Its applications to immunoglobulin superfamily and rhodopsin family (H. Hayashida)
318th —	Oct. 23	Hominoid evolution inferred from DNA sequence data (N. Saito)

FOREIGN VISITORS IN 1986

April 9, 1984– October 10, 1986	Pascale Barbier, Université des Sciences et Techniques du Languedoc Montpellier, France
February 26, 1985– July 25, 1986	Pierre Boursot, Université Montpellier, France
March 1, 1985–	Yi-de Huang, Shanghai Institute of Plant Physiology, China
September 11, 1985– September 10, 1986	Won Ho Lee, Pusan National University, Korea
October 15, 1985–	Yuan shen Qiu, Guangdong Microbiology Research Institute, China
January 14	Don Dennis, University of Delaware, U.S.A.
January 24	Sydney Brenner, MRC Laboratory of Molecular Biology, U.K.
January 30– March 7	Jozefa Styrna, Jagiellonian University, Poland Robert Fujimura, Oak Ridge National Laboratory, U.S.A.
March 15–June 12	Tjan Kiauw Nio, Bandung Institute of Technology, Indonesia
April 3	George Sugihara, Scripps Institute of Oceanography, UCSD, U.S.A.
April 22– April 30	Nguyen Xuan Hong, Hanoi University, Vietnam Susumu Ohno, Beckman Res. Inst. of the City of Hope, U.S.A.
June 11	Roy H. Doi, University of California, Davis, U.S.A.
July 7	R. H. Crozier, University of New South Wales, Australia
July 29	Andrew Czeizel, National Institute of Hygiene, Hungary
July 30–31	W. D. Hamilton, Oxford University, U.K.
September 1–2	Jo Hilgers, Netherlands Cancer Institute, Netherlands
September 2–29	Robert E. Glass, University of Nottingham, U.K.

September 4	R. T. Walker, University of Birmingham, U.K.
—	J. A. Richards, British Council, Tokyo
September 10–24	Songkran Chitrakon, Department of Agriculture, Thailand
September 29–30	Arne Hagberg, The Swedish University of Agricultural Sciences, Sweden
September 10–October 9	Peng Luo, Sichuan University, China
October 1–2	Chi Yen, Sichuan Agricultural University, China
October 2–5	Qi quan Shao, Institute of Genetics, Academia Sinica, China
October 3–7	Rusdy E. Nasution, National Biological Institute, Indonesia
October 8	Tibor Sik, University of Agricultural Sciences, Hungary
October 15–16	Bengt O. Bengtsson, University of Lund, Sweden
October 15–24	Ganesh Prasad, University of Gorakhpur, India
October 15	Li ming Shi, Kunming Animal Research Institute, Academia Sinica, China
October 15	Ulfur Arnason, University of Lund, Sweden
October 17–18	Udda Lundquist, Swedish University of Agricultural Sciences, Sweden
October 18–19	F. Scholz, Zentralinstitut für Genetik und Kulturpflanzenforschung, German Democratic Republic
November 10	Georges Pasteur, Université Montpellier-II, France
November 12	Shozo Yokoyama, Washington University School of Medicine, U.S.A.
November 27	James L. Manley, Columbia University, U.S.A.
December 9	Nai Ai Liang, Academia Sinica Guangzhou Branch, China
December 12	David M. Lambert, University of Auckland, New Zealand; Anthony J. Huges, Auckland Hospital, New Zealand
December 19–20	Masatoshi Nei, The University of Texas, U.S.A.

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国立遺伝学研究所年報　第37号
昭和62年8月25日　印刷
昭和62年8月31日　発行
発行者　松　永　　英
国立遺伝学研究所内
編集者　今村　孝・高畑尚之
国立遺伝学研究所内
印刷者　笠　井　康　弘
東京都新宿区高田馬場 3-8-8
印刷所　株式会社 国際文献印刷社
東京都新宿区高田馬場 3-8-8
発行所　国立遺伝学研究所
〒411　静岡県三島市谷田 1111
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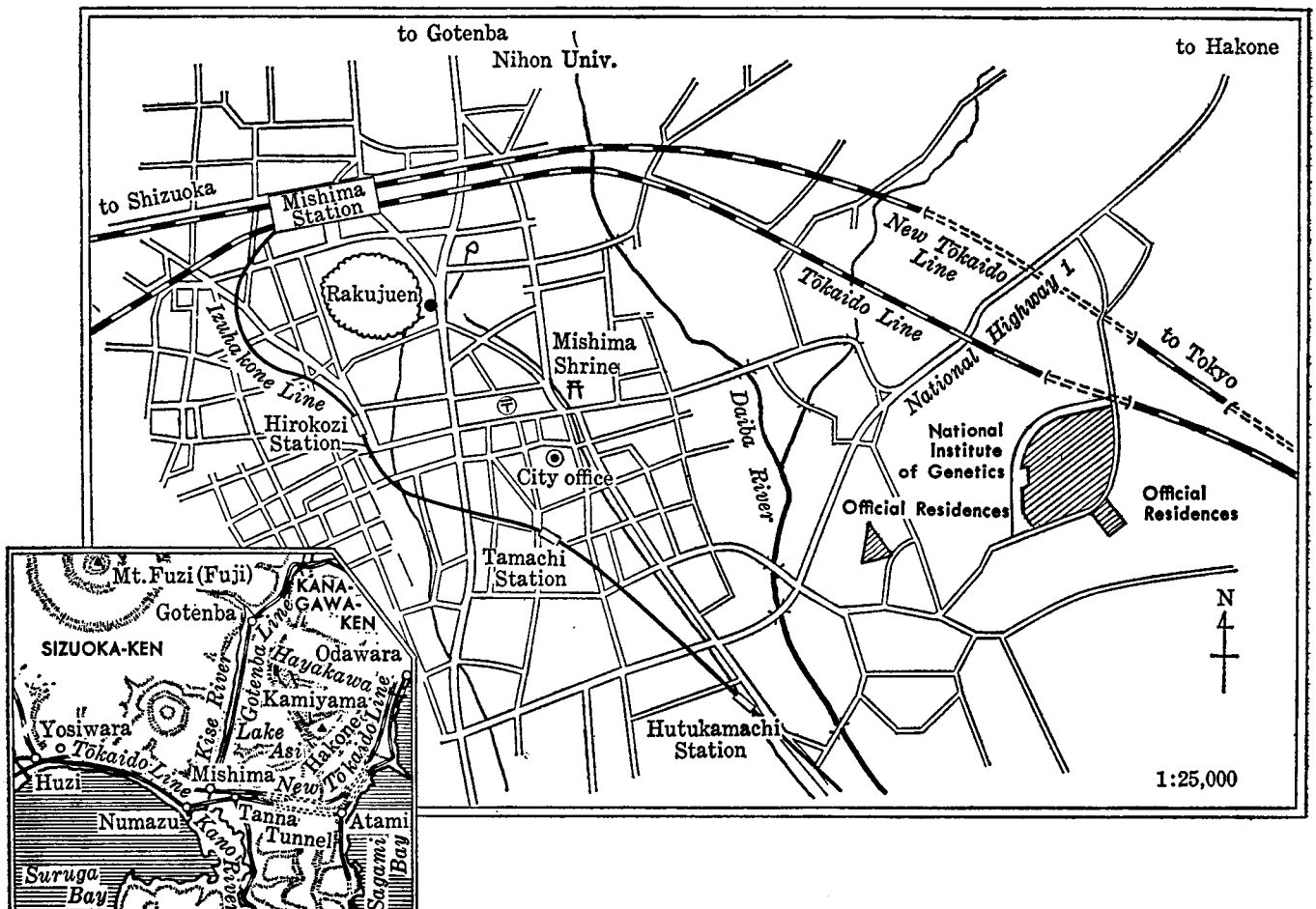
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