ISSN 0077-4995

NATIONAL INSTITUTE OF GENETICS

JAPAN

ANNUAL REPORT

No. 32 1981

Published by
THE NATIONAL INSTITUTE OF GENETICS
Misima, Sizuoka-ken, Japan
1982

Annual Report of the National Institute of Genetics

No. 32, 1981

Published by

The National Institute of Genetics, Japan

1982

CONTENTS

General statement 1
Staff 3
Council 6
Association for propagation of the knowledge of genetics........ 7
Projects of research for 1981........ 8
Researches carried out in 1981........ 12

I. Molecular Genetics

Methylation of the cap structure in mRNA by vaccinia associated enzyme system. URUSHIBARA, T., KAMIMURA, T., YAMAGUCHI, K., HATA, T. and MIURA, K. -I 12

On the base-stacking in the 5′-terminal cap structure of mRNA: a fluorescence study. NISHIMURA, Y., TSUBOI, M., YAMAGUCHI, K., HATA, T., HATTORI, M. and MIURA, K. -I........ 13

The nucleotide sequence of the tobacco chloroplast gene for the large subunit of ribulose-1,5-bisphosphate carboxylase/oxygenase. SHINOZAKI, K. and SUGIURA, M........ 14

The complete nucleotide sequence of a 23S rRNA gene from tobacco chloroplasts. TAKAIWA, F. and SUGIURA, M........ 14

Nucleotide sequence of the 16S–23S spacer region in an rRNA gene cluster from tobacco chloroplast DNA. TAKAIWA, F. and SUGIURA, M........ 17

The complete nucleotide sequence of a 16S ribosomal RNA gene from a blue-green alga, *Anacystis nidulans*. TOMIOKA, N. and SUGIURA, M........ 17

Cloning and characterization of a plasmid DNA from *Anacystis nidulans* 6301. SHINOZAKI, K., TOMIOKA, N., YAMADA, C. and SUGIURA, M........ 17

Transformation with the DNA fragments encompassing the polyoma virus promoter. SOEDA, E., MAKI, Y., NAKANO, Y. and TASHIRO, H........ 20

A total nucleotide sequence of murine papovavirus K DNA. JIKUYA, H., TAKEMOTO, K. K., and SOEDA, E........ 20

Genomic structure of human polyomavirus JC: nucleotide se-

quence of the region containing replication origin and small T antigen gene. MIYAMURA, T., JIKUYA, H., SOEDA, E. and YOSHIIKE, K. 21

Complete nucleotide sequence of Harvey murine sarcoma virus genome. FURUICHI, M., NAKANO, Y., CHAN, H. W., MARTIN, M. A. and SOEDA, E. 21

Construction of a gene library of silkworm and cloning its rRNA genes. KUSUDA, J. 22

II. Microbial Genetics

Physical map of *nrdA-nrdB-ftsB-glpT* region of the chromosomal DNA of *Escherichia coli*. YAMADA, M., TAKEDA, Y., OKAMOTO, K. and HIROTA, T. 24

Inhibitory effect of adenosine 3′, 5′-phosphate on cell division of *Escherichia coli* K-12 mutant derivatives. UTSUMI, R., TANABE, H., NAKAMOTO, Y., KAWAMUKAI, M., SAKAI, H., HIMENO, M., KOMANO, T. and HIROTA, Y. 24

Cell surface charge and cell division in *Escherichia coli* after X irradiation. SATO, C., KOJIMA, K., NISHIZAWA, K. and HIROTA, Y. 25

Structure of replication origin of the *Escherichia coli* K-12 chromosome: the presence of spacer sequences in the *ori* region carrying information for autonomous replication. ASADA, K., SUGIMOTO, K., OKA, A., TAKANAMI, M. and HIROTA, Y. 26

Conjugative transfer of pBR322 carrying the chromosomal DNA of *Escherichia coli* mediated by an Hfr. YAMADA, M. and HIROTA, Y. 26

Escherichia coli origin of replication: structural organization of the region essential for autonomous replication and the recognition frame model. HIROTA, Y., OKA, A., SUGIMOTO, K., ASADA, K., SASAKI, H. and TAKANAMI, M. 27

On the process of cellular division in *Escherichia coli*: nucleotide sequence of the gene for cellular division. NAKAMURA, M., MARUYAMA, I., SOMA, M., KATO, J.-I., SUZUKI, H. and HIROTA, Y. 28

Overproduction of *Escherichia coli* replication proteins by the use of the runaway-replication plasmids. YASUDA, S.

and TAKAGI, T. 28
Possible repressor of flagellar regulon in *Escherichia coli* K-12 KOMEDA, Y. 29
Coordination of flagellar formation and cell division in *Escherichia coli*. 1. Flagellar formation and transcription of 'hag' gene in cell division mutants of *Escherichia coli*. NISHIMURA, A. and HIROTA, Y. 31
Coordination of flagellar formation and cell division in *Escherichia coli*. 2. Transcription of *fla*-operons in the mutants of cell division in *E. coli*. NISHIMURA, A., KOMEDA, Y. and HIROTA, Y 34

III. Biochemical Genetics and Immunogenetics

A wild-derived new H-2 haplotype enhancing K-IA recombination. SHIROISHI, T., SAGAI, T. and MORIWAKI, K. 36
Effect of major histocompatibility (H-2) gene complex on the susceptibility to urethan induced lung tumor in mice. MORIWAKI, K. and MIYASHITA, N. 38
The relationship between immune response genes and the sesceptibility to 4NQO-induced lung tumor. MIYASHITA, N. and MORIWAKI, K. 39
Suppression of urethan-induced lung tumor development in the hybrid between A strain and Japanese wild-derived MOL. TEN2 strain. MORIWAKI, K. and MIYASHITA, N. 40
Genetic divergence between European wild mice with Robertsonian translocations from view point of mitochondrial DNA. MORIWAKI, K., YONEKAWA, H., GOTOH, O., WINKING, H. and GROPP, A. 42
Effect of major histocompatibility gene complex (MHC) on the growth of embryo-derived teratoma. TAYA, C. and MORIWAKI, K. 44
Allelic constitution of Hbb locus in Chinese wild mice, *Mus musculus*. MIYASHITA, N., SAKAIZUMI, M., WANG, C. H., MIGITA, S. and MORIWAKI, K. 45
Genic differentiation in the wild populations of the fish *Oryzias latipes*. SAKAIZUMI, M., EGAMI, N. and MORIWAKI, K. 46
Two-dimensional electrophoretic profiles of seed proteins from

one grain in wild and cultivated rice. SUN, C. R. and ENDO, T. 48

IV. *Developmental Genetics and Somatic Cell Genetics*

In vitro differentiation of adult structures from embryonic cells of *Drosophila melanogaster*. KURODA, Y. 49

Effect of chick endogenous lectin on cartilage differentiation of chick limb-bud cells in culture. KURODA, Y., MATSUTANI, E. and YAMAGATA, T. 50

Mutagenic activity of quercetin on embryonic human diploid cells in culture. KURODA, Y. 51

Co-mutagenic activity of non-mutagens in cultured Chinese hamster cells. KURODA, Y. and ASAKURA, M. 52

Effect of TPA on cell-to-cell adhesion of Chinese hamster cell in culture. KURODA, Y. and MIKAMI, H. 53

An autosomal, non-maternal effect sex-differential lethal mutation in *Drosophila melanogaster*. OISHI, K. and WATANABE, T. K. 53

Nematocyte differentiation and positional information in hydra. FUJISAWA, T., NISHIMIYA, C. and SUGIYAMA, T. 55

Changes of the head activation and head inhibition potentials during hydra regeneration. SUGIYAMA, T., ACHERMANN, J. and TAKANO, J. 56

Chimeric mouse embryos produced by aggregating testicular teratocarcinoma cells and 8-cell stage embryos. HANAOKA, K. and NOGUCHI, T. 57

Freeze preservation of mouse embryos. NOGUCHI, T. and SATO, M. 58

Growth of nervous tissue of a *Notch* lethal, *Df(1)N8* in *Drosophila melanogaster*. MINATO, K. 59

V. *Cytogenetics*

Hypo-pentaploid chromosome constitution of a spontaneous tumor developed in the Oceanian type black rat. YOSIDA, T. H. 60

Hypotrichotic mutant (*ba*) appeared in the inversion stock (LEM) of the Norway rat. YOSIDA, T. H. 61

Quantitative changes of nucleolar organizer regions (NORs) in 3-methylcholanthrene induced tumors of the Indian spiny mouse. YOSIDA, T. H. 62
Shift of the karyotypes in Indian spiny mouse tumor MPMC-53 after storage in deep freezing. YOSIDA, T. H. 63
Geographical variation of chromosomes numbers in the house shrew, *Suncus murinus* (Soricidae) and its distribution in the world. YOSIDA, T. H. 64
Geographical variation of Y-chromosomes in the house shrew, *Suncus murinus*, and its distribution. YOSIDA, T. H. 65
Karyotype of a hybrid male between the domestic cat and the leopard cat. YOSIDA, T. H. 66
Effect of metabolic perturvation on induction of the sister chromatid exchange (SCE). SUZUKI, H., YOSIDA, T. H., KUSANO, H. 67
A re-examination of chiasma terminalization and chiasma frequency in male mice. IMAI, H. T. and MORIWAKI, K. 68
X-Y chromosome dissociation in wild derived *Mus musculus* subspecies, laboratory mice, and their F_1 hybrids. MATSUDA, Y., IMAI, H. T., MORIWAKI, K., KONDO, K. and BONHOMME, F. 69
Chromosome observations of some Uruguayan ants (Hymenoptera: Formicidae). GONI, B., de ZOLESSI, L. C. and IMAI, H. T. 69
Chromosome observations of tropical ants in western Malaysia and Singapore. GONI, B., IMAI, H. T., KUBOTA, M., KONDO, M., YONG, H. -S. and THOW, Y. P. 71
Supernumerary Y chromosomes and its polymorphism in *Drosophila punjabiensis*. HANAKATA, A. and YAMAMOTO, M. 73
New series of segmental aneuploidy using transpositions between X and Y chromosomes. HANAKATA, A. and YAMAMOTO, M. 74
Cytogenetical studies on autosomal meiotic pairing in *Drosophila malanogaster* male. YAMAMOTO, M. 76
Constructions of T(2; 4), T(3; 4) autosomal translocations in *Drosophila melanogaster*. YAMAMOTO, M. 77
Cytogenetical analyses of the gene altering sensitivity to trehalose

in *Drosophila melanogaster*. YAMAMOTO, M. and ISONO, K. 79
The effects of the variaition in heterochromatin content on recombination frequencies in *Drosophila melanogaster*. YAMAMOTO, M. 80

VI. Mutation and Mutagenesis in Animals

The mutagenic activity of N-ethyl-N-nitrosourea in various germ-line cells of the silkworm. MURAKAMI, A. 83
Difference in mutability by the different administration routes of chemical mutagens in the silkworm. MURAKAMI, A. 84
Relationship between chemical-dose and specific-locus mutations by a mycotoxin aflatoxin B_1 in pupal oocytes of the silkworm. MURAKAMI, A. 86
Specific-locus mutations induced by β-emitter [methyl-^{3}H] thymidine in primordial germ-cells of dormant silkworm embryos. MURAKAMI, A. 88

VII. Radiation Genetics and Chemical Mutagenesis in Microorganism and Plants

Enzymatic comparison of DNA damages induced by beta-rays from tritiated water and by gamma-rays. INOUE, T. and KADA, T. 91
Mechanisms of environmental antimutagenesis. KADA, T. 92
The effect of septum-initiation mutations on sporulation and competent cell formation in *Bacillus subtilis*. SADAIE, Y. and KADA, T. 93
Purification and characterization of a human placental factor which enhances the priming activity of γ-irradiated DNA. INOUE, T. and KADA, T. 94
Inhibition of DNA-repair and radiosensitization. KADA, T., MOCHIZUKI, H. and YOKOIYAMA, A. 94
Mutagenic effect of ekatin (thiometon) in soybean test system. FUJII, T. and INOUE, T. 95

VIII. Population Genetics (Theoretical)

Was globin evolution very rapid in its early stages?: A dubious

case against the rate-constancy hypothesis. KIMURA, M..... 97
Possibility of extensive neutral evolution under stabilizing selection with special reference to nonrandom usage of synonymous codons. KIMURA, M. 97
Estimation of evolutionary distances between homologous nucleotide sequences. KIMURA, M. 98
Some calculations on the amout of selfish DNA. OHTA, T. and KIMURA, M. 98
Two-locus problems in transmission genetics of mitochondria and chloroplasts. OHTA, T. 99
Genetic variation in small multigene families. OHTA.......... 99
Genetic variability and rate of gene substitution in a finite population under mutation and fluctuating selection. TAKAHATA, N. 100
A model of evolutionary base substitutions and its application with special reference to rapid change of pseudogenes. TAKAHATA, N. and KIMURA, M.......... 101
A mathematical model of extranuclear genes and the genetic variability maintained in a finite population. TAKAHATA, N. and MARUYAMA, T.......... 101
A mathematical study on the distribution of the number of repeated genes per chromosome. TAKAHATA, N.......... 102
Algebra of inclusive fitness. AOKI. K.......... 103
Estimating evolutionary distance from restriction maps of mitochondrial DNA with arbitrary G+C content. AOKI, K., TATENO, Y. and TAKAHATA, N.......... 103
One- and two-locus models of the origin of worker behavior in Hymenoptera. AOKI, K. and MOODY, M.......... 103

IX. Population Genetics (Experimental) and Evolutionary Genetics

Association of chromosome- and enzyme-polymorphism in natural populations of *Drosophila melanogaster*. INOUE, Y., TSUNO, K. and WATANABE, T. K. 105
Typical hybrid dysgenesis is absent from the natural population of *Drosophila melanogaster* in Japan. YAMAMOTO, A. and WATANABE, T. K. 107
Biochemical phylogeny of the *Drosophila montium* species sub-

group. OHNISHI, S., KIM, K. W., WATANABE, T. K. 107

X. Human Genetics and Human Cytogenetics

Retinoblastoma: mutational mosaicism or host resistance? MATSUNAGA. E. 109
Genetics of Wilms' tumor. MATSUNAGA, E. 109
The "loss" of kinetochores from chromosomes of aged individuals. NAKAGOME, Y. and ABE, T. 110

XI. Behavioral Genetics and Ecological Genetics

The behavioral changes in mice continuously reared under noise condition. FUJISHIMA, T. 113
Weed-mediated natural selection in a segregating population of rice. MORISHIMA, H. 113
Demography and populational variability of *Rumex obtusifolius* conditioned by biotic environment. TERAI, K. and MORISHIMA, H. 114
Isozyme variation in *Altingia* forest at Ciwidey, West Java. ENDO, T. 115
Cross resistance to organophosphorous insecticides in *Drosophila*. INOUE, Y. 116

XII. Applied Genetics

Numerical evaluation of the Indica-Japonica differentiation in rice varieties. MORISHIMA, H. and OKA, H. I. 118
Associative nitrogen fixation of rice plants with isolated bacteria. FUJII, T. and OHTA, K. 118
Diallel analysis of nitrogen fixation in the rhizosphere of rice. IYAMA, S., SANO, Y. and FUJII, T. 119
Genetic variation of herbicide tolerance among F_5 lines of barnyard grass. IYAMA, S. 120

Books and papers published in 1981 by staff members 121
Abstracts of diary for 1981 126
Foreign visitors in 1981 127
Author index 131

GENERAL STATEMENT

The most memorable event in 1981 was the 3rd International Conference on Environmental Mutagens held in Mishima, following Tokyo meeting. At the 2nd ICEM Conference held in Edinburgh in 1977, we assumed a heavy task to act as the host to the next Conference. Since then, we discussed about the Conference site, either to be in Kyoto, Mishima or Tokyo. Finally we decided to hold the meeting in two places, first in Tokyo for four days and then in Mishima for the following two days. We earnestly desired to hold all meetings throughout in Mishima, but because of the insufficiency of hotel accommodation we were obliged to split the meeting site. Main events were scheduled in Tokyo, and special lectures and symposia related to genetic effects were arranged in Mishima. Here two plenary lectures were given by Honorary Presidents: Dr. A. Hollaender on "The quantitation of environmental mutagens" and Dr. Ch. Auerbach on "A view from the ivory tower". Both farsighted pioneers in this field gave through their enthusiastic talk deep impression to the audience, especially to young scientists. The reception was held in a friendly atmosphere at Plaza Hotel under the joint auspices of the Mayor of Mishima and the President of the Third International Conference on Environmental Mutagens, participated by about 400 eminent scientists from all over the world. Among them were Prof. P. Oftedal, the President of the International Environmental Mutagen Society, and Prof. F. H. Sobels, the President of ICPEMC. It was, indeed, one of the big events in the history of Mishima City. Thanks to the efforts of many staff members of our Institute and other people concerned, the Conference was closed in success.

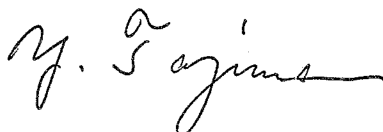
With regard to the reorganization of the Institute of the present system into an institute for joint use by the universities, the prospect is still dim because of a tight financial policy of the government. However, we wish to continue our appeal to the government to realize the reorganization as early as possible so as to keep pace with the rapid progress in the science of genetics in the world.

To our regret, we lost Dr. T. Kawahara, a staff member of the Department of Applied Genetics, on March 8 by a car accident. It is a great pity that the work on the domestication of wild quails has to be discontinued.

We had many visitors from abroad. I, myself, visited People's Republic of China, accepting the invitation from the Ministry of Agriculture, for three weeks from the middle of November for delivering lectures at the Southwestern Agricultural College near Chongquing. I also gave talks at Genetics Societies at Chongquing and Shanghai. Taking advantage of these opportunities I could get in touch with many young scientists of China. I am happy that I was able to contribute to the scientific and cultural exchange between China and Japan.

By the cooperation of the National Science Museum, the Institute held public lectures at Tokyo on November 14. The speakers were Dr. Tomoko Ohta, head of the 1st laboratory of the Department of Population Genetics, who spoke on "Evolution of multigene families," and Dr. Ei Matsunaga, head of the Department of Human Genetics, who talked on "Environment and heredity in carcinogenesis." I also had a chance to talk to general public at Tokyo Kyoiku Kaikan on a subject "What are genes?"

The budget for construction of sewage disposal was approved by the government, and the works started at the end of the year.



STAFF

Director

TAZIMA, Yataro, D. Ag.

Members

1. *Department of Morphological Genetics*

KURODA, Yukiaki, D. Sc., Head of the Department

The 1st Laboratory

MURAKAMI, Akio, D. Ag., D. Sc., Head of the Laboratory

The 2nd Laboratory

KURODA, Yukiaki, D. Sc., Head of the Laboratory
MINATO, Kiyoshi, M. Sc.

2. *Department of Cytogenetics*

YOSIDA, Tosihide H., D. Sc., Head of the Department

The 1st Laboratory

YOSIDA, Tosihide, H., D. Sc., Head of the Laboratory
YAMAMOTO, Masatoshi, Ph. D.

The 2nd Laboratory

MORIWAKI, Kazuo, D. Sc., Head of the Laboratory
IMAI, HIROTAMI, D. Sc.

3. *Department of Physiological Genetics*

MARUYAMA, Takeo, Ph. D., D. Sc., Head of the Department

The 1st Laboratory

WATANABE, Takao, K., D. Sc., Head of the Laboratory

The 2nd Laboratory

MARUYAMA, Takeo, Ph. D., D. Sc., Head of the Laboratory

4. *Department of Biochemical Genetics*

SUGIYAMA, Tsutomu, Ph. D., Head of the Department

The 1st Laboratory

NAWA, Saburo, D. Sc., Head of the Laboratory

YAMADA, Masa-Aki, M. Sc.

The 2nd Laboratory

OGAWA, Yoshito, D. Med., Head of the Laboratory
ENDO, Toru, D. Ag.

The 3rd Laboratory

SUGIYAMA, Tsutomu, Ph. D., Head of the Laboratory
FUJISAWA, Toshitaka, Ph. D.

5. *Department of Applied Genetics*

TAZIMA, Yataro, D. Ag., Head of the Department

The 1st Laboratory

TAZIMA, Yataro, D. Ag., Head of the Laboratory
FUJISHIMA, Tohru, D. Ag.

The 2nd Laboratory

IYAMA, Shin-ya, D. Ag., Head of the Laboratory

The 3rd Laboratory

MORISHIMA-OKINO, Hiroko, D. Ag., Head of the Laboratory

6. *Department of Induced Mutation*

KADA, Tsuneo, D. Sc., Head of the Department

The 1st Laboratory

TUTIKAWA, Kiyosi, Acting Head of the Laboratory
INOUE, Tadashi, D. Ag.

The 2nd Laboratory

KADA, Tsuneo, D. Sc., Head of the Laboratory

The 3rd Laboratory

KADA, Tsuneo, D. Sc., Head of the Laboratory
SADAIE, Yoshito, D. Sc.
TEZUKA, Hideo

7. *Department of Human Genetics*

MATSUNAGA, Ei, D. Med., D. Sc., Head of the Department

The 1st Laboratory

MATSUNAGA, Ei, D. Med., D. Sc., Head of the Laboratory

The 2nd Laboratory

NAKAGOME, Yasuo, D. Med., Head of the Laboratory

8. ***Department of Microbial Genetics***

HIROTA, Yukinori, D. Sc., Head of the Department

The 1st Laboratory

HIROTA, Yukinori, D. Sc., Head of the Laboratory
NISHIMURA, Yukinobu, D. Sc.

The 2nd Laboratory

YASUDA, Seiichi, D. Sc., Head of the Laboratory
YAMADA, Masao, D. Sc.

9. ***Department of Population Genetics***

KIMURA, Motoo, Ph. D., D. Sc., Head of the Department

The 1st Laboratory

OHTA, Tomoko, Ph. D., D. Sc., Head of the Laboratory

The 2nd Laboratory

KIMURA, Motoo, Ph, D., D. Sc., Head of the Laboratory
TAKAHATA, Naoyuki, D. Sc.
AOKI, Kenichi, Ph. D.

10. ***Department of Molecular Genetics***

MIURA, Kin-ichiro, D. Sc., Head of the Department

The 1st Laboratory

MIURA, Kin-ichiro, D. Sc., Head of the Laboratory
SOEDA, Eiichi, D. Ag.
SHIMOTOHNO, Kunitada, D. Pha.

The 2nd Laboratory

SUGIURA, Masahiro, D. Sc., Head of the Laboratory
SHINOZAKI, Kazuo, D. Sc.

11. ***Genetic Stocks Center***

YOSIDA, Tosihide H., D. Sc., Head of the Center

Animal Section

MORIWAKI, Kazuo, D. Sc., Head of the Laboratory
NOGUCHI, Takehiko, D. Sc.
INOUE, Yutaka, D. Sc.
KUSUDA, Jun, D. Ag.

Plant Section

FUJII, Taro, D. Ag., Head of the Laboratory

SANO, Yoshio, D. Ag.

Microbial Section

SUGIURA, Masahiro, D. Sc., Head of the Laboratory
KOMEDA, Yoshibumi, D. Sc.
NISHIMURA, Akiko

12. *Experimental Farm*

FUJII, Taro, D. Ag., Head of the Farm
MIYAZAWA, Akira

13. *Department of Administration*

KITAHARA, Kunio, Head of the Department
IORI, Toshiteru, Chief of the General Affairs Section
OOIDE, Yukio, Chief of the Finance Section

Honorary Members

KIHARA, Hitoshi, D. Sc., Director of the Kihara Institute for Biological Research, Member of Japan Academy, Emeritus Professor of Kyoto University
SAKAI, Kan-ichi, D. Ag.
MORIWAKI, Daigoro, D. Sc., Emeritus Professor of Tokyo Metropolitan University
OSHIMA, Chozo, D. Sc.
OKA, Hiko-Ichi, D. Ag.

COUNCIL

FUJII, Takashi, Chairman, Emeritus Professor of University of Tokyo
INOUYE, Eiji, Director of Research Institute of Handicap to Growth
IINO, Tetsuo, Professor of University of Tokyo
KONDO, Norio, Professor of Tokyo University of Agriculture
MISONOO, Keisuke, Member of Atomic Energy Commission
MORIWAKI, Daigoro, Emeritus Professor of Tokyo Metropolitan University
MOROHOSHI, Seijiro, President of Tokyo University of Agriculture and Technology
NAGAKURA, Saburo, Director of Institute of Molecular Sciences

OOSAWA, Fumio, Professor of Osaka University
SASA, Manabu, Emeritus Professor of University of Tokyo
SHINOZAKI, Nobuo, Director of Institute of Population Problems
TAKAHASHI, Man-emon, President of Musashi Women's Junior College
UMEZAWA, Hamao, Emeritus Professor of University of Tokyo
YAMAMURA, Yuichi, President of Osaka University

ASSOCIATION FOR PROPAGATION OF THE KNOWLEDGE OF GENETICS

MORIWAKI, Daigoro, President, Emeritus Professor of Tokyo Metropolitan University
MATSUNAGA, Ei, Managing Director, Head of the Human Genetics Department
YOSIDA, Tosihide H., Managing Director, Head of the Cytogenetics Department
SINOTO, Yosito, Manager, Professor of International Christian University
WADA, Bungo, Manager, Emeritus Professor of University of Tokyo
TAZIMA, Yataro, Manager, Director of the National Institute of Genetics
OSHIMA, Chozo, Manager

PROJECTS OF RESEARCH FOR 1981

Department of Morphological Genetics

Genetic studies on insect cells in tissue culture (KURODA and MINATO)
Developmental genetic studies on carcinogenesis in tissue culture (KURODA)
Genetics of somatic mammalian cells in culture (KURODA and MINATO)
Cytogenetics in the silkworm (MURAKAMI)
Studies on recombination in the silkworm (MURAKAMI)
Genetics of the silkworm (MURAKAMI, FUKASE and OHNUMA)
Radiation and chemical mutagenesis in the silkworm (TAZIMA and MURAKAMI)

Department of Cytogenetics

Studies on chromosome evolution and species differentiation in rodents and other small mammals (YOSIDA)
Chromosome study on experimental tumors (YOSIDA)
Experimental breeding and genetics of mice, rats and other wild rodents (YOSIDA and MORIWAKI)
Genetic study on the subspecies differentiation of mouse (MORIWAKI)
Immunogenetical study on the MHC functions in mice (MORIWAKI)
Cytogenetical study of ants (IMAI)
Cytogenetical studies on *Drosophila* (YAMAMOTO)
Cytogenetical study of fishes (YOSIDA)

Department of Physiological Genetics

Behavior genetics of *Drosophila* (WATANABE and OSHIMA)
Evolutionary and ecological genetics of *Drosophila* (WATANABE and KAWANISHI)
Population genetics of *Drosophila* (WATANABE)
Theory of population genetics and speciation (MARUYAMA)

Department of Biochemical Genetics

Studies on transformation in higher organisms (NAWA and YAMADA)
Genetical and biochemical studies of pteridine metabolisms in insects (NAWA)
Analysis of gene action on cell differentiation in higher organisms (NAWA and YAMADA)
Biochemical studies on the differentiation of muscle proteins in animals (OGAWA)
Genetical and biochemical studies of human serum proteins (OGAWA)
Genetical and biochemical studies on Japanese middle size dog (OGAWA)
Genetics of isozymes in plants (ENDO)
Genetic analysis of developmental mechanisms in hydra (SUGIYAMA and FUJISAWA)

Department of Applied Genetics

Quantitative genetic studies in poultry (KAWAHARA)
Genetic studies in wild populations of Japanese quails (KAWAHARA)
Behavioral genetic studies in mice (FUJISHIMA)
Theoretical studies on breeding techniques (IYAMA)
Genetic studies of trees in natural forest (IYAMA)
Evolutionary studies in wild and cultivated rice species (MORISHIMA and SANO)
Ecological genetic studies in weed species (MORISHIMA)
Genetic effects of environmental pollution on plant population (IYAMA and MORISHIMA)

Department of Induced Mutation

Molecular mechanisms of radiation- and chemical-induced mutations (KADA, SADAIE and TEZUKA)
Environmental mutagens, desmutagens and antimutagens (KADA)
Radiation genetics in mice (TUTIKAWA)
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)

Department of Human Genetics

Genetic studies on retinoblastoma and Wilms' tumor (MATSUNAGA)
Clinical cytogenetic studies of congenital abnormalities in man (NAKAGOME)
Molecular cytogenetic studies of human chromosomes (NAKAGOME)

Department of Microbial Genetics

Genetic regulatory mechanisms of DNA replication in *E. coli* (YASUDA)
Genetic regulatory mechanism of cellular division in *E. coli* (HIROTA, NISHIMURA and YAMADA)
Molecular genetics on DNA replication (YASUDA and HIROTA)
Genetic studies on bacterial cell envelope (HIROTA and NISHIMURA)
Synthetic bacterial plasmid (YASUDA and NISHIMURA)
DNA replication origin of *E. coli* (YASUDA, YAMADA and HIROTA)

Department 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 the evolution of altruism (AOKI)

Department of Molecular Genetics

Studies on the structure of genome RNA of viruses (SHIMOTOHNO and MIURA)
Studies on the primary structure of DNA (SOEDA and MIURA)
Studies on structure and function of messenger RNA (MIURA and SHIMOTOHNO)
Cloning of eukaryotic genes and their structural analysis (SUGIURA and SHINOZAKI)
Studies on molecular evolution of chloroplasts (SUGIURA and SHINOZAKI)

Genetic Stocks 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 Graminae (FUJII, SANO and IYAMA)
Studies on genetic differentiation in rice (SANO)
Cytogenetic studies of Norway rats and establishment of the chromosomal mutant stocks (YOSIDA)
Developmental genetic studies on mouse teratomas (NOGUCHI)
Studies on preservation of rodent embryos by deep freezing (NOGUCHI)
Studies on chromosomal polymorphism in Drosophila (INOUE)
Analysis of fibroin genes of silkworm and its relatives (KUSUDA)
Genetical study of flagellar formation in *Escherichia coli* K-12 (KOMEDA)
Basic studies on the gene purification and the construction of DNA banks (SUGIURA)
Genetical study on photosynthesis in blue-green algae (SUGIURA)
Coordination of flagellar formation and cell division in *E. coli* (NISHIMURA, KOMEDA and HIROTA)
Electron microscopic study of cell division in *E. coli* (NISHIMURA)

RESEARCHES CARRIED OUT IN 1981

I. MOLECULAR GENETICS

Methylation of the Cap Structure in mRNA by Vaccinia Associated Enzyme System

Toshiyuki URUSHIBARA, Takashi KAMIMURA, Kazuo YAMAGUCHI,
Tsujiaki HATA and Kin-ichiro MIURA

Process of formation of the cap structure attached to the 5′-termini of mRNAs synthesized *in vitro* by virion associated enzymes of vaccinia virus was reported in 1979 of this report series (No. 30). It was suggested that the cap formation by vaccinia virion proceeds in the definite order as a belt-conveyor system by using well orgarnized capping enzymes. However, when the enzymes solubilized from virion were used, the order of cap methylation was different. Here, substrate specificity of the 5′-5′ confronting dinucleotides was examined *in vitro* methylation by vaccinia virus particle itself.

The rates of methylation of various confronting dinucleotides by vaccinia virion depended on the number of the interposed phosphate groups. Maximum activity was observed in the material with three phosphate groups. This number coincides with the interposed phosphate groups in the confronting nucleotides structure at the 5′-terminus of vaccinia virus mRNAs synthesized *in vitro* as well as usual cap structure in a eukaryotic mRNA. The rates of methylation of $G^{5'}$ppA and $G^{5'}$ppppA were 0.52 and 0.35, respectively, compared with $G^{5'}$pppA. No methylation occurred for $G^{5'}$pA and $G^{5'}$pppppG. GpA (3′-5′) also was not methylated. Thus the methylation enzyme recognizes 2 to 4 phosphates in the 5′-5′ confronting dinucleotides.

After methylation reaction with [methyl ^{3}H] S-adenosyl-methionine, the added confronting nucleotides were split into nucleotides or nucleosides and analyzed. In any case only one radioactive material corresponding to 7-methylaguanosine or 7-methylguanosine-5′-monophosphate was detected. It is clear that methylation occurs only at the 7 position of guanosine in the

confronting dinucleotides.

Comparing $G^{5'}$pppA with $G^{5'}$pppG, or $G^{5'}$ppppA with $G^{5'}$ppppG, a G-G structure were more effective than G-A structure as a substrate for virion methylation. $G^{5'}$pppG and $G^{5'}$ppppG have a symmetrical structure. However, virion enzyme methylated only one guanine to yield 7-methylguanine. Methylation enzyme in a virion recognizes the 7-methylated guanine in the confronting nucleotides, and it does not advance further methylation.

On the Base-Stacking in the 5′-Terminal Cap Structure of mRNA: a Fluorescence Study

Yoshifumi NISHIMURA, Masamichi TSUBOI, Kazuo YAMAGUCHI,
Tsujiaki HATA, Masao HATTORI, Kin-ichiro MIURA

With the use of a synthetic model compound, $m^7G^{5'}$pppA and a few other $m^7G^{5'}$pppN's, conformation of the 5′-terminal cap structure of mRNA was stdied by fluorescence. The fluorescence at 370 nm of the 7-methylguanosine residue (m^7G) is found to be quenched when the base residue is involved in a stacking interaction with the adenosine residue in the cap structure $m^7G^{5'}$-pppA of a eukaryotic mRNA. On the basis of the observed degree of quenching, the amounts of the stacked and unstacked forms in the cap structure have been determined at various temperatures and pH's. It has been found that at pH 6.2 effective enthalpy and entropy in the unstacked→stacked change are $\Delta H=-4.4\pm0.1$ kcal/mole and $\Delta S=-14.3\pm0.2$ e. u., respectively. The pKa value for the m^7G residue is found to be 7.7 at 10°C and 7.3 at 30°C. The stacked structure seems to be less favourable in the deprotonated form that occurs in the higher pH solution. The similar results were also obtained from the studies by circular dichroism and ultraviolet absorption (M. Hattori, K. Miura: This report 1978 No. 29). A similar analysis of some other cap structures indicated that the stacked form in $m^7G^{5'}$pppN structure is favourable if N is a purine nucleoside or a 2′-0-methyl-pyrimidine nucleoside but not for an unmethylated pyrimidine nucleoside.

The Nucleotide Sequence of the Tobacco Chloroplast Gene for the Large Subunit of Ribulose-1,5-bisphosphate Carboxylase/Oxygenase

Kazuo SHINOZAKI and Masahiro SUGIURA

The gene for the large subunit of ribulose-1,5-bisphosphate carboxylase/oxygenase from tobacco has been cloned in pBR322 and sequenced (Fig. 1). The coding region contains 1431 base-pairs (477 codons). The deduced amino acid sequence of tobacco LS protein shows 90% homology with those of maize and spinach LS. The positions in the gene corresponding to the 5′ and the 3′ ends of tobacco LS mRNA have been located on the DNA sequence by a Sl nuclease mapping procedure. The LS gene promoter sequence has homology with *Escherichia coli* promoter sequences and its terminator sequence is capable of forming a stem and loop structure. A sequence GGAGG, which is complementary to a sequence near the 3′ end of tobacco chloroplast 16S rRNA and a putative ribosome binding site, occurs 6-10 bp upstream from the initiation codon (Gene, **20**, 91–102, 1982).

The Complete Nucleotide Sequence of a 23S rRNA Gene from Tobacco Chloroplasts

Fumio TAKAIWA and Masahiro SUGIURA

The nucleotide sequence of a tobacco chloroplast 23S rRNA gene, including the spacer between it and the 4.5S rRNA gene, has been determined (Fig. 1). The 23S rRNA coding region is 2804 base-pairs long. A comparison with the 23S rRNA sequence of *Escherichia coli* reveals strong homology and further shows a similarity between the chloroplast 4.5S rRNA and the 3′-terminal region of *E. coli* 23S rRNA. However, the 101 base-pair spacer sequence between the 23S and 4.5S rRNA genes has little homology with *E. coli* 23S rRNA. This work has been reported in Eur. J. Biochem. **124**, 13–19 (1982).

AATTCCG TATATTTTCA CATCTAGGAT TTACATATAC AACATATACC

ACTGTCAAGG GGGAAGTTCT TATTATTTAG GTTAGTCAGG TATTTCCATT TCAAAAAAAA AAAAAGTAAA AAAGAAAAAT TGGGTTGCGC TATATATATG

AAAGAGTATA CAATAATGAT GTATTTGGCA AATCAAATAC CATGGTCTAA TAATCAAACA TTCTGATTAG TTGATAATAT TAGTATTAGT TGGAAATTTT

GTGAAAGATT CCTATGAAAA GTTTCATTAA CACGGAATTC GTGTCGAGTA GACCTTGTTG TTGTGAGAAT TCTTAATTCA TGAGTTGTAG GGAGGGATTT

ATGTCACCAC AAACAGAGAC TAAAGCAAGT GTTGGATTCA AAGCTGGTGT TAAAGAGTAC AAATTGACTT ATTATACTCC TGAGTACCAA ACCAAGGATA

CTGATATATT GGCAGCATTC CGAGTAACTC CTCAACCTGG AGTTCCACCT GAAGAAGCAG GGGCCGCGGT AGCTGCCGAA TCTTCTACTG GTACATGGAC

AACTGTATGG TCCGATGGAC TTACCAGCCT TGATCGTTAC AAAGGGCGAT GCTACCGCAT CAGGCGTGTT GTTGGAGAAA AAGATCAATA TATTGCTTAT

GTAGCTTACC CTTTAGACCT TTTTGAAGAA GGTTCTGTTA CCAACATGTT TACTTCCATT GTAGGTAACG TATTTGGGTT CAAAGCCCTG CGCGCTCTAC

GTCTGGAAGA TCTGCGAATC CCTCCTGCTT ATGTTAAAAC TTTCCAAGGT CCGCCTCATG GGATCCAAGT TGAAAGAGAT AAATTGAACA AGTATGGTCG

TCCCCTGTTG GGATGTACTA CTAAACCTAA ATTGGGGTTA TCTGCTAAAA ACTACGGTAG AGTTGTTTAT GAATGTCTTC GCGGTGGACT TGATTTTACT

AAAGATGATG AGAACGTGAA CTTATTATTT ATGCGTTGGA GAGGTCGTTT CTTATTTTGT GCCGAAGCAC TTTATAAAGC ACAGGCTGAA ACAGGTGAAA

TCAAAGGGCA TTACTTGAAT GCTACTGCAG GTACATGCGA AGAAATGATC AAAAGAGCTG TATTTGCTAG AGAATTGGGC GTTCCGATCG TAATGCATGA

CTACTTAACG GGGGGATTCA CCGCAAATAC TAGCTTGGCT CATTATTGCC GAGATAATGG TCTACTTCTT CACATCCACC GTGCAATGCA TGCGGTTATT

GATAGACAGA AGAATCATGG TATCCACTTC CGGGTATTAG CAAAAGCGTT ACGTATGTCT GGTGGAGATC ATATTCACTC TGGTACCGTA GTAGGTAAAC

TTGAAGGTGA AAGAGACATA ACTTTGGGCT TTGTTGATTT ACTGCGTGAT GATTTTGTTG AACAAGATCG AAGTCGCGGT ATTTATTTCA CTCAAGATTG

GGTCTCTTTA CCAGGTGTTC TACCCGAGGC TTCAGGAGGT ATTCACGTTT GGCATATGCC TGCTCTGACC GAGATCTTTG GGGATGATTC CGTACTACAG

TTCGGTGGAG GAACTTTAGG ACATCCTTGG GGTAATGCGC CAGGTGCCGT AGCTAATCGA GTAGCTCTAG AAGCATGTGT AAAAGCTCGT AATGAAGGAC

GTGATCTTGC TCAGGAAGGT AATGAAATTA TTCGCGAGGC TTGCAAATGG AGCCCGGAAC TAGCTGCTGC TTGTGAAGTA TGGAAAGAGA TCGTATTTAA

TTTTGCAGCA GTGGACGTTT TGGATAAGTA AAAACAGTAG ACATTAGCAG ATAAATTAGC AGGAAATAAA GAAGGATAAG GAGAAAGAAC TCAAGTAATT

ATCCTTCGTT CTCTTAATTG AATTGCAATT AAACTCGGCC CAATCTTTTA CTAAAAGGAT TGAGCCGAAT ACAACAAAGA TTCTATTGCA TATATTTTGA

CTAAGTATAT ACTTACCTAG ATATACAAGA TTTGAAATAC AAAATCTAGA AAACTAAATC AAAATCTAAG ACTCAAATCT TTCTATTGTT GTCTTGGATC

Fig. 1. Nucleotide sequence of the tobacco chloroplast gene for the large subunit of ribulose-1,5-bisphosphate carboxylase/oxygenase.

TTCAAACGAGGAAAGGCTTACGGTGGATACCTAGGCACCCAGAGACGAGGAAGGGCGTAGTAATCGACGAAATGCTTCGGGGAGTTGAAAATAAGCATAG 100

ATCCGGAGATTCCCGAATAGGGCAACCTTTCGAACTGCTGCTGAATCCATGGGCAGGCAAGAGACAACCTGGCGAACTGAAACATCTTAGTGAGCCAGAG 200

GAAAAGAAAGCAAAAGCGATTCCCGTAGTAGCGGCGAGCGAAATGGGAGCAGCCTAAACCGTGAAAACGGGTTGTGGGAGAGCAATACAAGCGTCGTGCT 300

GCTAGGCGAAGACGCCCGAATGCTGCACCCTAGATGGCGAAAGTCCAGTAGCCGAAAGCATCACTAGCTTATGCTCTGACCCGAGTAGCATGGGGCACGT 400

GGAATCCCGTGTGAATCGACAAGGACCACCTTGCAAGGCTAAATACTCCTGGGTGACCGATAGCGAAGTAGTACCGTGAGGGAAGGGTGAAAAGAACCCC 500

ATCGGGGAGTGAAATAGAACATGAAACCGTAAGCTCCCAAGCAGTGGGAGGCCAGGGCTCTGACCGCGTGCCTGTTGAAGAATGAGCCGGCGACTCATAG 600

GCAGTGGCTTGGTTAAGGGAACCCACCGGAGCCGTAGCGAAAGCGAGTCTTCATAGGGCAATTGTCACTGCTTATGGACCCGAACCTGGGTGATCTATCC 700

ATGACCAGGATGAAGCTTGGGTGAAACTAAGTGGAGGTCCGAACCGACTGATGTTGAAGAATCAGCGGATGAGTTGTGGTTAGGGGTGAAATGCCACTCG 800

AACCCAGAGCTAGCTGGTTCTCCCCGAAATGCGTTGAGGCGCAGCAGTTGACTGGACATCTAGGGGTAAAGCACTGTTTCGGTGCGGGCCGCGAGAGCGG 900

TACCAAATCGAGGCAAACTCTGAATACTAGATATGACCTCAAAATAACAGGGGTCAAGGTCGGCTAGTGAGACGATGGGGGATAAGCTTCATCGTCGAGA 1000

GGGAAACAGCCCGGATCACCAGCTAAGGCCCCTAAATGATCGCTCAGTGATAAAGGAGGTAGGGGTGCAGAGACAGCCAGGAGGTTTGCCTAGAAGCAGC 1100

CACCCTTGAAAGAGTGCGTAATAGCTCACTGATCGAGCGCTCTTGCGCCGAAGATGAACGGGGCTAAGCGATCTGCCGAAGCTGTGGGATGTAAAAATAC 1200

ATCGGTAGGGGAGCGTTCCGCCTTAGAGAGAAGCCTCCGCGCGAGCGGTGGTGGACGAAGCGGAAGCGAGAATGTCGGCTTGAGTAACGCAAACATTGGT 1300

GAGAATCCAATGCCCCGAAAACCTAAGGGTTCCTCCGCAAGGTTCGTCCACGGAGGGTGAGTCAGGGCCTAAGATCAGGCCGAAAGGCTAGTCGATGGAC 1400

AACAGGTGAATATTCCTGTACTGCCCCTTGTTGGTCCCGAGGGACGGAGGAGGCTAGGTTAGCCGAAAGATGGTTATCGGTTCAAGAACGTAAGGTGTCC 1500

CTGCTTTGTCAGGGTAAGAAGGGGTAGAGAAAATGCCTCGAGCCAATGTTCGAATACCAGGCGCTACGGCCGGAAGTAACCCATGCCATACTCCCAGGAA 1600

AAGCTCGAACGACTTTGAGCAAGAGGGTACCTGTACCCGAAACCGACACAGGTGGGTAGGTAGAGAATACCTAGGGGCGCGAGACAACTCTCTCTAAGGA 1700

ACTCGGCAAAATAGCCCCGTAACTTCGGGAGAAGGGGTGCCTCCTCACAAAGGGGGTCGCAGTGACCAGGCCCGGGCGACTGTTTACCAAAAACACAGGT 1800

CTCCGCAAAGTCGTAAGACCATGTATGGGGCTGACGCCTGCCCAGTGCCGGAAGGTCAAGGAAGTTGGTGACCTGATGACAGGGGAGCCGGCGACCGAAG 1900

CCCCGGTGAACGGCGGCCGTAACTATAACGGTCCTAAGGTAGCGAAATTCCTTGTCGGGTAAGTTCCGACCCGCACGAAAGGCGTAACGATCTGGGCACT 2000

GTCTCGGAGAGAGGCTCGGTGAAATAGACATGTCTGTGAAGATGCGGACTACCTGCACCTAGACAGAAAGACCCTATGAAGCTTCACTGTTCCCTGGGAT 2100

TGGCTTTGGGCCTTTCCTGCGCAGCTTAGGTGGAAGGCGAAGAAGGCCTCCTTCCGGGGGGGCCCGAGCCATCAGTGAGATACCACTCTGGAAGGGCTAG 2200

AATTCTAACCTTGTGTCAGGACCTACGGGCCAAGGGACAGTCTCAGGTAGACAGTTTCTATGGGGCGTAGGCCTCCCAAAAGGTAACGGAGGCGTGCAAA 2300

GGTTTCCTCGGGCCGGACGGAGATTGGCCCTCGAGTGCAAAGGCAGAAGGGAGCTTGACTGCAAGACCCACCCGTCGAGCAGGGACGAAAGTCGGCCTTA 2400

GTGATCCGACGGTGCCGAGTGGAAGGGCCGTCGCTCAACGGATAAAAGTTACTCTAGGGATAACAGGCTGATCTTCCCCAAGAGCTCACATCGACGGGAA 2500

GGTTTGGCACCTCGATGTCGGCTCTTCGCCACCTGGGGCTGTAGTATGTTCCAAGGGTTGGGCTGTTCGCCCATTAAAGCGGTACGTGAGCTGGGTTCAG 2600

AACGTCGTGAGACAGTTCGGTCCATATCCGGTGTGGGCGTTAGAGCATTGAGAGGACCTTTCCCTAGTACGAGAGGACCGGGAAGGACGCACCTCTGGTT 2700

GACCAGTTATCGTGCCCACGGTAAACGCTGGGTAGCCAAGTGCGGAGCGGATAACTGCTGAAAGCATCTAAGTAGTAAGCCCACCCCAAGATGAGTGCTC 2800

TCCT

Fig. 1. Nucleotide sequence of tobacco chloroplast 23S rRNA gene.

Nucleotide Sequence of the 16S-23S Spacer Region in an rRNA Gene Cluster from Tobacco Chloroplast DNA

Fumio TAKAIWA and Masahiro SUGIURA

The nucleotide sequence of a spacer region between 16S and 23S rRNA genes from tobacco chloroplasts has been determined (Fig. 1). The spacer region is 2080 bp long and contains $tRNA^{Ile}$ and $tRNA^{Ala}$ genes which have intervening sequences of 707 bp and 710 bp, respectively. Strong homology between the two intervening sequences is observed. These spacer tRNAs are synthesized as part of an 8.2 kb precursor molecule containing 16S and 23S rRNA sequences. This work has been reported in Nucleic Acids Research **10**, 2665–2676 (1982).

The Complete Nucleotide Sequence of a 16S Ribosomal RNA Gene from a Blue-Green Alga, *Anacystis nidulans*

Noboru TOMIOKA and Masahiro SUGIURA

The complete nucleotide sequence of a 16S ribosomal RNA gene from *Anacystis nidulans* has been determined (Fig. 1). This nucleotide sequence has 83% homology with that of tobacco chloroplast 16S rRNA gene and 74% homology with that of *E. coli* 16S rRNA gene. The 3′-terminal region of this gene contains the sequence CCTCC which is complementary to sequence found at the 5′-termini of prokaryotic mRNAs.

Cloning and Characterization of a Plasmid DNA from *Anacystis nidulans* 6301

Kazuo SHINOZAKI, Noboru TOMIOKA, Chieko YAMADA and Masahiro SUGIURA

A plasmid DNA of *Anacystis nidulans* 6301 was isolated by CsCl density gradient containing ethidium bromide. The size of the plasmid DNA, named pBAl, was estimated to be 5.04 ± 0.26 Md by electron microscopic analysis and 5.2 Md by agarose gel electrophoresis. The pBAl was digested with BamHI and cloned in pBR322 vector propagated in *E. coli* HB101 cells. The recombinant DNA, named pBAS18, was digested with various restriction endonucleases and its cleavage map was constructed. The cleavage map of the pBAl plasmid DNA was made on the basis of the

16S -- CATG ... CATCC -- 23S

298 bp

64 bp

72 bp

707 bp

73 bp

710 bp

tRNA^{Ile}

tRNA^{Ala}

Fig. 1. Nucleotide sequence of the 16S–23S spacer region in an rRNA gene cluster from tobacco chloroplast DNA.

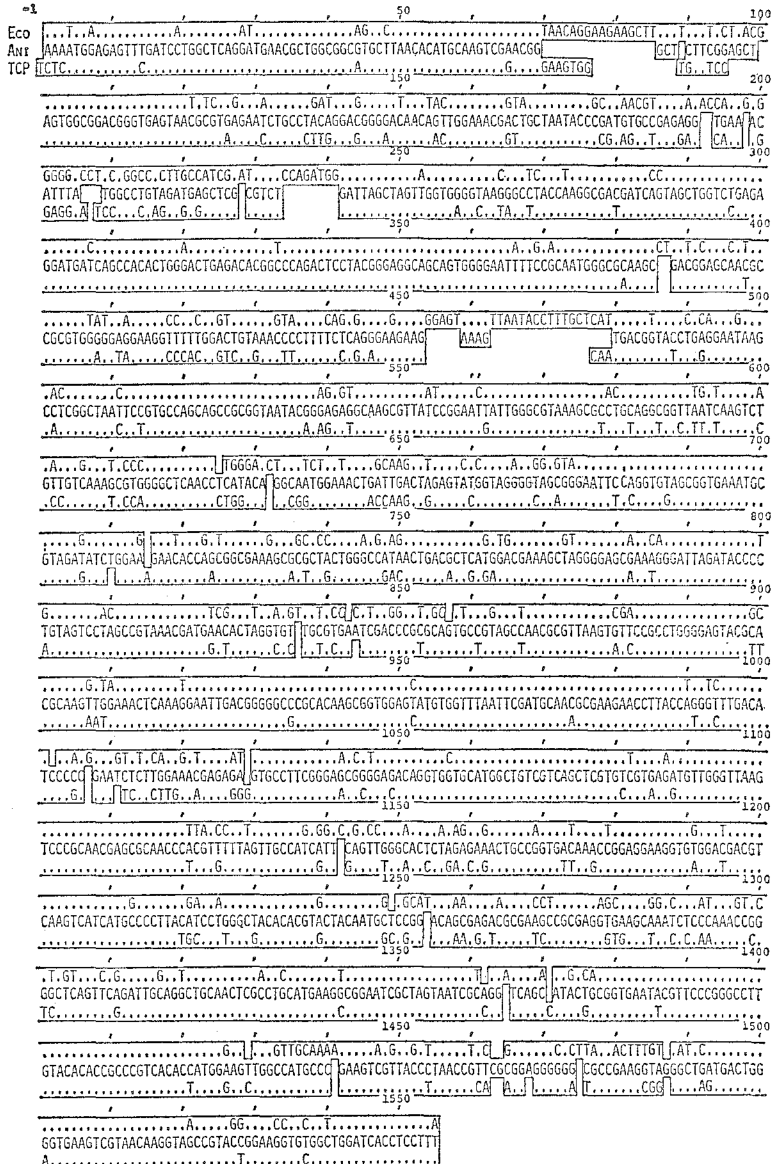


Fig. 1. Nucleotide sequence of *A. nidulans* 16S rRNA gene. The non-coding strand is shown (middle row). For comparison, sequences of *E. coli* rRNA gene (top row) and of tobacco chloroplast rRNA gene (bottom row) are presented.

cleavage map of the pBAS18 and pBR322 DNA. This work has been reported in Gene 19, 221–224 (1982).

Transformation with DNA Fragments Encompassing the Polyoma Virus Promoter

Eiichi SOEDA,, Yoshio MAKI, Yoshiro NAKANO and Hiroyuki TASHIRO

The polyoma Pst I-2 fragments encompassing the promoter but not encoding functional tumor antigens were cloned in pBR 322 and applied to 3Yl rat and NIH mouse fibroblast cells by using calcium phosphate technique. The recipient cells were induced foci and exhibited some properties of transformed cells. Four clones of the transformed rat cells were transplanted to new born Fisher rats to form tumors. The polyoma viral sequences were detected in the DNAs prepared from the cells by Southern blotting hybridization as discrete Eco RI fragments. Some of these were isolated by using λ phage vector technique. The transformed phenotypes were transmissible into normal recipients via DNA transfection.

These evidences have suggested that the polyoma virus promoter can induce cellular transformation.

A Total Nucleotide Sequence of Murine Papovavirus K DNA

Hiroyuki JIKUYA, Kenneth K. TAKEMOTO[1] and Eiichi SOEDA

A total nucleotide sequence of murine papovavirus K (KV) genome has been determined which consisted of 4994 nucleotides. Nucleotide comparison revealed that KV genome could code for small T antigen, large T antigen, VP1, VP2, and VP3, of which sequences were homologous to those of SV40 and polyoma virus, but not for the relevant of polyoma virus middle T antigen. These evidences suggested that these viruses diverged from a common origin, and enforced further that the polyoma virus middle T antigen was implicated in tumor formation of animals.

[1] Laboratory of Viral Diseases, National Institute of Allergy and Infectious Diseases, Bethesda, Maryland 20205 U.S.A..

Genomic Structure of Human Polyomavirus JC: Nucleotide Sequence of the Region Containing Replication Origin and Small T Antigen Gene

Tatsuo MIYAMURA[1], Hiroyuki JIKUYA, Eiichi SOEDA and Kunito YOSHIIKE[1]

The nucleotide sequence of the region of human polyomavirus JC (JCV) DNA between 0.5 and 0.7 map units from a unique *Eco* RI cleavage site was determined and compared with those of the corresponding regions of another human polyomavirus BK (BKV) and simian virus 40 (SV40) DNAs. Within this region consisting of 945 base pairs, we located the origin of DNA replication near 0.7 map units, the entire coding region for small T antigen, and splice junctions for large T antigen mRNA. The deduced amino acid sequences for small T antigen and the part of large T antigen markedly resembled those of BKV and SV40. The results strongly suggest that JCV has the same organization of early genome as those of BKV and SV40 on the physical map with the *Eco* RI site as a reference point.

Complete Nucleotide Sequence of Harvey Murine Sarcoma Virus Genome

Masato FURUICHI, Yoshiro NAKANO, Hardy W. CHAN[2], Malcolm A. MARTIN[2] and Eiichi SOEDA

The complete nucleotide sequence of Harvey murine sarcoma virus (Ha-MuSV) was determined and genetic elements were located on the nucleotide sequence mainly by comparison with that of Moloney murine leukemia virus (Mo-MuLV), progenitor of Ha-MuSV.

The viral genome is composed of 4,376 nucleotides inserts derived from rat sequences flanked on the 5′ and 3′ ends by 141 and 907 nucleotides the sequences derived from Mo-MuLV, respectively. A topology of the genomic organization has suggested that Ha-MuSV was generated as results of multiple recombinations between Mo-MuLV and rat cellular sequences, and between rat cellular sequences. The recombination sites between them were tentatively assigned on the nucleotide sequence. The sequences close

[1] Department of Enteroviruses, National Institute of Health, Kamiosaki, Shinagawa-ku, Tokyo 141.

[2] DNA Recombinant Research Unit, National Institute of Allergy and Infectious Diseases, National Institute of Health, Bethesda, Maryland 20205 U.S.A.

to the recombination sites were characterized by the presence of some direct repeat sequences.

The viral genome has a coding capacity for a transformation specific protein, $p21^{src}$ in only one open reading frame of the non-homologous region to 30S RNA downstream from the left long terminal repeat (LTR) derived from Mo-MuLV terminal sequences, intervening the non-coding region of 938 nucleotides in length which is highly homologous to 30S RNA. A topology of the genomic organization of Ha-MuSV suggests that activation elements for RNA synthesis resided in the LTR induce the enhanced expression of the $p21^{src}$ gene via approximately 1 kb non-coding region.

Construction of a Gene Library of Silkworm and Cloning its rRNA Genes

Jun KUSUDA

It has not been possible to extract intact whole genomic DNA from eukaryote cells using conventional biochemical methods because of its large size. Recent advance of gene cloning techniques made possible to isolate DNA fragments containing specific genes from genomic DNA.

This paper presents construction of a gene library of silkworm (*Bombyx mori*). Genomic DNA from posterior silkglands of 5th instar larvae (standard strain C-108) was digested partialy with restriction endonuclease Sau3A and subjected to electrophoresis in 1% agarose gel. The DNA fragments of $1\sim2\times10^4$ bp long eluted from agarose gel were joined to λ phage vector. Charon30 DNA was cleaved into four fragments with BamHI and removed two internal fragments with genes nonessential for phage viability. The Charon30 arms consist of BamHI fragments containing the λ cos site were ligated with genomic DNA fragments digested with Sau3A which produced the same cohesive end as BamHI. Ligated DNAs were packaged *in vitro*. Yield was about 1.4×10^5 infectious phages per μg silkworm DNA. Assuming an average size of 1.3×10^4 bp for replaceable fragments in Charon30 and a genome size of 1×10^9 bp for silkworm, the number of independent recombinant phage needed to find any given single copy sequence in the library with a probability of 0.99 is calculated to be 3.5×10^5 phages on the basis of an equation by Clarke and Carbon (Clarke, L. and Carbon, J. 1976 Cell 91–99). Approximately 4×10^5 recombinant phages were produced for the gene library of silkworm. In order to examine the faithfullness for

representation of silkworm genome in this library, the size of inserts and the probability of finding clones carrying rRNA gene were determined. The average size of insert DNAs from 35 independent plaques was 1.4×10^4 bp as judged by restriction analysis. From this size, the number of clones were proven to be sufficient for the gene library of silkworm. It is known that the silkworm genome code for 240 copies of rRNA genes and the repeat unit of this gene is 1×10^4 bp long. We expected to detect one to two rDNA clones in 400~500 plaques from above values. The silkworm library was screened for rDNA sequence using ^{32}P labeled rRNA as a probe.

Positive signals were observed at the frequency of one per 360 plaques and this agreed well with the estimated value. The size of inserts and a recovery of clones containing rRNA gene suggest that this gene library is complete and available as a gene pool of silkworm.

II. MICROBIAL GENETICS

Physical Map of *nrdA-nrdB-ftsB-glpT* Region of the Chromosomal DNA of *Escherichia coli*

Masao YAMADA, Yutaka TAKEDA, Kazuo OKAMOTO and Yukinori HIROTA

Seven pLC plasmids (pLC 3-46, 8-12, 8-24, 8-29, 14-12, 19-24, and 42-17) which complemented *nrdA*, *nrdB*, *ftsB* and/or *glpT* mutations of *Escherichia coli* were analyzed. A restriction map of each plasmid was constructed and restriction fragments were subcloned into pBR322. A physical map of approx. 15×10^6 Md segment of the chromosomal DNA was deduced from the overlapping region of the pLC plasmids. The pLC plasmids and newly constructed plasmids were examined for the ability to rescue the mutations. The complementation tests defined the location of the genes in the 15×10^6 Md segment in the following order: *nrdA-nrdB-ftsB-glpT*. Functional *nrdA*, *B* and *ftsB* genes were located in the 3.1×10^6 Md *EcoRI-PstI* fragment. (For detail, see Gene, **18**, 309–318, 1982).

Inhibitory Effect of Adenosine 3′, 5′-phosphate on Cell Division of *Escherichia coli* K-12 Mutant Derivatives

Ryutaro UTSUMI*, Hiroyuki TANABE*, Yumiko NAKAMOTO*,
Makoto KAWAMUKAI*, Hiroshi SAKAI*, Michio HIMENO*,
Tohru KOMANO*, and Yukinori HIROTA

Cell division of *Escherichia coli* K-12 strain PA3092 was inhibited by the addition of adenosine 3′,5′-phosphate (cAMP), and the cellular morphology was changed from rods into filaments. Nucleoids in the filaments were regularly distributed and septum formation was perfectly inhibited. This inhibition of cell division by cAMP was reversed by the addition of guanosine 3′,5′-monophosphate. To examine whether the inhibitory effect of cAMP on cell division in *E. coli* PA3092 was specific, its effect in several parental strains was investigated. Induction of cell filamentation by cAMP was observed in *E. coli* PA309 and P678, but not in *E. coli* W595, W1, Y10, or the

* Laboratory of Biochemistry, Department of Agricultural Chemistry, Kyoto University, Kyoto 606.

wild type strain. This result suggests that filamentation by cAMP in *E. coli* PA3092, PA309, and P678 was due to the mutagenesis by which *E. coli* P678 was derived from *E. coli* W595. (For detail, see Bacteriol. 147(3), 1105–1109, 1981).

Cell Surface Charge and Cell Division in *Escherichia coli* after X Irradiation

Chikako SATO*, Kiyohide KOJIMA*, Kimiko NISHIZAWA*, and Yukinori HIROTA

Simultaneous detection of electrophoretic mobility (EPM) and morphology of individual irradiated *Escherichia coli* cells under the phase microscope revealed a concurrent decrease in EPM and arrest of cell division. EPM decreased with time and reached a minimum 15 min after irradiation with doses ranging from 100 R to 80 kR. Cells elongating due to the division block retained the minimum EPM. After a recovery phase, separated small-sized daughter cells and some long filamentous cells, which had a few cleavages at the termini, returned to the normal EPM. This finding indicates that recovery in EPM, which represents recovery in the surface architecture, precedes or coincides with the resumption of cell division. Nuclear staining of the recovering cells leads to the suggestion that the cleavage of the cell takes place whenever the EPM has recovered, irrespective of the segregation of DNA, which gives rise to anuclear cells having normal EPM. It is suggested that the mechanism of EPM decrease is Ca^{2+}-dependent conformational change of the mambrane accompanying vertical translocation of (For detail, see Radiation Research, *87*, 646-656, 1981)

* Laboratory of Experimantal Radiology, Aichi Cancer Center Research Institite, Chikusa-ku, Nagoya 464, Japan.

** Laboratory of Oncological Pathology, Nara Medical University, Kashihara 634, Japan.

Structure of Replication Origin of the *Escherichia coli* K-12 Chromosome: the Presence of Spacer Sequences in the *ori* Region Carrying Information for Autonomous Replication

Kiyozo ASADA*, Kazunori SUGIMOTO*, Atsuhiro OKA*, Mituru TAKANAMI*, and Yukinori HIROTA

The replication origin region of the *Escherichia coli* K-12 chromosome has been cloned, and a region of 245 base-pairs has been shown to contain all the information for autonomous replication (defined *ori*). In order to obtain further information on the sequence organization in the defined *ori* stretch, various types of mutation were introduced by *in vitro* techniques as a restriction site (*Ava*II site) which is located near the middle of *ori*. When the correlation between these mutations and replicating junction was examined, different effects were obtained with the types of mutation: the replicating function was completely destroyed by either insertion or deletion of short sequences, but not by base- substitutions. Based on these observations and on the fact that multi-gene products are involved in the initiation of replication, we assume that two categories of sequences are present within the *ori* stretch, one specifying interaction with initiation factors (recognition sequences) and the other spacing the recognition sequences in appropriate distances (spacer sequences), and that the *Ava*II site is located in the spacer region. (For detail, see Nucleic Acid Research **10**(12), 3745–3754, 1982)

Conjugative Transfer of pBR322 Carrying the Chromosomal DNA of *Escherichia coli* Mediated by an Hfr

Masao YAMADA and Yukinori HIROTA

Hybrid plasmids consisting of a non-mobilized plasmid, pBR322, and a segment of chromosomal DNA of *Escherichia coli* could be transferred from an Hfr donor to recipient cells by a bacterial mating. When the chromosomal DNA in the plasmid corresponded to the early transfer region of the Hfr, the frequency of the transfer was high. The *recA* function of both donor and recipient cells was required in the tansfer. The physical association of the hybrid plasmid with the transferring Hfr chromosome between the homologous sequence may cause the transfer of the non-mobilized

* Institute for Chemical Research, Kyoto University, Uji, Kyoto.

plasmid. This phenomemon is useful for the determination of the chromosomal site of an unidentified fragment cloned in a non-mobilized plasmid. (For detail, see Gene, 1982).

Escherichia coli Origin of Replication: Structural Organization of the Region Essential for Autonomous Replication and the Recognition Frame Model

Yukinori HIROTA, Atsuhiro OKA*, Kazunori SUGIMOTO*, Kiyozo ASADA*, Hitoshi SASAKI*, and Mituru TAKANAMI

The replication origin region of the *E. coli* chromosome has been cloned, and the precise location of the region carrying autonomous replicating function (defined *ori*) has been determined. The left boundary of *ori* is between positions 23(A) and 35(T) and the right boundary is between positions 267(C) and 268(A). The maximum size of the *ori* segment is therefore 245 base pairs (bp) long.

A series of mutations having short sequences inserted or deleted was introduced in the vicinity of *Bgl*II, *Bam*HI, *Ava*II, mutations C to T (or G to A) were also introduced by *in vitro*, mutagenesis which used bisulfite, and the correlation between the phenotypes and nucleotide sequences of mutant was analyzed. We could identify two categories of regions: one is the region in which even a single base substitution destroys *ori* function and the other is the region in which insertion or deletion of short sequences, but not base-substitutions, destroys *ori* function.

On the basis of these observations, we propose the following model for the structural organization of *ori* (recognition frame model): the recognition sequences that determine the sites where the components involved in the replication initiation recognize and the spacer sequences (or distance sequences) that maintain the recognition saquences at precise destances are connected with each other in a chimeric fashion.

According to this model, the striking effect caused by the shft of recognition sequences within *ori* can be explained. The *ori* stretch should provide precise topological information for the formation of an initiation complex by multiple components which are involved in the initiation of replication. (For detail, see "The initiation of DNA replication, Academic Press., Ed. Ray., pp 1–12.)

* Institute for Chemical Research, Kyoto University, Kyoto 611, JAPAN.

On the Process of Cellular Division in *Escherichia coli*: Nucleotide Sequence of the Gene for Cellular Division

Masataka NAKAMURA, Ichiro N. MARUYAMA, Masaaki SOMA, Jun-ichi KATO*, Hideho SUZUKI*, and Yukinori HIROTA

We determined the nucleotide sequence of a DNA fragment containing the *fts*I gene coding for penicillin-binding protein 3 (PBP-3) which is indispensable for the cell division of *Escherichia coli*. The DNA segment necessary for expression of the *fts*I gene was contained in the *Pvu*II restriction fragment 2.8 kilobase long derived from the chromosomal segment on pLC26-6. The nucleotide sequence analysis showed that only one open reading frame, with this restriction fragment, allowed a region enough to code for the size of PBP-3 (60 kdal). The coding region for the PBP-3 was determined according to the N-terminal amino acid sequence of the *in vitro* synthesized PBP-3, wich was most likely to be the primary translational product and had the N-terminal sequence of (N) Met-Lys-Ala-Ala-Ala-Lys..... The region of the structural gene for *fts*I consisted of 1,764 base-pairs coding for a polypeptide of 588 amio acid residues and of 63,876 daltons in molecular weight. The *in vitro* synthesized PBP-3 showed slower mobility in SDS-gel electrophoresis than the authentic PBP-3 and it was suggested that the translational product of the *fts*I gene may be processed to yield the mature PBP-3. The deduced amino-acid sequence indicates that the polypeptide of PBP-3 has a typical characteristic of a membrane protein, i. e. 79% of amino-acid residues is nonpolar or uncharged polar, and there are several hydrophobic regions. A possible promoter sequence and ribosome binding sequence of the *fts*I gene are suggested. The pattern of codon usage for the *fts*I gene was similar to that reported for the ribosomal proteins in *E. coli*.

Overproduction of *Escherichia coli* Replication Proteins by the Use of the Runaway-Replication Plasmids

Seiichi YASUDA and Tsutomu TAKAGI

A derivative of the runaway-replication plasmid was constructed. This plasmid, pSY343, has the gene for kanamycin-resistance and single sites for *Eco*RI, *Bam*HI, *Hin*dIII, *Kpn*I and *Xho*I which can be used as cloning sites

* Laboratory of Genetics, Department of Biology, Faculty of Sciences, University of Tokyo, Hongo, Tokyo, 113 Japan.

without inactivating the kanamycin gene or the replication genes. The replication genes of *Escherichia coli* were cloned on the plasmid. Activity of *dnaA*, *dnaZ* and *ssb* gene products were 200, 90 and 60 fold greater, respectively, in the cells containing these plasmids than in normal cells.

Possible Repressor of Flagellar Regulon in *Escherichia coli* K-12

Yoshibumi KOMEDA

We have examined Fla⁻ effects onto transcription of each flagellar operon using *fla-lacZ* fusion technique. Summarizing the results, operon "A" was situated after operon "B" whose gene products were responsible for transcription of operon "A" using arrow (as B→A, see this report of 1979 edition). When we obtained results showing C→B, B→A, and C→A, we described as C→B→A. This qualitative analysis did not specify molecular mechanism between alternative manners: (i) "C" interacts with both "A" and "B" and (ii) "C" interacts with "B" but not with "A", and "B" interacts with "A", consequently "C" indirectly interacts with "A".

This article focuses on the interaction between group (3) operons and (4), (5), (6) operons (designation, see 1979 report). The transcription of *hag* gene, for example, requires all of flagellar gene products except *flaS*, *flaT*, *flaU* and *flbC* gene products. There are three possible ways to carry out this control: (i) All of the gene products are positive factors. (ii) There is one (or a few) repressor of *hag* transcription and the others are anti-repressors. (iii) Complex mechanism including the methods (i) and (ii). Double mutants were constructed in order to examine these possibilities. If the positive interaction is the only way as in (i), the transcription of *hag*, *flaS*, Mocha and *flaZ* operons is independent to *flaU* and *flbC* gene products and only depends upon the existence of (3) operon gene products.

This mechanism predicts that *flaU flaE* or *flbC flaE* double mutants, for example, would show the phenotype of single *flaE* mutant for transcription of (4), (5), (6)- operon- transcription. The phenotype of the *flaE flbC* double mutant was the same as that of the *flaE* mutant itself; the phenotype of the *flaE flaU* double mutant was the same as that of the *flaU* mutant itself. This result showed that the function of *flaE* gene is not independent to *flaU* gene product. In order to extend this observation a whole set of double mutants carrying *flaU4511*: Tn5 mutation was constructed. They were used as hosts

for examination of Lac phenotype of each *λfla-lac* phage. Almost all double mutants had the same phenotype as that of *flaU4511* mutant. *flaU4511 flaD*, *flaU4511 flaI*, and *flaU4511 flbB* double mutants showed the phenotypes of the latter mutations. Next, the *flaD4512*:: Tn5 mutation was introduced to each *fla* mutant and its effect was examined. The result showed that *flaD* activity was required for transcription of (4), (5), (6) operons.

The simplest explanation of these data was that *flaU* coded for the repressor of (4), (5), (6) operons and that it had interaction with (3) operon products for its repressor action. The *flaU* product amy repress (4), (5), (6) operon in the case of mutations in (3) operons (except *flaD*, *flaE* and *motD* genes). If a *flaU* mutation occurs and leads to absence of repressor molecules, (4), (5), (6) operons are transcribed even if there is a mutation in (3) operon (except *flaD* product).

As already known, all of the *fla* genes are positive factors for the appearance of a flagellum. The *flaU* gene thus should be not only a repressor but also a positive factor. Since we have identified all of the *fla* genes by collection of flagellum-less phenotype mutants so far, this dual function may mutate to have novel repressor function. Accordingly, all of the *flaU* mutants were examined for the capacity to support the *lacZ* expression after *λfla-lacZ* infection. Out of five *flaU* mutants, strain YK4435 was different to the other mutants. It had incomplete flagella as the others, but it did not permit transcription of (4), (5), (6) operons. Insertion mutants, strains YK4511 and YK3440, were supposed to be null-phenotype and did not permit transcription of above mentioned operons. Being different to these mutants, strain YK4435 had a defect of flagellar formation still retaining repressor function. The *flaU4435 flaB* double mutants did not permit transcription of (4), (5), (6) operons. Other double mutants carrying (3) operon defects behaved as same way. The *flaU4435 flaD4512*:: Tn5 double mutants did not promote transcription of *λfla-lac* phages as expected. The existence of this mutant (YK4435) favoured the idea that *flaU* gene product has dual role: Positive factor for appearance of flagellum and repressor of *hag*, *flaS*, Mocha (*motA* and *che*) and *flaZ* operon transcription.

Coordination of Flagellar Formation and Cell Division in *Escherichia coli*

1. Flagellar Formation and Transcription of 'hag' Gene in Cell Division Mutants of *Escherichia coli*

Akiko NISHIMURA and Yukinori HIROTA

The molecular process of cell division in *E. coli* was investigated by the following experimental system. We document here a phenomenon that flagellar formation of thermosensitive mutants of *E. coli* defective in cell division is arrested when the mutant strain was grown under the non-permissible temperature. We used ten thermosensitive mutants defective in cell division at the different genes. Those are *ftsB*, C, D, E, F, G, I, or Z (defective in cell division at 40° and form multinucleated filaments) and *par*A, B (defective in partition of daughter nucleus at 40° and form filaments). These mutants were first grown in broth at 30° and then the incubation temperature was shifted to 40°. Cell division of the mutant was immediately arrested upon temperature shift.

Flagellar Formation

Flagellar formation of the mutant strains at non-permissible temperature was measured by counting the number of flagellar filaments per cell length. Samples were taken from a culture after various times of incubation at 40°. The number of flagella per cell and cell length in each sample were measured by the electron microscopic observation (50–150 cells were examined in each sample). The results showed that the seven *fts* mutant strains defective in either *fts*B, C, D, E, F, G, or Z immediately arrested flagellar formation at 40°. The two mutant strains defective in partition of daughter nuclei, *par* mutant, either *par*A or *par*B, arrested flagellar formation. However a mutant class, *fts*I, continued flagellar formation at the same rate to wild type strain, PA3092. The *fts*I mutant is a thermosensitive mutant defective in the penicillin binding protein 3 (PBP-3) which is involved in the final step of cell division (Suzuki, H. *et al.* 1978: Proc. Natl. Acad. Sci. USA **75**: 664)

Penicillin G inhibits cell division of *E. coli* by binding to PBP-3. The effect of penicillin G on flagellar formation was therefore measured. A wild type strain, PA3092, was cultivated at 40° in nutrient broth containing 2 units per ml of penicillin G. The flagellar formation of the cell, thus inhibited cell division, was continued as in the case of the mutant strain, *fts*I, under

the non-permissible growth condition. This means that penicillin G inactivates PBP-3, the product of *fts*I gene, by binding, and inhibition of flagellar formation did not take place.

From these results it is concluded that the seven *fts*-genes or the two *par*-genes acts on the regulatory processes of cell division and flagellar formation. Penicillin G or the TS-mutation occured in the *fts*I gene inhibits cell division but the flagellar formation is not inhibited.

To elucidate the regulatory process acting in the coordination between flagellar formation and cell division, the recovery of flagellar formation in the mutant strain, *fts*D, E or Z, at 30° was examined as follows. The cell division and flagellar formation was arrested by growing the mutant cells at 40° for 60 min; the culture was then brought back to 30°. At various times, aliqotes of sample were taken and flagellar formation was measured. The results showed two kind of responses in those mutants. When a mutant culure of *fts*E or *fts*Z was shifted to 30°, cell division resumed after a short lag ($\simeq$15 min) and flagellar formation was immediately recovered. However cell division and flagellar formation of the mutant strain, *fts*D, did not resume over 90 min incubation after the incubation temperature was shifted to 30°. In conclution, the recovery of flagellar formation in those mutant strains at 30° accompany the recovery of cell division. These results support a mechanism of coordination is acting on the regulatory processes of cell division and flagellar formation.

Transcription of flagellin messanger RNA (fln-mRNA)

Flagellar filament is composed of monomer proteins, flagellin, which is the gene products of a gene, hag. Transcription of *fln-m*RNA in the *fts*-mutant strains was therefore measured at 40° by DNA-RNA hybridization (Gillespie, D. and Spiegelman, S. 1965: J. Mol. Biol. **12**: 829). The mutant strain was grown at 30° and then the incubation temperature was shifted to 40°. The culture was incubated at 40° and aliqotes of sample were teken at various time and a 2 min pulse of ^{3}H-uridine (10 μCi/ml final) was given to label RNA. RNA was extracted by phenol extraction from the sample. A hag specific transducing λ DNA (λ hag DNA) was prepared and it was denatured and fixed on cellulose-nitrate disc. Each disc carryed 10 μg of denatured λ hag DNA. The disc was incubated (63° 24 hours) with RNA-^{3}H in 0.25 ml 2$\times$SSC half saturated with phenol. Nonspecifically bound RNA was digested with RNase. The disc was then dried and the radioactivity was

counted. The result showed that *fln-m*RNA synthesis of the nine mutants (*fts*B through G, Z, *par*A and B) was inhibited at 40°. However *fln-m*RNA of *fts*I mutant was continued to be synthesized under the non-permissible temperature.

Two possibilities are involved in the result; when the incubation temperature was shifted from 30° to 40°, the cell division of these mutant strain and transcription of *fln-m*RNA is arrested. Alternatively, rate of degradation of *fln-m*RNA is accelerated. Rate of degradation of *fln-m*RNA in the mutant strain, *fts*Z, and wild type strain, PA3092, was therefore compared, as follows. The cells were grown at 30°, then a 2 min pulse of ^{3}H-uridine was given to a culture and rifampicine (100 μg/ml final) was added to inhibit synthesis of *fln-m*RNA; the culture was then divided into two parts. The one was brought to 40° and the other was kept at 30°. After various times of incubation, aliqotes of sample were taken. Radioactivity of *fln-m*RNA fraction of the samples was counted by the method mentioned above. The results showed that the rate of degradation of *fln-m*RNA in the mutant strain, *fts*Z, and wild type strain, PA3092, at 40° was approximately 6.5–8 min. The rate of degradation of *fln-m*RNA in both strains at 30° was 9 min. These results agreed with the reported half life of *fln-m*RNA at 37° as 7.5 min (Suzuki, H. *et al.* 1974: Ann. Rep. Natl. Inst. Genet. (Japan) **24**: 14). Thus significant difference was not found in the degradation rate of *fln-m*RNA between the mutant strain, *fts*Z, and wild type strain, PA3092. From these results it is concluded that the arrest of flagellar formation in the *fts*-mutant was due to the sessation of the transcription of *fln-m*RNA.

Accounting for the all of the experimental results, the following regulatory mechanism was proposed. The nine *fts*-gene products (*fts*B through G, Z, *par*A and B) act on the transcription of genes of cell division and flagellar formation. As a consequence of thermal inactivation of the *fts*-gene products at the non-permissible temperature, both cell division and flagellar formation are arrested. In the case of the *fts*I mutant it is known that the function of *fts*I gene product is to act on the enzymatic process of cell division, and that the thermal inactivation of the *fts*I gene product does not affect the processes of transcription.

Coordination of Flagellar Formation and Cell Division in *Escherichia coli*

II. Transcription of *fla*-Operons in the Mutants of Cell Division in *Escherichia coli*

Akiko NISHIMURA, Yoshibumi KOMEDA and Yukinori HIROTA

In the accompanying paper (in this issue), it is reported that the nine genes (*fts*B, C, D, E, F, G, Z and *par*A, B) regulate transcription of *fln*-*m*RNA. In this paper, we report the expression of a series of flagellar operons, *fla*Z, *hag* and *mot*A, are controlled under the same regulatory process.

In *E. coli*, over 40 genes are known to be required for synthesis of flagella and functions of flagella including motility and chemotaxis of this organism. Recently, Komeda (1982: J. Bact. **150**: 16) analyzed these genes coding for the components of flagella at the level of the transcription. He used "lac-gene fusion technique" and 6 classes of flagellar operons were fused with *lac*Z gene. In these strains, transcription-translation system of the fused genes of *lac*Z operate *via fla* gene promoter so that the measurement of β-galactosidase activity directly reflected the transcription of the fused operon, in question.

A series of double mutant strains carrying *fts*-mutation also containing these *lac*-fusion genes were constructed by bacterial mating experiment. Therefore expression of *fla*-*lac* gene of the *fts*-mutant strains under non-permissible temperature can be quantitated by measuring the β-galactosidase activity of these strains. The β-galactosidase activity of the *fts* derivatives thus constructed was measured as follows.

The cells were grown at 30° and then incubation temperature was shifted to 40°. Cell density was recorded by measuring the light absorbance at 600 mμ. Aliqotes of cultures were taken after the temperature shift from 30° to 40°, then immediately one drop of toluene was added to permealize the cell. The toluene was evaporated at 37° and β-galactosidase activity of the sample was measured (Miller, J. H. 1972). A 0.1 ml of samples were added to a 0.9 ml of assay medium (Z-buffer), then 0.2 ml of o-nitrophenyl-β-D-galactoside (4 mg/ml) was added. After 15 min of incubation at 28°, reaction was arrested by adding 0.5 ml of a 1M-Na_2CO_3 solution and optical density at 420 mμ was measured.

The result showed that β-galactosidase activity of the *fts*-derivatives carrying both the *fts*-mutation (*fts*Z, *fts*D or *par*A) and the fused *lac*Z with either

*fla*Z, *hag* or *mot*A operon was decreased under the non-permissible temperature. However the derivative of *fts*I mutant strains carrying the fused *lac*Z with either *fla*Z, *hag* or *mot*A operon had β-galactosidase activity under the non-permissible temperature. In conclusion, *fts*Z, *fts*D and *par*A genes regulates the expression of *fla*Z, *hag* and *mot*A operons. However TS-mutation occured in the *fts*I gene does not effect on the expression of those three genes. Expression of *flb*B, *fla*D, *fla*G, *fla*A or *flb*C operons in the *fts*-derivatives were examined. The result showed that the TS-mutation occured in the *fts*Z, D, I and *par*A genes does not effect on the expression of those five *fla*-operons. These five *fla*-operons (*flb*B, *fla*D, *fla*G, *fla*A and *flb*C) are shown to be required for the completion of the basal structure of flagella, and it is also required in the early process of the flagellar formation. The expression of the three operons (*fla*Z, *hag* and *mot*A) are shown to function at the late process of flagellar formation, and the expression of these operons requires the expression of the other five *fla*-operons (Komeda, Y. 1982: J. Bact. **150**: 16).

The *fts*-gene products are required for transcription of genes essential for cell division and flagellar formation and affect on the expression of the *fla*-operons (*fla*Z, *hag* and *mot*A) which are the components operating on the late process of the flagellar formation.

Thus, we present here two alternative hypothesis: One hypothesis is that the *fts*-gene products act as catabolite activater proteins and control directly the transcription of genes essential for cell division and flagellar formation (such as *fla*Z, *hag* and *mot*A). Alternatively, it can be supposed that the thermal inactivation of *fts*-gene products, which are involved in the cell division, changes the physical character of membrane of the mutants. As the result, formation of basal structure of flagella can not be formed on the mutant membrane and then transcription of the *fla*-genes, such as *fla*Z, *hag* and *mot*A, are arrested at the non-permissible temperature.

III. BIOCHEMICAL GENETICS AND IMMUNOGENETICS

A Wild-Derived New H-2 Haplotype Enhancing K-IA Recombination

Toshihiko SHIROISHI, Tomoko SAGAI and Kazuo MORIWAKI

It is generally accepted that the overall recombination frequency between H-2K and H-2D loci is about 0.4%. However, the value fluctuates depending upon the combinations of H-2 haplotypes in the heterozygote. We re-examined the intra-H-2 recombination frequency, using three B10.W congenic strains which carry the H-2 complex of Japanese wild mice. Of the three, one strain, B10.MOL-SGR ($H\text{-}2^{wm7}$), showed an extremely high incidence of recombination (about 3%) in all combinations with three different inbred B10 congenic strains (Table 1). Twenty-nine recombinants originated in the heterozygotes of these H-2 combinations. The serological typing of these recombinants to map the crossing-over points revealed that most of the recombination events occurred between H-2K and IA loci (Table 2). These results firmly suggest that the wm7 haplotype enhances the H-2K end specific recombination irrespective of H-2 partners in heterozygous parents.

Thus the B10.MOL-SGR strain will provide a powerful tool for investigating the structure and functions of H-2 genes, because we can obtain the recombinant derived from K-end specific crossing-over by screening at most 30–40 mice, probably irrespecitive of H-2 haplotypes. Moreover, the resultant recombinants would be very useful in searching for unknown Class I and Class II loci if they are in the position of the H-2K end, especially

Table 1. Frequency of intra-H-2 recombination

H-2 haplotype of heterozygous parents	Method of screening	No. of mice screened	No. of recombinants	Recombination frequency (%)
a/wml	HA	294	1	0.3
a/wm11	HA	299	0	—
a/wm7	HA	539	16	3.0
k/wm7	CT	174	5	2.9
b/wm7	CT	204	8	3.9
Total		1510	30	1.9

HA: Hemagglutination test.
CT: Cytotoxicity method.

Table 2. Genetic composition of the recombinant H-2 haplotypes

Parental H-2 haplotype	Established strain	Recombinant H-2 haplotype	Origin of H-2 subregions				
			K	A	E	S	D
a/wm7	B10.A (R201)	aw 1	k	w	w	w	w
	B10.A (R202)	aw 2	k	k	k	d	w
	B10.A (R203)	aw 3	k	k	k	w	w
	B10.A (R204)	aw 4	w	k	k	d	d
	B10.A (R205)	aw 5	w	w	w	d	d
	B10.A (R206)	aw 6	w	k	k	d	d
	B10.A (R207)	aw 7	w	k	k	d	d
	B10.A (R208)	aw 8	k	k	k	d	w
	B10.A (R209)	aw 9	w	k	k	d	d
	B10.A (R211)	aw11	k	k	k	w	w
	B10.A (R212)	aw12	w	w	w	d	d
	B10.A (R213)	aw13	w	w	w	d	d
	B10.A (R214)	aw14	w	k	k	d	d
		aw15	w	k	k	d	d
		aw16	k	?	?	?	w
	B10.A (R217)	aw17	w	w	w	d	d
k/wm7		kw1	k	?	?	?	w
		kw2	w	k	k	?	k
		kw3	w	k	k	?	k
		kw4	w	k	k	?	k
		kw5	k	w	w	?	w
b/wm7		bw1	b	w	w	?	w
		bw2	b	w	?	?	w
		bw3	b	w	?	?	w
		bw4	b	w	?	?	w
		bw5	b	w	?	?	w
		bw6	b	w	?	?	w
		bw7	w	b	?	?	b
		bw8	b	w	?	?	w

between the H-2K and IA loci.

Effect of Major Histocompatibility (H-2) Gene Complex on the Susceptibility to Urethan Induced Lung Tumor in Mice

Kazuo MORIWAKI and Nobumoto MIYASHITA

In addition to a ptr (pulmonary tumor resistance) gene, H-2 gene complex seems to function in the processes of tumorigenesis in lung. This study aims to reveal the effect of H-2 gene complex on the incidence of urethan induced lung tumor in mice. A single subcutaneous injection of 15% urethan solution was carried out in a dose of 1.5 mg/g body weight using 21 day-old mice. Number of tumor foci were scored after 5 months on the lungs fixed with formaldehyde-ethanol (1:9). During the five months, the mice treated with urethan were bred in the lamina flow rack occasionally monitored by microbiological assay.

At first we examined A H-2 congenic strains. As shown in Table 1, the lung tumor incidence is clearly suppressed in A.SW (H-2^s), A.CA (H-2^f) and A.BY (H-2^b) comparing to A strain (H-2^a). This H-2 effect was further demonstrated in B10 H-2 congenic mice (Table 2). B10 (H-2^b) exhibited lower susceptibility but B10.A (H-2^a) showed rather higher susceptibility. B10.A (3R) which H-2 genes on K, A, E, S, D regions are b b k d d instead of k k k d d in B10.A has lower susceptibility. B10.A (5R), b b k d d, showed lower incidence as well. B10.A (4R), k k b b b, is as similar as B10.A (5R). These data suggest that H-2 IA^k and IE^k genes are related to the high incidence of urethan induced lung tumor. The similar effect of IA^k and IE^k genes was also observed as to the higher incidence of 4NQO-induced lung tumor (Miyashita & Moriwaki, this Annual Report). In the spontaneous lung tumor in mice, Feraldo *et al.* (Immunogent. **9**: 383, 1979) demonstrated the possible relevance of H-2 IB^k gene to the higher incidence.

So far, urethan effect on the lung tumor development has been rather considered to be an augmentation of spontaneous incidence (Shimkin and Stoner, Adv. Cancer Res. **21**: 1, 1975). But nobody knows the causation of spontaneous lung tumor at this moment. Instead, the good accordance in the effect of H-2^k genes on the higher incidence of lung tumors between the chemical-induced and spontaneous ones leads us to assume that certain chemical factors in the environment may cause the spontaneous tumor as well.

Table 1. Effect of H-2 complex on the incidence of urethan-induced lung tumors

H-2 congenic A strains	H-2 complex K	A	E	S	D	Lung tumor foci/mouse
A	k	k	k	d	d	30.6 ± 1.3 (24)
A.TL	s	k	k	k	d	25.8 ± 2.8 (8)
A.TH	s	s	s	s	d	17.0 ± 0.6 (26)
A.CA	f	f	f	f	f	17.0 ± 0.6 (58)
A.SW	s	s	s	s	s	12.3 ± 0.7 (51)
A.BY	b	b	b	b	b	13.8 ± 0.6 (58)

( ): Number of mice observed.

Table 2. Effect of H-2 complex, especially I-region, on the susceptibility to the urethan-induced lung tumors

Strains	Tumors/lung	Susceptibility to lung tumors	H-2 complex K	A	E	S	D	H-2 haplotype
B10.BR	1.3 ± 0.2 (16)	High	k	k	k	k	k	k
B10.A	1.2 ± 0.2 (26)	High	k	k	k	d	d	a
B10.A(2R)	1.2 ± 0.2 (37)	High	k	k	k	d	b	h2
B10	0.7 ± 0.2 (37)	Low	b	b	b	b	b	b
B10.A(3R)	0.7 ± 0.2 (31)	Low	b	b	k	d	d	i3
B10.A(5R)	0.6 ± 0.1 (59)	Low	b	b	k	d	d	i5
B10.A(4R)	0.5 ± 0.1 (42)	Low	k	k	b	b	b	h4
B10.D2	0.3 ± 0.1 (15)	Low	d	d	d	d	d	d
B10.M	0.5 ± 0.1 (46)	Low	f	f	f	f	f	f
B10.S	0.7 ± 0.1 (24)	Low	s	s	s	s	s	s
B10.RIII	0.4 ± 0.1 (21)	Low	r	r	r	r	r	r

( ): Number of mice observed.

The Relationship between Immune Response Genes and the Susceptibility to 4NQO-Induced Lung Tumor

Nobumoto MIYASHITA and Kazuo MORIWAKI

We have previously reported (Moriwaki and Miyashita, A. R. N. I. G. **31**: 37, 1981) that the H-2 gene complex is related to the development of urethan-induced lung tumor in mice. But *in vivo* metabolic pathway of urethan is still unknown. In the present study we examined A. H-2 congenic strains of mice for their response to 4NQO in studying the effect of H-2 gene complex to lung tumor induction. An avarage number of lung tumor foci was 9.0 for H-2^a haplotype, and 2.6 to 5.0 for the other H-2 haplotypes (Table 1). These results suggest that the I region genes control neither the metabolism nor the activation of the specific chemical carcinogen, but function as

Table 1. Effect of H-2 complex on the susceptibility to urethan- and 4NQO-induced lung tumors in A.H-2 congenic mice

Strain	H-2 haplotype	Ae:Eα complex	Number of lung tumor foci per mouse	
			4NQO	Urethan
A	a	E^k	9.0±2.1 (13)	30.6±1.3 (24)
A.TL	t1	E^k	Not Tested	25.8±2.8 (8)
A.TH	t2	—	2.6±1.1 (8)	17.0±1.1 (26)
A.SW	s	—	5.0±0.5 (25)	12.3±0.7 (51)
A.CA	f	—	4.8±0.9 (37)	17.0±0.6 (58)
A.BY	b	—	5.0±0.9 (10)	13.8±0.6 (58)

Urethan dose : 1.5 mg/g body weight.
4NQO dose : 12.5 μg/g body weight.

the regulatory system for the more common steps in the growth of lung tumor.

Suppression of Urethan-Induced Lung Tumor Development in the Hybrid between A Strain and Japaness Wild-Derived MOL.TEN2 Strain

Kazuo Moriwaki and Nobumoto Miyashita

Bloom and Falconer (J. N. C. I. 33: 607, 1964) have suggested the presence of a single recessive major gene (ptr) conferring low susceptibility to urethan-induced lung tumor in C57BL strain. In their experiment, F_1 hybrid between A and C57BL exhibited nearly similar level of the higher incidence as that of the susceptible parent, A strain. This tendency was also shown in our study. To eliminate the effect of H-2 complex, we examined F_1 hybrids between A and B10.A which has C57BL background and H-2^a haplotype as A. Their lung tumor incidence induced by urethan was almost the same as A strain, which definitely confirmed the higher susceptibility to lung tumor in $(A \times B10)F_1$ (Table 1).

On the other hand, we have already observed that the susceptibility to urethan-induced lung tumor of MOL.TEN2 strain derived from Japanese wild mouse (Mus musculus molossinus) is significantly lower than that of C57BL/10 (B10). In the present study, we further found a novel mode of inheritance in the hybrids between A and MOL.TEN2 strains. Differing from $(A \times B10)F_1$, $(A \times MOL.TEN2)F_1$ exhibited the apparently lower

Table 1. Suppression of urethan-induced lung tumor development in the intersubspecies hybrids

Strain	Ptr gene	H-2 haplotype	No. of mice observed	No. of tumor foci per mouse
A	Ptr	a	24	30.6±1.3
B10	Ptr	b	37	0.7±0.2
(A×B10)F_1	Ptr/ptr	a/b	28	19.9±0.9
(A×B10.A)F_1	Ptr/ptr	a/b	10	26.2±1.6
MOL.TEN2	?	wml	13	0.0
(A×MOL.TEN2)F_1	Ptr/?	a/wml	12	0.8±0.2
A× (A×MOL.TEN2)F_1	Ptr, ?	a, a/wml	{ 7 { 8	6.3±0.8 22.6±4.0

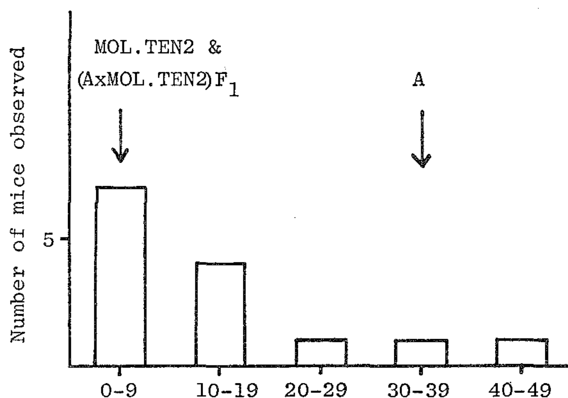


Fig. 1. Distribution of the lung tumor incidence in A× (A×MOL.TEN2)F_1 backcrosses.

susceptibility to the lung tumor (Table 1). Pulmonary tumor resistance (ptr) seems to be dominant in this case. Backcross experiment between A×(A×MOL.TEN2)F_1 demonstrated unexpected segregation. Though the number of N_2 progenies with the lower susceptibility are seemingly equal to those with the higher susceptibility as seen in Table 1, the frequency distribution shown in Fig. 1 obviously deviates to the lower susceptibility, instead of the bimodal peaks expected for a single locus.

These data possibly imply that in the hybrids between genetically remote subspecies such as laboratory inbred mice (M. m. domesticus) and Japanese wild mice (M. m. molossinus), certain regulatroy locus or loci other than

'ptr' could function to suppress tumorigenesis. These loci seem to work only under the heterozygous condition in the intersubspecies hybrids. The lower susceptibility to lung tumor in (A × MOL.TEN2)F_1 hybrids is probably explained by this notion. This phenomenon may not be generalized to the hybrids between laboratory inbred and wild mice, because F_1 hybrids between A and BRV.MPL which is derived from European subspeices, M. m. brevirostris, and relatively closer to the laboratory mice genetically. If we assume the mumber of such loci are more than two, the higher frequency of resistant individuals in the backcross (N_2) progenies could be well understood.

The reason why these genetic loci could work only in the inter-subspecies hybrids remains to be explored.

Genetic Divergence between European Wild Mice with Robertsonian Translocations from View Point of Mitochondrial DNA

Kazuo Moriwaki, Hiromichi Yonekawa[1], Osamu Gotoh, Heinz Winking[1] and Alfred Gropp[2]

To estimate the genetic divergence between the European wild mice with Robertsonian (Rb) translocations, their mitochondrial DNAs (mtDNAs) isolated from liver were analysed by the agarose slab-gel electrophoresis after digestion with eight kinds of restriction endonucleases, BamHI, EcoRI, HindII, HindIII, PstI, HpaI, HpaII and BglI. These mtDNA preparations were further subjected to make restriction map, from which approximate divergence times between each Rb variations were estimated as the order of 10^5 years (Table 1). Comparing these data with the divergence times between various Mus musculus subspecies in Europe and Asia, the Rb variations are apparently included within Mus 1 group. Five lines of Rb variations were examined in the present study: Those from Poschiave Valley (Pos), Yougoslavea (Zadar), Milano (Mil-II), and two lines from Apennine (CD and CB). A linear relationship was observed between the divergence times and the number of shared translocations in those Rb variations, suggesting that the Rb translocation commonly observed in the Rb variant populations should have occurred in a fairly old age such as 10^5 years ago.

1) Saitama Cancer Center Research Institute, Saitama-ken.
2) Medizinische Hochschule Lübeck, F. R. G.

Table 1. Sequence divergence and time of divergence estimated from mtDNA among five Robertsonian variations and six subspecies of *Mus musculus*

	Robertsonian variations				M. m. subspecies						
	Zadar	Mil-II	CD	CB	dom	brv	mus (D)	mus (B)	mol	cas	bac
					Sequence divergence (%)						
Pos	1.5	0.8	1.5	1.1	0.8	0.0	1.8	4.0	3.4	6.1	7.5
Zadar		2.2	2.1	1.8	1.4	1.5	2.4	4.9	4.0	6.1	7.4
Mil-II			1.4	0.4	1.5	0.8	1.0	4.8	4.2	4.9	6.1
CD				1.0	1.4	1.5	0.3	4.5	4.0	4.6	5.9
CB					1.1	1.1	0.7	4.2	3.6	4.3	5.5
					Time of divergence ($\times 10^6$ years)						
Pos	0.53	0.28	0.53	0.39	0.28	0.0	0.63	1.40	1.19	2.13	2.63
Zadar		0.77	0.74	0.63	0.49	0.53	0.84	1.72	1.40	2.13	2.59
Mil-II			0.49	0.14	0.52	0.28	0.35	1.68	1.47	1.72	2.14
CD				0.35	0.49	0.53	0.11	1.58	1.40	1.61	2.06
CB					0.39	0.39	0.25	1.47	1.26	1.51	1.93

Effect of Major Histocompatibility Gene Complex (MHC) on the Growth of Embryo-Derived Teratoma

Choji TAYA and Kazuo MORIWAKI

Mouse teratoma can be experimentally induced by the transplantation of early embryos under the kidney capsule. In order to find out the genetic factors governing the *in vivo* growth of embryo-derived teratoma, 6.5 and 7 day-old embryos of several strains were transplanted under the kidney cpasule of syngeneic recipients. After 40 days the grown tumors were weighed from the view point of the immune reaction of host aminals.

Strain A mouse was the greatest in the tumor weight among three kinds of A. H-2 congenic mice. A. BY and A. SW strains developed rather smaller weight of tumors. Teratoma produced in B10.A strain was the smallest in the weight. Tumors obtained from $[A \times B10.A]F_1$ were larger than those produced in A strain (Table 1). This may imply that the susceptibility to teratoma in A strain mice is a dominant trait against that of B10 strain when H-2 is the same.

These results suggest the genetic background of A strain plays an important role in the growth of embryo-derived teratoma and MHC seems to affect partially on the tumor growth in this system.

Table 1. Effect of H-2 complex on the growth of embryo-derived teratoma

Strains	H-2 haplotypes	Weight of tumors (mg)±S. E.	
		6.5 day-old embryo	7 day-old embryo
A	a	633±134 (34)	2033±567 (8)
A.BY	b	369± 74 (9)	1562±417 (15)
A.SW	s	N. D.	852±251 (13)
$[A \times B10.A]F_1$	a	881±193 (24)	N. D.
B10.A	a	193± 29 (8)	657±316 (5)

Allelic Constitution of Hbb Locus in Chinese Wild Mice, *Mus musculus*

Nobumoto MIYASHITA, Mitsuru SAKAIZUMI, Cheng Hai WANG[1], Shunsuke MIGITA[2] and Kazuo MORIWAKI

In order to investigate whether the high frequency of Hbb^p allele is unique to Japanese wild mice, *Mus musculus molossinus*, a total of 49 mice were collected at 9 localities of China. The restriction enzyme cleavage patterns of mitocondrial DNA of these mice were also examined (Yonekawa *et al.* unpublished data). Electrophoretic survey of Hbb alleles in the Chinese wild mice revealed a high frequency of Hbb^p (93.9%), whereas Hbb^d was rare (6.1%) and Hbb^s could not be found (Table 1.). Especially, in the seven localities which mtDNAs were MOL type, Hbb^p was solely observed. But Hbb^d allele was common (50.0%) in the two localities which mtDNAs were not MOL type. On the other hand, Hbb^p allele is prevalent in the central regions of Japan (Minezawa *et al.*, Jap. J. Genetics 54: 165, 1979), while wild mice in Philippines and Taiwan, *Mus musculus castaneus*, carries the lower frequency of Hbb^p in addition to predominant Hbb^d. These results

Table 1. Phenotypes of hemoglobin beta-chain (Hbb) of wild mice from China

Locality of collection	No. of mice	No. of phenotype			Type of* mtDNA
		P	PD	D	
Urumuchi	10	10			MOL
Turfan	1	1			MOL
Jiayuguang	3	3			MOL
Lanzhou	13	13			MOL
Chengtu	5	5			MOL
Changchun	7	7			MOL
Beijing II	4	4			MOL
Beijing I	2	1	1		BRV
Shanghai	4		3	1	CAS
Total	49	44	4	1	

* Yonekawa *et al.* unpublished.
MOL : Mus musculus molossinus (Japanese wild mouse) type.
BRV : Mus musculus brevirostris type.
CAS : Mus musculus castaneus type.

1) Institute of Biological Products, Lanzhou.
2) Cancer Research Institute, Kanazawa University.

suggest the close genetic relationship between *M. m. molossinus* and Chinese wild mice.

Genic Differentiation in the Wild Populations of the Fish *Oryzias latipes*

Mitsuru SAKAIZUMI, Nobuo EGAMI[1], and Kazuo MORIWAKI

Allozyme studies at 21 loci revealed an extensive genic differentiation in Japanese wild populations of the freshwater fish *Oryzias latipes*. By means of unique alleles at the *Adh*, *Pgm*, *Idh*, and *Sod* loci, the Japanese wild populations could be divided into two major groups, the 'Northern Population' and the 'Southern Population'. Crosses have shown that these four loci are not closely linked. The Southern Population was further divided into several subpopulations by the geographically specific alleles at the *Aat*, *Acp*, *Amy*, *Me*, and *Pgd* loci. Concerning these nine loci, little clinal variation was observed. The boundary of allelic distribution was distinct and well correlated with mountain limit.

Two Chinese populatios, Beijin and Shanghai, were studied. Although they were distinct from Japanese populations by the unique allele at *Ck-A* locus, they had the same alleles as the Southern Population had at the *Adh*, *Idh*, and *Pgm* loci, and shared the common allele with the northern Kyushu populations at *Sod*, and with populations of San-in district at *Amy* locus. Beijin and Shanghai populations were similar (Table 1) in spite of the geographic distance between these two sites (ca. 1000 km). No large mountain barrier exists between these localities, and it is known that the Yellow River has changed its lower reach several times in these two thousand years.

Clinal variation at enzyme loci has been reported for many fish species. These authors have argued for a correlation with water temperature. In case of *Oryzias latipes* little clinal distribution is observed. The distribution of allelic variation rather well coincides with geographic features. This phenomenon may be due to random drift and restricted migration, rather than to selection or the difference in environmental conditions.

The genetic distance value was calculated by the method of Nei (1972). The estimates were presented in Table 1. The average D values for all pairs of localities within a group were nearly 0. This shows that the gene constitution is fairly homogeneous within a group. On the other hand, the

[1] Zoological Institute, Faculty of Science, University of Tokyo.

Table 1. Estimates of gnetic distance among wild populations of medaka based on 21 loci

		Southern Population					
	Northern Population	Eastern Subpop.	Inland Sea Subpop.	San-in Subpop.	Ryukyu Subpop.	Shanghai population	Beijin population
Northern Population	(.01)						
Eastern Subpop.	.35	(.03)					
Inland Sea Subpop.	.33	.07	(.03)				
San-in Subpop.	.29	.14	.11	(.03)			
Ryukyu Subpop.	.43	.19	.16	.19	(.01)		
Shanghai population	.47	.24	.21	.18	.39	—	
Beijin population	.63	.24	.27	.24	.43	.03	—

Mean values of genetic distance for all pairs of localities within populations are in parenthesis.

D values among groups were markedly larger than them. Especially the values were large among the Northern Population, the Ryukyu Subpopulation, and the Chinese populations. These results suggest that this species has much variation, and considerably differentiated regionally.

Two-dimensional Electrophoretic Profiles of Seed Proteins from One Grain in Wild and Cultivated Rice

Chong-rong SUN and Toru ENDO

It should be noted that protein profiles of dormant seeds are highly stable qualitatively and quantitatively, though they are slightly affected by environmental conditions such as seasonal fluctuations during the protein synthesis. Thus the profiles must be a useful tool for phylogenetic differentiation as well as breeding problems in view point of nutritional improvement.

One grain, about 20 mg in cultivated and about 15 mg in wild rice, was macerated with 0.2 ml of the extractant containing 8 M urea, 1% Nonidet P-40 and 2% DTT, and resultant slurry was centrufuged. During the procedure, more than half amounts of whole proteins are generally possible to be solubilized. The supernatant was subjected to the two-dimensional polyacrylamide gel electrophoresis according to Brown *et al.* (1979, PNAS **76**: 2381).

The extracts from one grain gave about 75 to 108 spots counted, most of which were minor protein spots on the gels after staining with coomassie blue. The major spots were classified arbitrarily into three groups, I, II and III, which are located in the corresponding areas of the gels, respectively. Group I consisted of five to eight major spots, but is not identified yet. Group II comprised six to ten major spots, and was identified as glutelin. Group III was composed of only one major spot, identified as globulin. Six Asian *perennis* and 108 (Indica type of *Oryza sativa*) showed to have group I, II and III. T65 (Japonica type of *O. sativa*) showed to have group II and III, but lacked group I. W025 (*O. glaberrima*) showed to have group I and II, but lacked group III.

The present result suggests that Indica type is phylogenetically much nearer to Asian *perennis* than Japonica type. Also, it may suggest that Japonica type has a genetic margin for incorporation of genes specifying the group I proteins.

IV. DEVELOPMENTAL GENETICS AND SOMATIC CELL GENETICS

In Vitro Differentiation of Adult Structures from Embryonic Cells of *Drosophila melanogaster*

Yukiaki KURODA

In *Drosophila* cells which will differentiate into adult tissues and organs are predetermined at the stage of blastoderm formation in embryonic development. These cells proliferate in an undifferentiated state during embryonic and larval development and form imaginal discs. The final differentiation of the adult organs occurs under influence of ecdysterone secreted from the ring gland during the pupal development. Recently I have succeeded in forming the adult structures from embryonic cells of *D. melanogaster* in culture with ecdysterone-containing medium.

The wild-type Oregon-R strain was used. Newly laid eggs were dechorionated by treatment with sodium hypochloride solution. Embryos at the stage of post-gastrulation were surface-sterilized in 70% ethyl alcohol, dissociated into small pieces of tissue fragments and cells, and cultured in T-5 flasks in medium K-17 supplemented with 15% fetal calf serum and 0.1 mg/ml fetuin at pH 6.7 at 28°C.

When tissues and cells from embryos were cultured in medium without ecdysterone, various embryonic and larval tissues or cells differentiated. Muscle cells, epithelial cells and nerve cells showed their characteristic morphology and function under these culture conditions employed. However, no adult tissues or organs were formed without ecdysterone.

When ecdysterone was added to culture medium at the concentrations of 1–10 μg/ml, some characteristic adult tissues and organs developed. Among them the structures which were very similar to the imaginal wing and antennal discs were remarkable. These imaginal disc-like structures showed a typical feature, *i. e.* multiple concentric folded layer of thin cells. The eye disc-like structures with a regular arrangement of ommatidium-forming cells also developed. The bristles and trachea structures also differentiated very well. In nerve cells many multiple branched nerve fibers were produced.

The most striking example of the development of adult organs was the

leg-like structures. After one day of cultivation, small leg disc-like buds appeared, then they extended gradually, and finally every segments of the adult leg developed. They consisted of segments that corresponded to the adult segments of the legs, such as the coxa, the femur, the tibia and the tarsus. At the tip of the tarsus claw-like structures also developed.

Effect of Chick Endogenous Lectin on Cartilage Differentiation of Chick Limb-Bud Cells in Culture

Yukiaki KURODA, Etsuya MATSUTANI and Tatsuya YAMAGATA[1)]

It has been reported that endogenous lectins occurred in a wide variety of animal tissues. Among them, galactoside-specific lectins are widely distributed in a variety of chick embryonic tissues, such as skeletal muscle, brain, heart, lung, liver, retina, spinal cord, kidney and sterna. These are also found in chick embryos at the earliest stage, blastoderm. However, their role in the development and differentiation of embryonic tissues still remains unclear.

Mesenchyme cells obtanied from the limb-buds of chick embryos are known to differentiate into cartilage cells *in vitro*. We reported that plant lectins enhanced cartilage differentiation when added to the culture of mesenchyme cells dissociated from quail embryos (Matsutani, E. and Y. Kuroda, 1982 Develop. Biol. 89: 521–526). Since various chick embryo tissues so far tested contained β-D-galactoside-specific lectin, we examined the activity of the chick embryonic lectin on the chondrogenesis of mesenchyme cells.

β-D-galactoside-specific lectin was purified from 14 day chick embryos using a lactamyl Sepharose column. The lectin preparation eluted from the second affinity column was analyzed by SDS polyacrylamide gel electrophoresis, and it showed one protein band with a molecular weight of 16,000. Mesenchyme cells were dissociated from the limb-buds of White Leghorn chick embryos at the stage 24. Cells were incubated in medium 199 with 10% serum, using a modified micro-mass culture method. An inoculum of 1×10^5 cells was incubated for 12 hours in 0.5 ml of culture medium containing 5×10^4 units/ml of lectin.

The lectin-treated cells showed a slight increase in cell proliferation, formed a greater number of cartilage nodules, and incorporated about twice as much as ^{35}S-sulfate per cell than cell in control cultures.

1) Mitsubishi-Kasei Inst. of Life Sci., Machida, Tokyo.

The results of this study indicates that the chick endogenous lectin, which may have the activity to recognize the specific site(s) on cell membrane, promotes the chondrogenesis of mesenchyme cells *in vitro*. We are now carrying out more detailed study to clarify the mechanism by which this lectin promotes the chndrogenesis both *in vitro* and *in vivo*. For details, see Develop. Biol. 92: 544–548, 1982.

Mutagenic Activity of Quercetin on Embryonic Human Diploid Cells in Culture

Yukiaki KURODA

In the series of experiments, the mutagenicity of various chemicals on cultured human diploid cells was compared with those in other test systems, to find out the characteristics of human cells for these chemicals. In the present experiment, quercetin, which is a carcinogenic flavonoid in the bracken and had a strong mutagenic activity in the *Salmonella* test system, was examined for its activity on mutation induction in cultured human diploid cells.

The effect of quercetin on cell survival was examined by the colony forming activity of cells treated with quercetin at various concentrations for 4 hours. The surviving fraction was 0.73 in cells treated with 30 μg/ml quercetin, and 0.64 in cells treated with 100 μg/ml, showing that quercetin had not so strong cytotoxicity. With higher concentrations of quercetin, effect of ethyl alcohol used as a solvent exceeded that of quercetin on cell survival.

The frequency of 8-azaguanine resistant mutations induced by treatment with 1 μg/ml quercetin for 4 hours was 0.36 per 10^5 survivors. With 3 μg/ml quercetin, the mutation frequency was 0.66 per 10^5 survivors. With higher concentrations than 10 μg/ml, no more increase in induced mutation frequency was detected.

These results indicate that quercetin had a week mutagenicity, which was about one tenth of that of tryptophan pyrolysis products, on cultured human diploid cells. This is interesting with reference to the fact that quercetin had no mutagenic activity in insect systems such as silkworm.

Co-Mutagenic Activity of Non-Mutagens in Cultured Chinese Hamster Cells

Yukiaki KURODA and Masumi ASAKURA

Carcinogens present in human environment have been effectively detected by their mutagenic activity in bacterial test systems. Results obtained in such systems, however, can not directly apply to the assessment of these chemicals on human being. In the present experiment, some chemicals, which have not been detected for their mutagenicity in the *Salmonella* test systems, were examined for their activity to induce 8-azaguanine (8AG)- or uabain (OUA)-resistant mutations in Chinese hamster V79 cells. Chemicals examined were aniline and norharman.

Aniline had a cytotoxic effect on V79 cells. The value of D_{50} for 4 hour treatment was 1.7 mg/ml, and 1.2 mg/ml with metabolic activation by S-9 Mix. For mutation experiments, cells were treated with chemicals for 4 hours, cultured for 6 or 2 days in normal medium for mutation expression time, and then replated on fresh dishes. The numbers of 8AG- or OUA-resistant mutant colonies were scored after cultivation for another 14 or 10 days in respective selection medium.

The induced 8AG-resistant mutation frequency of cells treated with aniline at concentrations of 0.2–2.0 mg/ml was 7–10 per 10^5 survivors. No increase in the mutation frequency was observed with addition of S-9 Mix. OUA-resistant mutations were not significantly induced both by aniline alone or with S-9 Mix. Norharman had a weak mutagenic activity on V79 cells. When cells were treated with both aniline and norharman simultaneously at low concentrations, which did not induce mutations by exposure of either one chemical, 8AG-resistant mutations were effectively induced. The induced mutation frequency increased by addition of S-9 Mix.

To analyze the mechanism of co-mutagenic effect of these two chemicals, cells were separately treated with norharman before aniline treatment. The induced mutation frequency was lower in cells treated with norharman 4 hours or immediately before aniline treatment than that in cells treated with norharman and aniline simultaneously. This suggests that the co-mutagenic effect of aniline and norharman may be due to the inhibitory effect of norharman on the inactivation of aniline rather than due to the intercalation of norharman into the DNA molecule.

Effect of TPA on Cell-to-Cell Adhesion of Chinese Hamster Cells in Culture

Yukiaki KURODA and Hirokazu MIKAMI

A phorbol ester purified from seeds of *Croton tiglius* L., 12-0-tetradecanoyl phorbol-13-acetate (TPA), had a strong promoter activity on the induction of mouse skin tumor. TPA also had a variety of activities in the induction of ornithine dehydogenase activity in the cell membrane, the inhibition of the metabolic cooperation in cultured cells, and the modification of the cellular differentiation. These results suggest that the site of action of TPA may be the cell membrane. In the present experiment, the effect of TPA on the cell-to-cell adhesion of Chinese hamster V79 cells.

Cell suspensions containing 10^6 cells in 3 ml medium were rotated on a gyratory shaker at 38°C. Under these culture conditions, cells in suspensions come together, adhere to each other and form aggregates after 24 or 48 hours. The shape and size of aggregates are constant and highly reproducible, and can be used for examining quantitatively the effect of various chemicals on the cell-to-cell adhesion.

In control cultures, V79 cells formed many spherical aggregates with a diameter of approximately 230 μm, after rotation culture for 24 hours. When TPA was added to the culture medium at concentrations of 1 to 100 ng/ml, the average diameter of aggregates formed increased, indicating that TPA enhanced the cell-to-cell adhesion. This enhancing effect of TPA on aggregation continued for another 24 hours in rotation culture, even when TPA was removed from the medium. Phorbol had no effect on aggregate formation of Chinese hamster cells.

The adhesiveness of cell to the plastic surface of petri dishes was no affected by TPA. The colony-forming activity and the population doubling time of cells were also not affected by TPA. These results indicated that TPA had a specific effect on the cell-to-cell adhesion.

An Autosomal, Non-maternal Effect Sex-differential Lethal Mutation in *Drosophila melanogaster*

Kugao OISHI and Takao K. WATANABE

Recent studies on the sex-specific lethals of *D. melanogaster* have indicated that all these lethals so far characterized are involved in dosage com-

pensation and/or sex determination in one way or another. They can be classified into several groups by their modes of action: (1) A sex-linked, non-maternal effect sex-specific locus (Sex-lethal, *Sx1*, which has both male-specific and female-specific alleles and plays a central role in sex determination and dosage compensation), (2) An autosomal, maternal-effect female-specific locus (daughterless, *da*, which regulates the expression of $Sx1^+$ locus), (3) Autosomal, non-maternal effect, male-specific loci (e. g., maleless, *mle*; four loci on the second chromosome and a locus on the third have been characterized. These are involved in the regulation of dosage compensation in males, and also regulate the expression of $Sx1^+$ in females), and (4) A sex-linked, maternal-effect male-specific locus (sonless, *snl*, which interacts with sex-transformation mutants).

We have recovered an autosomal, non-maternal effect, sex-differential lethal which apparently represents a new class. Whether this mutant is involved in dosage compensation and/or sex determination, and if so how, is thus of considerable interest. Preliminary results of examinations are presented here. A third chromosome (♯100) extracted from a natural population at Katsunuma, Yamanashi Prefecture, was found to be female lethal and male semi-lethal when made homozygous. The gene responsible for this lethal effect, *fle(3)100* (femaleless), was mapped at 3–45. Homozygous females were completely lethal, but males appeared at about 50% the expected number. It is not clear whether the male semi-lithality is due to *fle(3)100* or to another gene tightly linked. Since the map position was close to sex-transformation mutants transformer (*tra*, 3–45) and doublesex (*dsx*, 3–48.1), a possibility was raised that homozygous males were lethal and the males that appeared represented female-to-male sex-transformed flies. This was negated as *fle(3)100/tra* and *fle(3)100/dsx* were normal and fertile flies. At least some homozygous *fle(3)100* females survived to pharate adults but never eclosed. They had external morphology completely differentiated and normal. No idication of abnormal dosage compensation was obtained in the preparatins of salivary gland chromosomes of third-instar female larvae. Further characterizations are underway.

Nematocyte Differentiation and Positional Information in Hydra

Toshitaka FUJISAWA, Chiemi NISHIMIYA and Tsutomu SUGIYAMA

Nematocytes are hydra's stinging cells used for capturing preys and for protection. There are 4 types of nematocytes (stenotele, desmoneme, holotrichous isorhiza and atrichous isorhiza) classified by the morphology of a stinging structure present in the cytoplasm of the cell.

The spatial distribution of the differentiating nematocyte along the body column is very characteristic. For example, desmonemes are differentiated mainly in the distal part of the body column and stenoteles are differentiated predominantly in the proximal part of the body (Bode and Flick, 1977). This position dependency of nematocyte differentiation suggests the possibility that positional information which controls pattern formation of hydra is also involved in regulating nematocyte differentiation. In order to test this possibility, 2 different sets of experiments were done: 1) The axial distribution of differentiating nematocytes was examined in the hydra strains which have different levels of positional information. 2) An assay system was devised to identify and characterize a factor(s) which controls nematocyte differentiation in the hydra tissue.

In the first experiments the patterns of nematocyte differentiation along the body column were examined by the thiolacetic acid-lead nitrate method (David and Challoner, 1974) which specifically stains the nematocytes in their final stage of differentiation from the interstitial stem cells. In the standard wild type (105), stenoteles occupy 0–7% of the total nematoblasts in the regions near the head, 5–20% in the middle and 50–60% near the foot, whereas desmonemes occupy 65–75%, 55–65% and 30–40% in these regions, respectively. These patterns of nematocyte differentiation are essentially unchanged in the mutant strains (reg-16 and mh-1) which have significantly altered levels of both head activation and inhibition potentials. This suggests that, although nematocyte differentiation is position dependent, positional information involved in the head formation has no influence on the regulation of nematocyte differentiation.

In the second experiments, we found that the hydra tissue contains a factor(s) which preferentially inhibits stenotele differentiation when applied to hydra externally. The factor is released into the culture solution by the head regenerating animals. The kinetics of the release of the factor matches

well with the known kinetics of the release of "head inhibitor" from the head regenerating hydra. This finding is consistent with the view that stenotele differentiation is normally regulated by head inhibitor. However, the purified head inhibitor (gift of Dr. S. Berking) showed no inhibitory activity on the stenotele differentiation. In addition, the factor which is present in the homogenates of normal hydra tissue was not found in the homogenates of epithelial hydra (hydra consiting of only epithelial cells and lacking interstitial stem cells, their derivatives (nerves and nematocytes) and gland cells) which has normal positional information. The identity of the factor is presently unknown.

Changes of the Head Activation and Head Inhibition Potentials during Hydra Regeneration

Tsutomu SUGIYAMA, Josef ACHERMANN* and Jun TAKANO**

Hydra has a strong regenerative capacity. After amputation of the head and foot, a new head regenerates from the end of the body column which originally had the head and a new foot regenerates from the other end of the body column. This polarity of regeneration has been thought to be governed by some sort of polarity "gradient" which exists along the body axis (Child, 1941).

Wolpert *et al.* (1974) and Gierer and Meinhardt (1974) proposed similar models on the polarity gradient. According to them hydra tissue possesses 2 antagonistic morphogenetic potentials which control the formation of head structure. They are the potential to activate the head formation (head activation potential) and the potential to inhibit the head formation (head inhibition potential). Their levels are high in the head region and become low in the foot region forming the "gradients" along the body column.

In order to test the validity of these models, we have examined the changes of the morphogenetic potentials during head regeneration of mutant strains and compared them to those of the wild type strain (105). The relative strengths of morphogenetic potentials were measured by the method of the lateral tissue grafting of Webster (1966). Mutant strains used were reg-16 which is defective in head regeneration and L4 which has a low budding rate. Both mutants were shown to have lower head activation potentials and higher

* Zoological Institute, University of Zurich, Switzerland.
** Department of Biology, Nagoya University.

head inhibition potentials comparing to 105 under the steady state growth conditions (Achermann and Sugiyama, 1982; Takano and Sugiyama, 1982).

After head amputation, 105 polyp shows an immediate and drastic decrease of the head inhibition potential and a gradual increase of the head activation potential at the distal regenerating tip. In reg-16, the decrease of the head inhibition potential occurs very slowly and in steps, and the increase of the activation potential does not take place. L4 shows a gradual and stepwise decrease of the head inhibition potential (similar to reg-16), and small and gradual increase of the head activation potential (intermediate between 105 and reg-16).

These observations suggest that the head activation and inhibition potentials play crucial roles in hydra head regeneration, and that whether or not even a small increase of the head activation potential takes place at the regenerating tip determines success (in L4) or failure (in reg-16) of the subsequent head regeneration.

Chimeric Mouse Embryos Produced by Aggregating Testicular Teratocarcinoma Cells and 8-cell Stage Embryos

Kazunori HANAOKA and Tahehiko NOGUCHI

We reported in the last year that chimeric aggregates formed between cleaving stage embryos and embryonal carcinoma cells (ECC) of a testicular teratocarcinoma (STT-2) developed at a high rate into blastocysts.

This year we studied the capacity of these chimeric blastocysts to develop into normal conceptuses *in utero*. One hundred blastocysts formed between cleaving stage embryos and ECC of STT-2, and 130 blastocysts formed between the embryos and ECC of 311 (a clonal line derived from STT-2) were transfered into uterus of pseudopregnant females of random bred ICR mice. As STT-2 has type "a" Gpi-1, strains of mice having type "b" Gpi-1 were chosen as the embryo donors so that chimerism may be determined by the allozyme marker. They were sacrificed at 6 and 7 days after transfer (corresponding 8 and 9 day of gestation). Chimerism of the embryos was examined by electrophoretic analysis of Gpi-1 allozymes using the whole homogenate of the embryos. The results are summarized in Table 1.

Seven (30%) of the 24 embryos with normal morphology, and 35(82%) of the 43 abnormal ones were chimeras. Contribution rate of ECC-derived cells in the normal chimeric embryos were estimated by densitometrical

Table 1. Developmental capacity *in utero* of blastocysts made between ECC and 8-cell stage embryos

Teratocarcinoma	No. transfered	No. implanted			
		Total	Dead	Abnormal (Chimeric)	Normal (Chimeric)
STT-2	100	66	31	21 (17)	14 (4)
311	130	58	26	22 (18)	10 (3)

analysis of electrophoregram of PGI-1. The contribution rates of ECC-derived cells in the seven chimeric embryos were estimated to be 1.0, 0.89, 0.42, 0.33, 0.24, 0.20, 0.16.

Freeze Preservation of Mouse Embryos

Takehiko NOGUCHI and Masahiro SATO

Mouse embryos similar to tissue cells show no deterioration in viability during prolonged storage in liquid nitrogen (Whittingham, and Whitten, 1974). Freeze preservation of mouse embryos has been applied as insurance against loss of valuable and irrevocable mouse strains and mutants. It has been also applied as a method to prevent genetic drifts which may occur during maintenance of mice in live state. In addition, it offers great economic benifit in the husbandry of mice.

As these benifits of freeze preservation of embryos meet well to the purposes of this laboratory, we started freezing embryos from this year. Eight hundreds of 8-cell stage embryos obtained from inbred strains including C57BL/6, C57BL/10, 129/Sv and A/He were frozen essentially by the method of Whittingham, Leibo and Mazur (1972). To estimate the viability, 366 embryos were thawed 1 to 3 months after freezing, and cultured in Biggers medium Within 2 days in culture, 232 embryos (63%) developed into expanded blastocysts. To check the developmental capacity *in utero* of these blastocysts, 80 of them were transfered into uterus of pseudopregnant ICR mice. Twenty two (30%) new born mice were obtained. They grew into fertile adults.

Growth of Nervous Tissue of a *Notch* Lethal, *Df(1)N8* in *Drosophila melanogaster*

Kiyoshi MINATO

The lethal alleles of *Notch* locus in *Drosophila melanogaster* are known to be embryonically lethal, producing an extensive hypertrophy of nervous tissue at the expense of hypoderm. Using the recently developed techniques of more facilitated fixation (Zalokar, 1976) and embedding resins enabling easy orientation of embryo, one of *Notch* lethals, *Df(1)N8* was alayzed quantitatively for the growth of nervous and other tissues with the histological preparations.

As the results, some abnormalities were observed in the first time in embryos at 6-hours of development, in which neuroblasts as about four times in number as those in the normal embryos were seen, probably segregated from the ectoderm and leaving fewer hypodermal cells (about a half of normal embryos). These hypertrophied neuroblasts were accompanied with the typical assymetrical mitosis giving rise to relatively smaller ganglionic cells so frequently as in normal embryos. At more advanced stage of 8-hours, still many neuroblasts were observed in lethal embryos, while the numbers of those in normal embryos decreased conciderably through divisions producing ganglionic cells. While, at this stage, few mitoses of neuroblast themselves were seen in normal embryos, more frequent mitoses were observed in abundant neuroblasts in lethal embryos.

At the succeeding stages of 10- and 12 hours, the fully condensed central nervous system was formed in normal embryos, while only some groups of loosely condensed ganglionic cells were observed in lethal embryos. Although these poorly differentiated ganglionic cells occupied much more areas of the body cavity in lethal embryos, they showed, on the basis of the cell number, only less extent of hypertrophy (at most, two times or less of normal ones) than expected from the presence of far many neuroblasts in the initial phase. These results suggest that the accumulation of neuroblasts in the initial phase may be produced by a delayed and prolonged process of mitoses which might also explain more mitotic figures observed at the later stage in lethal embryos.

V. CYTOGENETICS

Hypo-pentaploid Chromosome Constitution of a Spontaneous Tumor Developed in the Oceanian Type Black Rat

Tosihide H. YOSIDA

A tumor (fibrosarcoma) developed spontaneously in the dorsal side of one male in the Oceanian type black rats (*Rattus rattus rattus*) obtained from our breeding colony. This black rat was originally obtained from the United States, and at the third generation in the sister-brother mating the tumor developed. The chromosomes were observed in the primary culture by conventional and G-banding stainings. The normal karyotype of the tumor bearing rat was observed from cells of the tail culture by our routine technique.

The normal karyotype of this rat showed 38 chromosomes in all 50 cells analysed. Among them two large metacentric autosome pairs, M_1 and M_2 were identified as the Oceanian type black rat. The large M_1 chromosome is consisted of the centromeric fusion of pair nos. 4 and 7, and the other M_2 chromosome by pair nos. 11 and 12.

Chromosome numbers counted in 50 tumor cells were distributed from 38 to 150. Among them cells with 91 to 95 chromosomes occurred most frequently (60%), those with 71 to 75 chromosomes were 12% in occurrence, and the other cells were very few (6 to 2%). This tumor, thus, is characterized by having the hypo-pentaploid stemline. Karyotype of the tumor cells is not always constant in cells under study, eventhough they have the same chromosome number. Interesting is that the number of the chromosomes differs according to the chromosome pair. For instance, in one hypo-pentaploid cell with 93 chromosomes, 8 chromosome pairs were pentasomic, 4 pairs were hexasomic, 6 pairs were tetrasomic and X and Y were disomic. In another cell with a hypo-tetraploid constitution (72 chromosomes), 13 pairs were tetrasomic, 3 were disomic, one was trisomic and the remaining one was pentasomic. Another interesting feature in this tumor was that the centromeric fission was observed in the large metacentric M_2 chromosomes. For example, hypo-pentaploid cells are expected to have 6 M_2 chromosomes, but they had 3 metacentric M_2, 3 acrocentric no. 11 and

3 acrocentric no. 12 by fission of 3 M_2 chromosomes. In contrast, M_1 metacentric chromosomes were always stable. By the polyploidization, the number of pair no. 1 showed 4.6 ± 1.00. The number of M_2 chromosomes, however, showed 2.3 ± 0.62 in average. This suggests strongly that about the half number of the metacentric M_2 elements dealt with the centromeric fission.

Hypotrichotic Mutant (*ba*) Appeared in the Inversion Stock (LEM) of the Norway Rat

Tosihide H. YOSIDA

A rat stock "LEM" has been derived from the rats with inversion chromosome no. 1 (Yosida 1980, Jap. J. Genet. 55: 397). These rats are characterized by the presence of the large metacentric pair no. 1, though each of which is subtelocentric in the normal karyotype of the other strains. A hypotrichotic mutant occurred spontaneously in the course of inbreeding in this stock. The present paper deals with a process of establishment of the mutant shock, and the genetical and chromosomeal analyses of the new mutant.

The LEM-stock here concerned originated from the hybrids between the Lewis and Long Evans strain rats. In a female of the Lewis-strain rat (LEW-1c), a translocation occurred between pair nos. 1 and 12. This rat was mated to a normal Lewis male. One female out of the offspring was a mosaic consisting of two cell types, such as translocation/normal pair no. 1 and translocation/inversion pair no. 1. This female was crossed to a normal Long Evans-strain rat. In the offspring thus obtained the rats carrying inversion/normal pair no. 1 appeared by segregation. Among hybrids between these rats the inversion homozygotes were obtained. Two female hypotrichotic rats appeared in the third generation from a mating between a inversion homologous female and a male. Further, 3 females and 3 males of a similar appearance were yielded in the other two litters. The hypotrichotic rats were easily identified soon after birth because of slightly smaller size than the normal ones. They did not show normal hair growth in the young stage, though some fuzzy hairs were often observed in young and adult stages showing somewhat an irregular periodic cycle from fuzzy hair to almost complete hairless. The hairs, however, sometimes remained thinly on the face appearing as striped pattern in the adult rat. The hypotrichotic rats were similar in general appearance to the nude rat, but the thymus was

present in both new born and adult rats. The thymus, however, was slightly smaller in size than the normal one, being remarkable by rather yellowish colouration. The male and female hypotrichotic rats were fertile, and the female parents were able to lactate to the new born, though its ability seemed to be slightly inferior. Based on the genetical nalysis, it is shown that the present hypotrichotic character is due to a simple and recessive mutant gene bared, (*ba*). In the cross between the hypotrichotic female and male, five rats were obtained, all of which showed the hypotrichosis. The chromosome constitution of the bared rats studied so far was characterized by a typical inversion pair no. 1, the pattern being the same as that of other LEM-stock rats. Except the no. 1 chromosome inversion, all other chromosomes of the bared rats were normal. This means that the bared (*ba*) rats should be produced by gene mutation in the LEM-stock, but is not due to the new chromosomal mutation.

Quantitative Changes of Nucleolar Organizer Regions (NORs) in 3-methylcholanthrene Induced Tumors of the Indian Spiny Mouse

Tosihide H. YOSIDA

Indian spiny mouse, *Mus platythrix*, is characterixed by 26 acrocentric chromosomes, and the size of the chromosome is generally larger than that of the house mouse. The nucleolar organizers of this animal are located near centromeres of three chromosome pairs, nos. 5, 8 and 12 (Yosida 1980, Cytologia **45**: 753). In the methylcholanthrene induced tumors (fibrosarcomas), Yosida (Cancer Genet. Cytogenet. 3: 211, 1981) reported the frequent occurrence of chromosome anomalies in the autosome pairs with the nucleolar organizer regions (NORs). In order to inquire into the problem as to whether the quantitative variation of the NORs occurs in these tumor cells, the NORs were observed in two tumors (MPMC-53 and MPMC-55) newly induced in the animals by subcutaneous injection of 3-emthylcholanthrene. The tumor cells in early culture passages were used for the chromosome study. Cells of the tail tip cultured from the animal bearing MPMC-55 were used as the normal control in the present study.

The NORs in the normal cells were found to occur in chromosome nos. 5, 8 and 12 with considerably different frequencies, being 88.3% in pair no. 5, 14.2% in no. 8 and 47.2% in no. 12. The tumor MPMC-55 was charac-

terized by a near-diploid stemline karyotype. In 22 karyotypes analysed, 16 cells (73%) had 26 chromosomes, leaving two cells with 25 chromosomes, three cells with 27 chromosomes and one cell with 28 chromosomes. Among them 12 cells showed translocation. They are consisted of nos. 5 and 12 (8 cells), nos. 5 and 8 (one cell), and some other chromosomes (3 cells). The frequencies of the NORs observed in 22 cells were 84.0% in no. 5 and 13.6% in no. 8, while 109.1% in no. 12. The latter high frequency value is due to the occurrence of trisomy in this chromosome. In 26 cells of the MPMC-53 tumor examined, 15 cells (57.6%) were in a diploid range (24 to 27 chromosomes). Among them 10 cells showed translocations. The trnslocation occurring between no. 5 and no. 8 was found in 10 cells, and that between no. 8 and no. 12 in 2 cells. The latter translocation was found to be duplicated with the fomer one. The number of the NOR in nos. 5, 8 and 12 increased generally to 86.6, 76.6 and 80.0%, respectively. They are also higher than the control frequencies.

Shift of the Karyotypes in Indian Spiny Mouse Tumor MPMC-53 after Storage in Deep Freezing

Tosihide H. YOSIDA

Indian spiny mouse tumor, MPMC-53, which induced by 3-methylcholanthreene was characterized by having diploid (57.7%), triploid (3.8%) and tetraploid ranged cells (38.5%) in *in vitro* cultivation. Among 15 diploid ranged cells which analysed their katyotype by G-bandings, 10 cells had translocation between chromosome pairs 5 and 8, and 2 cells between nos. 8 and 12. One batch of cultures of this tumor was stored in the deep freezer (−80°C). Ten days after storage the cells were again cultured *in vitro* and the katyotypes of these cells were analysed.

A shift of the ploidy in tumor cell population was found to be diploid (83.8%), triploid (1.5%) and tetraploid ranged cells (14.7%). It is clearly shown that the frequencies of diploid and tetraploid ranged cells reversed from those in non-freezing bathes. The karyotype of tumor cells after freezing also changed markedly from that in non-frozen batches. A new translocation between homologous chromosomes of no. 6 was found in all 25 cells analysed, and the translocation between nos. 5 and 8, which were frequent in the non-frozen cells, was never observed in the cell population developed after freezing.

The frequencies of the necleolar organizer in pair nos. 5, 8 and 12 in the non-frozen cells were 86.6, 76.6 and 80.8 per cent, respectively, but those after freezing changed to 100.0, 8.0 and 90.0%, respectively. The shift of karyotypes seems to be attributable to a preferential selection in cell population due to deep freezing.

Geographical Variation of Chromosomes Numbers in the House Shrew, *Suncus murinus* (Soricidae) and its Distribution in the World

Tosihide H. YOSIDA

Karyotypes of the house shrew (*Suncus murinus*) collected from 8 different localities in East, Southeast and Southwest Asia were examined with an interest of the geographical variation and distribution of this animal. The chromosome number of the house shrews collected from 7 localities; Okinoerabu in East Asia, Tainan, Bogor and Makassar in Southeast Asia and Kanpur, Kalyani and Bhubaneswar in Southwest Asia; was constantly 40 in the somatic cells. This seems to be the basic number in this species. Karyotypes of all specimens with 40 chromosomes were identical except the shape of Y chromosomes. The autosomes were classified into 3 groups; acrocentrics (nos. 1 to 12), subtelocentrics (nos. 13 to 15) and meta- or submentacentric (nos. 16 to 19). The X chromosome was the largest submetacentric, while the Y chromosome was a rather smaller submeta- or metacentric which showed apparent variations in its size and shape among specimens collected from different localities.

Specimens collected from Trincomalee in Sri Lanka had 32 chromosomes, among which 4 autosome pairs (nos. 1 to 4) were acrocentrics, three autosome pairs (nos. 5 to 7) were subtelocentrics, and the other 8 autosome pairs (nos. 8 to 15) were metacentric or submetacentric. Among the latter group 4 pairs (nos. 8 to 11) were conspicuously large meta- or submetacentrics. The other 4 pairs (nos. 12 to 15) appeared to correspond to the pair nos. 16 to 19 seen in the basic karyotype with 40 chromosomes. Similarly, nos. 5 to 7 in the Sri Lanka karyotype were thought to be homologous to nos. 13 to 15 in the basic karyotype. The X chromosome was a large submetacentric, and the Y a smaller submetacentric. Based on the comparison between the karyotypes of the house shrew in Sri Lanka and the specimens collected from the other localities, it is strongly suggested that the 4 pairs of

large biarmed elements in the 32-chromosome specimens were diviated through Robertsonian fusions among 8 acrocentric pairs in the animal with 40 chromosomes. Variation of chromosome numbdrs from 40 to 36 has been reported by Yong (1974) in specimens collected in the central part of West Malaysia. House shrews with 32 and 30 chromosomes have been reported by Satya Parakash and Aswathanarayana (1976) in the specimens collected from southern India.

According to some taxonimists the wild form of the house shrew has been distributed originally in the forest of central India, and then they moved to the other parts of Southwest, Southeast and East Asia with the migration of man. They are suggested to be the original type which should be characterized by having the basic karyotype with $2n=40$. On the other hand, the sequential Robertsonian rearrangements should have taken place in the house shrews moved to southern India from the central India. The animals with 32 chromosomes have developed by the Robertsonian fusion between 8 chromosome pairs, and those with 30 chromosomes by the Robertsonian rearrangement of two more chromosome pairs than the above karyotype. The former animals seemed to have migrated from southern India to Sri Lanka. These animals, would migrate to some islans in the Indian Ocean and to the west coast of the Malayan peninsula. The Malayan population with 40 to 36 chromosomes seems to have arisen through hybridization between the animals with 40 chromosomes originally migrated there and those with reduced chromosome numbers which migrated through the Indian Ocean.

Geographical Variation of Y-Chromosomes in the House Shrew, *Suncus murinus*, and its Distribution

Tosihide H. YOSIDA

Basic karyotype of the house shrew, *Suncus murinus*, was $2n=40$. The chromosome numbers of this animal distributed in southern India, however, showed 30 to 32 by Robertsonian translocation between acrocentrics included in the basic karyotype. The X-chromosome in this animal was invariably large metacentric, while the Y-chromosome showed a wide geographical variation in its size and shape.

The Y-chromosome in specimens from Java and Celebes ($2n=40$) was a considerably small submetacentric, which was shorter than the long arm of

the X. The Y in the Sri Lanka taxon with $2n=32$ was similar in size and shape to that in Indonesia, but that in the Okinoerabu specimens was the metacentric, though its size was similar to the above specimens. The Kalyani specimens had a slightly larger Y, nearly comparable to the long arm of the X. In contrast, that in the Kanpur population was an unusually large submeta- or subtelocentric element, being much longer than the long arm of the X. The Y chromosomes, thus, are classified roughly into 5 types; (A), the large submentacentric (or subtelocentric); (B), the median sized submetacentric; (C), the small submetacentric; (D), the small metacentric and (E), the small acrocentric. The type A found in Kanpur and Delhi seemed to show a distribution in northern India. The type B was observed in northern (Kalyani) and central India (Hyderabed) to southern Malaysia (Malacca). The type C and D were also found rather widely in Southeast and East Asia. The type E was found in northern India (Kalyani) and South Vietnam. If a suggestion on the evolution of the Y is allowed, the largest type A which distributed widely in northern India is the original form, and the types B and C occurred by sequential deletions of the long arm. Type D is resulted from an inversion in the type C, and type E from a deletion of one arm in type C or D.

Karyotype of Hybrid Male between the Domestic Cat and the Leopard Cat

Tosihide H. Yosida

As a hybrid male born from mating between the female domestic Siamese cat (*Felis catus*) and a male leopard cat (*F. bengalensis*) was obtained, the hybrid was cytogenerically examined. The chromosomes were studied based on leukocytes cultured by the routine procedure.

The chromosome number and the karyotype of the domestic cat were fully consistent with those by the previous studies. Eighteen autosome pairs were classified into A to F groups; the group A is consisted of 3 large submetacentric pairs (A-1 to A-3); the group B of 4 large subtelocentric pairs (B-1 to B-4); the group C of 2 large metacentric pairs (C-1 and C-2); the group D of 4 considerably small subtelocentric pairs (D-1 to D-4); the group E of 3 small metacentric pairs (E-1 to E-3) and the group F of 2 small acrocentric pairs (F-1 and F-2). The E-1 pair was remarkable by having the secondary constriction. The X was metacentric corresponding in size to

the C group, while the Y was of small subtelocentric structure.

Although karyotype of the leopard cat could not be examined, it has been studied by Makino and Tateishi (1952) and Hsu and Benirschke (1968). According to them, the leopard cat had 38 chromosomes similar to the domestic cat, but the karyotype is slightly different between them. The karyotype of the leopard cat was similarly classified into A to F, but the number in the groups B and F was different between these two species. In the leopard cat the B group was consisted of 5 pairs (B-1 to B-5), and the F group was represent by a single pair (F-1). The other autosome pairs were apparently similar between the two species. The X and Y chromosomes were identical between them, except the size of Y which is slightly smaller in the leopard cat.

The chromosome number of the hybrid between the domestic cat and the leopard cat was the same as that of the parents ($2n=38$), but its karyotype was slightly different from those of both parents. The autosomes were classified into A- to F-groups corresponding to parents. Three A-pairs (A-1 to A-3), 2 C-pairs (C-1 and C-2), 4 D-pairs (D-1 to D-4) and 3 E-pairs (E-1 to E-3) were closely identical with parent karyotypes, but the chromosome numbers of the groups B and F were different in the hybrid. In the group B, 4 pairs (B-1 to B-4) were the same as those of both parents, while B-5 was single without homologous pair in the hybrid. Apparently the single B-5 was of the leopard cat origin. In the group F, 3 small acrocentics were observed. The F-1 was characterized by normal pair, but the F-2 was single without homologue in the hybrid. Comparison revealed that the unpaired F-2 element was a derivative from the domestic cat.

Effect of Metabolic Perturvation on Induction of the Sister Chromatid Exchange (SCE)

Hitoshi SUZUKI, Tosihide H. YOSIDA and Toshihisa KUSANO*

Although 5-bromodeoxy uridine (BrdU) is incorporated into DNA strands, the effect of BrdU on SCE induction seems to be due to the metabolic perturbation of DNA nucleotide synthesis (Davidson *et al.* 1980). It is interesting to know whether metabolic perturbation induced by other drugs can also increase the SCE frequency. SCE induction by treatment with 5-fluorodeoxyuridine, known as a inhibitor of thymidine kinase, could be com-

* Dept. of Life Science, Hiroshima Women's University.

pletely suppressed by addition of thymidine or BrdU, but not suppressed by deoxycytidine, deoxyuridine or uridine. Thus the effect of 5-flurodeoxy-uridine on SCE induction may be due to depletion of dTTP pool.

The SCE frequency increased by treatment with excess amount of deoxy-ribonucleosides such as dT, dA and dG. These chemicals are known to inhibit the ribonucleotide reductase and cause the metabolic perturbation in DNA nucleotide synthesis. Induction of SCE by these chemicals was partially suppressed by the addition of the other deoxyribonucleosides such as dC, which could rescue the metabolic perturbation.

Hydroxyurea also known as a inhibitor of the ribonucleotide reductase can induce SCE (Ishii and Bender, 1981), but the induction could not be suppressed by the addition of the four deoxyribonuleosides. Thus, it is questionable to say that the SCE induction by HU is depended on metabolic perturbation.

We also examined whether the SCE was induced in the uridine requiring mutant UR-216 cells (Kusano et al., 1976) when they were cultured in uridine insuficient medium. When the UR-216 cells were cultured in low concentration of uridine for two cell cycles, the SCE frequency was about two times higher than that of control. In these studies a conclusion may be drown that metabolic perturbation in DNA synthesis is one of the factors of SCE induction.

A Re-Examination of Chiasma Terminalization and Chiasma Frequency in Male Mice

Hirotami T. IMAI and Kazuo MORIWAKI

The distribution and frequency of chiasmata have been analyzed in male BALB/c mice. Bivalents were classified in terms of the number of interstitial chiasmata (CH) and terminal associations (TA) present as follows; 1CH, 2CH, 1TA, 1CH·1TA, 1CH·2TA, 2CH·1TA, 2CH·2TA and 0. We provide evidence that the TA frequently dissociates during 1st meiotic prophase. Consequently six of the observed bivalents may be derived from three basic bivalent types (namely 2CH·2TA, 1CH·2TA and 2TA) by dissociation of the TA according to the following schemas: (1) 2CH·2TA→2CH·1TA→2CH, (2) 1CH·2TA→1CH·1TA→1CH, and (3) 2TA→1TA→0. We also provide evidence that interstitial chiasmata do not move, which implies that a TA can not be formed by chiasma terminalization. The

chiasma frequency estimated by assuming that terminal associations do not result from terminalized chiasmata is 17.2±2.4 compared to a value of 25.4±2.2 calculated on the assumption of chiasma terminalization. For details, see Chromosoma (Berlin) 85: 439–452, 1982.

X-Y Chromosome Dissociation in Wild Derived *Mus musculus* Subspecies, Laboratory Mice, and Their F_1 Hybrids

Yoichi MATSUDA[1], Hirotami T. IMAI, Kazuo MORIWAKI, Kyoji KONDO[1], and F. BONHOMME[2]

The frequencies of X and Y chromosome dissociation in primary spermatocytes of wild-caught specimens of six *Mus musculus* subspecies, fifteen inbred strains, and their F_1 hybrids were examined. Three different types (I, II, and III) of X-Y dissociation were identified. Type I was found in inter-subspecies hybrids (BALB/c × *M. m. molossinus, M. m. bactrianus, M. m. castaneus,* or *M. m. urbanus*), in which the X-Y dissociation was less than 20% in each subspecies but 60–80% in the F_1 hybrids. Type II was seen in the DBA/2J and DDK strains, in which the dissociation frequency was about 30% in the homoxygous condition and increased up to 50% in inter-strain hybrids (C57BL/6J×DBA/2J or DDK). Type III found in the WB/Re strain also showed high dissociation frequency (51.1%) in the homozygous condition but the value was remarkably lower (11.3%) in (C57BL/6J× WB/Re)F_1s. Some Asian wild mice seem to have type II or III as well as type I. For details, see Cytogenetics and Cell Genetics 34: 241–252, 1982.

Chromosome Observations of Some Uruguayan Ants (Hymenoptera: Formicidae)

Beatriz GOÑI, Lucrecia C. de ZOLESSI[3], and Hirotami T. IMAI

Although more than 300 species of ants including European, Indian, Japanese, Australian and New World fauna have been cytologically investigated (Crozier 1975, Anim. Cytogenet. 3, Insect 7; Imai *et al.* 1977, Chromosoma 59: 341–393; Imai and Kubota 1981, Nucleus 24: 93–96),

[1] Faculty of Agriculture, Nagoya University, Nagoya.

[2] Laboratoire d'Evolution des Vertébrés, Université des Sciences et Techniques du Languedoc, Mnotpellier.

[3] Departmento de Artrópodos, Facultad de Humanidades y Ciencias, Universidad de la República O. del Uruguay, Montevideo, Uruguay.

chromosomes of neotropical ants have rarely been observed (Crozier 1970, Can. J. Genet. Cytol. **12**: 109–128). As the Neotropical region is one of the centers of ant's distribution and differentiation, and as a lot of endemic species are found in there, karyological survey of neotropical ants is very important for analysing the karyotype evolution of ants. Recently, we had an oppportunity to observe Uruguayan ants. This paper is a preliminary report on chromosomes of thirteen Uruguayan ants involving three subfamilies (Myrmicinae, Dolichoderinae and Formicinae).

Myrmicinae: Four species of *Pheidole*, one species of *Solenopsis*, and three species of *Acromyrmex* were examined. It was revealed that all the species of *Pheidole* (*cornutula*, *fallax*, *spinninodis* and *strobeli*) had the same chromosome number (2n=20 or n=10, and 2AN=40) and showed a nearly similar karyotype. According to Crozier (1975) and Imai *et al.* (1977), most members of the genus *Pheidole* reported (more than thirty) have 2n=20. Our present observations also suggest that *Pheidole* is a karyotypically conservative genus. A large fire ant species *Solenopsis saevissima* had 2n=32 (or n=16) and 2AN=62, which is almost identical with those of *S. aurea* (Crozier, 1970) and *S. invicta* (Glancey *et al.* 1976, Ann. Ent. Soc. Am. **69**: 469–470) having many metacentrics and submetacentrics. Two New World attine ants *Acromyrmex* (*Acromyrmex*) *ambiguus* and *A.* (*A.*) *hispidus* had the same karyotype 2n=38 and 2AN=62. Another attine species belonging to a different subgenus *Acromyrmex* (*Moellerius*) *heyeri* also showed a very similar karyotype to the previous two species mentioned above (i. e., 2n=38 and 2AN=62), suggesting that they are karyologically closely related. This is the first report on the chromosomes of the tribe Attini.

Dolichoderinae: One species (*Conomyrma pyramica*) was examined. This species is one of the common ants in South America, but this is the first cytological report of the genus. The diploid chromosome number was 2n=18. As all chromosomes excepting one pair were bi-armed, the diploid arm number was 2AN=34.

Formicinae: Four species of the cosmopolitan genus *Camponotus* were observed. The results were as follows: *C. mus* (2n=26, 2AN=42), *C. bonariensis* (2n=40, 2AN=48), *C. punctulatus* (2n=40, 2AN=44), and *C. rufipes* (2n=40, 2AN=44). The karyotypes of the latter three species were almost the same excepting a minor change in arm number. On the other hand, the karyotype of *C. mus* was quite different from the other members in chromosome number. In spite of such a remarkable difference

in chromosome number, their arm numbers were fairly conservative (2AN=42–48), suggesting that Robertsonian rearrangements would have played a significant role in their karyotype differentiation.

Chromosome Observations of Tropical Ants in Western Malaysia and Singapore

Beatriz GOÑI, Hirotami T. IMAI, Masao KUBOTA[1], Masaki KONDO[2], Hoi-Sen YONG[3], and Yow Pong THO[4]

In order to investigate the karyotype evolution of ants by using the karyograph method devised by Imai and Crozier (Am. Nat. **116**: 537–569, 1980), the second author is collecting ants from all over the world. As a part of the project Imai, Kubota and Kondo had recently the chance to collect tropical ants of Malaysia and Singapore. This is a preliminary report of chromosome numbers of 73 ant species placed in 36 genera, 20 tribes and 5 subfamilies. As the precise identification of species name is practically difficult in ants, we tentatively classified them as sp. 1, 2, 3, . . . based on external morphologies and karyotypes. For the convenience of researchers who are interested in the taxonomy of these ants, we deposited one set of the dry specimens to the Australian National Insect Collection (CSIRO, Canberra, Australia) and also to the Basel Museum (Switzerland). The results are summarized in Table 1.

Table 1. Chromosome numbers of Malaysian ants

Taxa	Chrom. number (n) 2n	Taxa	Chrom. number (n) 2n
SUBFAMILY PONERINAE		*Leptogenys* sp. 1	38
Tribe Platythyreini		*Leptogenys* sp. 2	48
Probolomyrmex sp.	28	*Diacamma* sp.	(18) 36
Tribe Ectatommini		*Pachycondyla* sp.	(11,11+1B)/
Gnamptogenys sp. 1	42		22+2B
Gnamptogenys sp. 2	36		(B-chrom. polymorphism)
Tribe Ponerini		*Mesoponera* sp. 1	28

[1] Nakasone 13, Odawara, Kanagawa-ken, 250.
[2] Shiraume Gakuen College, Kodaira, Tokyo.
[3] Department of Genetics and Cellular Biology, Universiti Malaya, Kuala Lumpur, Malaysia.
[4] Forest Research Institute, Kepong, Malaysia

Table 1. Continued

Taxa	Chrom. number (n) 2n
Mesoponera sp. 2	22
Mesoponera sp. 3	36
Hypoponera sp.	38
Brachyponera sp.	22
	(Reciprocal translocation)
Tribe Odontomachini	
Anochetus sp. 1	24
Anochetus sp. 2	(19)
Odontomachus sp. 1	(22) 44
Odontomachus sp. 2	30,30+1B
	(B-chrom. polymorphism)
Odontomachus sp. 3	(22) 44
Tribe Cerapachyini	
Cerapachys sp.	(25) 50
SUBFAMILY PSEUDOMYRMICINAE	
Tribe Pseudomyrmini	
Tetraponera sp.	44
SUBFAMILY MYRMICINAE	
Tribe Pheidolini	
Aphaenogaster sp.	30
Pheidole sp. 1	20
Pheidole sp. 2	(10) 20
Pheidole sp. 3	20
Pheidole sp. 4	20
Pheidole sp. 5	(16, 17)
	(Robertsonian polymorphism)
Pheidole sp. 6	20
Pheidole sp. 7	16
Pheidole sp. 8	38
Pheidole sp. 9	18
Pheidole sp. 10	20
Tribe Cardiocondylini	
Cardiocondyla sp.	40
Tribe Crematogastrini	
Crematogaster sp. 1	26
Crematogaster sp. 2	36
Crematogaster sp. 3	36
Crematogaster sp. 4	24
Tribe Solenopsidini	
Monomorium sp. 1	22
Monomorium sp. 2	22
Diplorhoptrum sp.	38
Oligomyrmex sp. 1	36
Tribe Myrmecinini	
Acanthomyrmex sp. 1	(11)
Acanthomyrmex sp. 2	22
Pristomyrmex sp.	22
Tribe Myrmicariini	
Myrmicaria sp. 1	44
Myrmicaria sp. 2	44
Myrmicaria sp. 3	44
Tribe Tetramoriini	
Triglyphotrix sp. 1	20
Triglyphotrix sp. 2	18
Tetramorium sp. 1	22
Tetramorium sp. 3	26
Tetramorium sp. 4	14
Tribe Attini (?)	
Proatta sp.	32
Tribe Dacetini	
Smithistruma sp.	16
SUBFAMILY DOLICHODERINAE	
Tribe Dolichoderini	
Dolichoderus sp.	18
Tribe Tapinomini	
Iridomyrmex sp.	18
Tapinoma sp.	(5) 10
Technomyrmex sp.	30
SUBFAMILY FORMICINAE	
Tribe Plagiolepidini	
Anoplolepis sp.	(17) 34
Acropyga sp.	(15)
Tribe Camponotini	
Camponotus sp. 1	(19)
Camponotus sp. 2	(20) 40
Camponotus sp. 3	38
Camponotus sp. 4	(18) 36
Camponotus sp. 5	(20) 40
Camponotus sp. 6	44
Polyrhachis sp. 1	(21)

Table 1. Continued

Taxa	Chrom. number (n) 2n	Taxa	Chrom. number (n) 2n
Polyrhachis sp. 2	(21)	*Paratrechina* sp. 3	28
Polyrhachis sp. 3	20	*Paratrechina* sp. 4	16
(Pericentric inversion polymorphism)		*Paratrechina* sp. 5	28
Tribe Lasiini		*Prenolepis* sp.	34, 36
Paratrechina sp. 1	16	(Robertsonian polymorphism)	
Paratrechina sp. 2	26		

Supernumerary Y Chromosomes and its Polymorphism in *Drosophila punjabiensis*

Akiko HANAKATA and Masatoshi YAMAMOTO

Extra sex chromosomes are normally detrimental to the individuals carrying them. In *Drosophila melanogaster* an extra X chromosome causes inviability, on the other hand extra Y chromosomes are less deleterious. The presence of an extra Y chromosome in both sexes hardly causes any clear phenotypic effect. A female can endure two extra Y chromosomes to be fertile, while a male having two extra Ys (XYYY) become sterile. Their fitness is lower than normal males and females and further they show meiotic drive through abnormal meiotic segregation of sex chromosomes. Thus it could be suspected that the number of Y chromosome aneuploids in natural condition should be rare. In fact there has been no report to date on the variation in Y chromosome numbers in natural populations of any organisms even those having sex determination mechanisms similar to *Drosophila*. We now report here the first finding of polymorphism of Y chromosome numbers in *Drosophila punjabiensis* isofemale lines collected from natural populations.

D. punjabiensis is classified into a *Drosophila montium* subgroup which belongs to *Drosophila melanogaster* group. This species is widely spread and commonly found in South East Asia. Ten isofemale lines collected from seven local populations in India, Malaysia and Burma were cytologically examined. The genome of *D. punjabiensis* cosists of two pairs of metacentric chromosomes (2nd & 3rd), one pair of dot-shaped chromosomes (4th) and sex chromosomes of acrocentric X and acrocentric Y chromosome. Two lines among ten, namely Texas 3033.4 from Malaysia and MYS 172 from

India, had supernumerary chromosomes. These supernumerary chromosomes were not distinguishable from the Y chromosome found in XY karyotypic male, judging from either conventional staining of Giemsa or Quinacrine-Hoechst staining. A variety of Y chromosome numbers was observed in the two lines. They were XX, XXY, XXYY in the female and XY, XYY, XYYY in the male. The frequencies of individuals carrying extra Y chromosome(s) were ca. 70% in MYS172 and ca. 40% in Texas3033.4. This extremaly high frequency of supernumerary Y chromosomes should indicate that the additional Y chromosomes are very stable in the labolatory conditions at least. Although similar observation concerned with Y chromosome-number polymorphism has been made in a wild population of tsetse fly (Maudlin, I. 1979), the frequency of Y chromosome aneuploids was very low both in males and in females.

In order to understand the mechanism by which such high frequency of Y chromosome naeuploids can be maintained in *Drosophila punjabiensis*, the following questions are now being examined.

1) Meiotic segregation of XYY, XYYY in the male and XXY, XXYY in the female.
2) The effect of extra Y chromosomes on viability and fertility.

New Series of Segmental Aneuploidy Using Transpositions between X and Y Chromosomes

Akiko HANAKATA and Masatoshi YAMAMOTO

In *Drosophila melanogaster*, chromosomal duplications and deficiencies are of great use for not only cytological gene mapping, studies of gene functions and its dose effect, but also molecular biological studies. Many efforts have been made for their production and application for genetical reseach since the begining of *Drosophila* genetics. With regard to X chromosome, a lot of deficiencies and free duplications have already been produced. But there are at least ten regions where no deficiency has been obtained because of haplo-lethal or halpo-sterile in the female, and most of the free duplications are limited in either proximal or distal regions.

Translocations between X and Y [$T(X;Y)$] are the most powerful tools to generate deficiencies and duplications in the X chromosome. When flies possessing $T(X;$Y) translocations with different X chromosome breakpoints are crossed, they produce aneuploids of deficiency and duplication besides

the euploids. Although the progenies carry extra Y chromosome fragments in their genome, it can be assumed that the Y chromosome aneuploidy does not give a serious damage to the individual because of the inertness of the heterochromatic Y chromosome. By this method, it is theoretically possible to produce adequate deficiencies and duplications in any region of the X. Although the system of segmental aneuploidy is very efficient and useful in *Drosophila* genetics, it certainly have the following two disadvantages for higher techniques of chromosome manipulation. (1) Because two translocation fragments are newly combined to synthesize a duplication and a deficiency as a segmental aneuploidy, a portion or all of the Y chromosome is duplicated in the genome. Though an Y chromosome hardly affects the external morphological phenotype, it reduces viability and fertility of the fly. (2) A duplication can be made at any region of the X with segmental aneuploidy, but the chromosomes used must be the ones of T(X ; Y). It is impossible at present to make a duplication between a particular X chromosome and a small segment which covers a speicfic regions in question.

The most useful and simple chromosomal rearrangement for this purpose could be a free Y chromosome carrying a small portion of the X. We have tried to generate such chromosomes as follows: males carrying *T(X ; Y)*, y^+B^S/Y were irradiated with 3600R of X-rays and were crossed to *C(1)DX*, *ywf* females. y^+B^S progeny males were individually crossed to *C(1)DX*, *ywf* females and their offsprings were examined. If a transposition of a X fragment into Y chromosome occurred, phenotypic changes can be easily observed. From a single cross, males carrying *T(X→Y)* transposition and females carrying the extra X fragment as a free duplication on the Y appeared as brothers and sisters. After the screening, they were examined cytologically. Up to date, the original *T(X ; Y)B146*, *T(X ; Y)*$B146^L B133^R$ (breakpoint 12E9) and *T(X ; Y)D12* (7C1) gave seven *T(X→Y)s* from 10,854 y^+B^S screened males. They are:

Transposition	Cytological breakpoints
T(X→Y)M04	12E9:14A
T(X→Y)M57	12E9:15A
T(X→Y)H4	12E9:15A
T(X→Y)H5	12E9:13A1
T(X→Y)H16	10D:12E9
T(X→Y)H17	12E9:13E2
T(X→Y)H31	7C1:8C

It is conceivable that free Y chromosomes carrying a X segment of any region of the X can be obtained by the same method.

Cytogenetical Studies on Autosomal Meiotic Pairing in *Drosophila melanogaster* Male

Masatoshi YAMAMOTO

Conventional pairing at meiosis is regarded as complete in the sense that partner homologues lie in close parallel alignment throughout their entire length. This assumes that pairing is by strict homology and that there are no pairing sites as such.

It has long been appreciated that conventional pairing does not apply to many sex chromosome systems. The clearest example is in *Drosophila* where X and Y chromosomes were argued for the occurrence of speicfic pairing sites within the heterochromatin. Here pairing is not by heterochromatic homology but rather by specialised entities called pairing sites or collochores.

Since the autosomes in *D. melanogaster* males do not engage in crossing over one can ask whether the autosomal pairing mechanisms are conventional. It is known that the conventional stages during which pairing normally occurs appear to be absent in meiosis of *D. melanogster* as too is synaptonemal complex. Male meiosis is thus thoroughly unconventional.

In the study of heterochromatin function on chromosome pairing, I found that the heterochromatin of autosomes are not involved in homologue recognition and subsequent pairing. The finding that euchromatic homology is of importance to normal meiotic pairing in the male leads to the next logical question; Is all euchromatin equally efficient in bringing about pairing and disjunction, or are some euchromatic segments are more important than others?

I have asked the above question on chromosomes 2, 3 and most extensively 4. I describe here the results obtained from the study of chromosome 4.

The translocation $T(X;4)B^s$ and $T(2;4)b$ both have breakpoints on the euchromatin of chromosome 4. The breakpoint of $T(X;4)B^s$ is at 1/4 proximal from the euchromatic distal end and that of $T(2;4)b$ is at the middle of euchromatin. In both cases the proximal portion containing heterochromatin is a partner for meiotic pairing with a nomral 4. *T(X;4)e15* is broken in the heterochromatin, but the size of translocated segment is minimal me-

asured by Q-H banding techniques. In this translocation too, the proximal fragment is involved in pairing. When, however, almost entire 4th chromosome heterochromatin is translocated onto chromosome 2 as is *T(2; 4)Y64* for example, a normal chromosome 4 always pairs with the translocated 4th fragment, and never pairs with the proximal portion of the 4th. Similarly, *T(X; 4)*w^{m5} and *T(X; 4)*$w^{m258-18}$ whose breakpoints are in the heterochromatin and the distal 1/3 is translocated onto the distal end of X chromosome were applied to cytological examination. As a result a normal chromosome 4 failes to pair with either distal or proximal fragments in almost all meiocytes. Interestingly enough however, at low frequency the translocated distal portion of 4 which is now on the X is involved in association with a normal 4.

This finding clearly indicates that there is a discrete priring site in the 4th chromosome heterochromatin as found in sex chromosome systems, and the site is located at the close visinity to the breakpoint of *T(X; 4)*w^{m5}. This is the first experimental demonstration to show the existence of pairing site in an autosome. It should be interesting to study the nature of this pairing site on the molecular basis and also ask if there exist similar entities as pairing sites in chiasmate organisms as well which show the conventional meiotic pairing processes as such.

Construction of *T(2; 4)*, *T(3; 4)* Autosomal Translocations in *Drosophila melanogaster*

Masatoshi YAMAMOTO

Meiotic chromosome pairing has been extensively studied in sex chromosomes because of ease in manipulating chromosomal abberations. Beginning the study of autosomal meiotic pairing, I faced the serious difficulty that not enough chromosomal rearrangements involving autosomes were available.

In order to carry out cytological studies of meiotic pairing of chromosomes 2 and 3, I firstly constructed *T(2; 4)* and *T(3; 4)* translocations. The screening procedure employed was as follows; Wild-type males of Canton-S strain were exposed to 3600R of X-rays. Then the X-rayed males were crossed to *ci*/*ci* virgin females in a mass and kept in 18°C incubator in order to enhance penetrance of the *ci* phenotype. Most F_1 ci^+/ci males showed wild-type phenotype, but at a low frequency males having *ci* phenotype appeared. The

Translocations and their breakpoints are listed below;

T(2; 4)*Y2*	35D3–4; 101F
T(2; 4)*Y7*	50C14–D1; 102C-D
T(2; 4)*Y22*	40–41; 101F
T(2; 4)*Y34*	36C–D; 101F
T(2; 4)*Y40*	56B–C; 101F
T(2; 4)*Y47*	31D–E; 101F
T(2; 4)*Y64*	35C; 101F
T(2; 4)*Y76*	40–41; 101F
T(2; 4)*Y86*	21E1–2; 101F
T(2; 4)*Y92*	57B4–6; 101F
T(2; 4)*Y100*	21D; 101F
T(2; 4)*Y106*	55A; 101F
T(2; 4)*Y109*	40–41; 59B; 101F
T(214)*Y141*	40–41; 101F
T(2; 4)*Y142*	52E; 102C
T(2; 4)*Y164*	33A; 101F
T(2; 4)*Y185*	52D; 101F
T(2; 4)*Y209*	36C–D; 101F
T(2; 4)*Y220*	56F6–8; 101F
T(2; 4)*Y231*	52F; 102D
T(2; 4)*Y241*	54D–E; 101F
T(2; 4)*Y308*	49F10–15; 101F
T(2; 4)*Y318*	40–41; 101F
T(2; 4)*Y325*	34E–F; 101F
T(2; 4)*Y344*	59F; 101F
T(2; 4)*Y375*	60F5; 101F
T(2; 4)*Y376*	33C; 101F
T(2; 4)*Y476*	29D; 101F
T(2; 4)*Y492* [*In*(2*LR*)]	30A7–9; 56F8–9; 101F
T(2; 4)*Y496*	22A1–2; 101F
T(2; 4)*Y517*	54F; 101F
T(2; 4)*BIII 17*	40–41; 101F
T(2; 4)*K10*	57F3–6; 101F
T(2; 4)*K12*	49B; 101F
T(2; 4)*K13*	25A3–4; 101F
T(3; 4)*Y9*	65B4; 101F
T(3; 4)*Y13*	80–81; 101F
T(3; 4)*Y25* [*In*(3*R*)]	90C; 98C; 101F
T(3; 4)*Y37*	80–81; 101F
T(3; 4)*Y57*	75F; 102C
T(3; 4)*Y61*	92A; 101F
T(3; 4)*Y63*	80–81; 101F
T(3; 4)*Y71*	81F; 101F
T(3; 4)*Y78*	100F; 102C14
T(3; 4)*Y81*	64E; 101F
T(3; 4)*Y104*	96A1–4; 101F
T(3; 4)*Y121*	80–81; 101F
T(3; 4)*Y140*	96A20–25; 101F
T(3; 4)*Y144*	88C; 101F
T(3; 4)*Y151*	78B; 101F
T(3; 4)*Y154*	72C; 101F
T(3; 4)*Y161*	99E; 101F
T(3; 4)*Y168*	76B1; 102D
T(3; 4)*Y175*	91D; 101F
T(3; 4)*Y177*	77B; 101F
T(3; 4)*Y183*	68D; 101F
T(3; 4)*Y210*	67E; 101F
T(3; 4)*Y226*	63D; 101F
T(3; 4)*Y249*	80–81; 101F
T(3; 4)*Y252*	80–81; 101F
T(3; 4)*Y255*	94B; 101F
T(3; 4)*Y262*	71F; 101F
T(3; 4)*Y285*	96D–E; 101F
T(3; 4)*Y291*	80–81; 101F
T(3; 4)*Y320*	88D; 101F
T(3; 4)*Y391*	98F11–12; 101F
T(3; 4)*Y403*	67E3–4; 101F
T(3; 4)*Y425*	83A; 101F
T(3; 4)*Y434*	80–81; 101F
T(3; 4)*Y446*	70D; 101F
T(3; 4)*Y449*	88E4–6; 101F
T(3; 4)*Y477*	75C; 101F
T(3; 4)*Y494*	98B; 101F
T(3; 4)*Y495*	70C; 101F
T(3; 4)*Y512* [*In*(3*R*)]	94B–C; 96E; 101F
T(3; 4)*BII8*	77A; 101F
T(3; 4)*BII 13*	93E; 101F

ci phenotype is known to come out when a breakage occured in the visinity of *ci* locus on chromosome 4. F_1 males showing *ci* phenotype, thus indicate that the individual was very likely to have a breakage on the 4th at least. The *ci* males were then individually allowed to mate with *yf*:=; *bw*; *e*; *ci* ey^R females. F_2 males heterozygous for the markers were testcrossed to the *yf*:=; *bw*; *e*; *ci* ey^R females and checked their segregation of marker characteristics. A translocation heterozygote can be easily identified since it shows new linkage groups. The translocations newly synthesized were kept as stocks over *SM1*, *Cy* and *TM3*, *Sb*.

Cytogenetical Analyses of the Gene Altering Sensitivity to Trehalose in *Drosophila melanogaster*

Masatoshi YAMAMOTO and Kunio ISONO

Various behaviours of organisms, such as locomotion, flight, mating, phototactics, circadian activity, eclosion and learning are believed to be under the genic control. In *D. melanogaster* feeding behaviour is primarily determined by the sensitivity at the chemosensory hairs which have taste receptor cells for water, salts, and sugars. Behavioural and electrophisiological studies have revealed that there are at least four groups of sugar receptor sites for glucose, fructose, sucrose and trehalose.

Recently Isono *et al.* found a feeding behaviour mutant which showed insensitivity to trehalose. It is already known about the mutant that (1) the gene Tre^+ controls sensitivity specific to trehalose not to other sugars and (2) Tre^+ gene functions at the *site* of sensory organs, chemosensory hairs, not in the nurvous system, because the mutant does not make any alterations on feeding behaviour and sensitivity to other sugars.

We have analysed the mutant by sucrose-trehalose feeding preference test. Wild type flies show similar degrees of sensitivity to 2 mM sucrose and 20 mM trehalose. Naturally the flies ingest approximately equal amount of each sugar when they are allowed to choose either 2 mM sucrose or 20 mM trehalose. Because sucrose solution contains red dye and trehalose does blue, wild type flies show purple (red+blue) abdomens after ingestion and the mutants show red abdomen.

Genetic dissections of *Tre* mutant using several X chromosome deficiencies and *T*(*X*; *Y*) translocations for segmental aneuploidy has given the following results;

(1) The sensitivity to trehalose is determined by the single gene Tre^+ on the X, differing from other behavioural genes that are often polygenic.
(2) The Tre^+ locus was mapped cytologically between 5A8–5B3 according to the salivary chromosome bands.
(3) The sensitivity to trehalose measured by the ratio between the number of flies ingested trehalose and that of those ingested sucrose showed a strong correlation with the gene dose of Tre^+. The order of sensitivity goes $Tre/0 = Tre/Tre < Tre^+/Tre \fallingdotseq Tre^+/0 < Tre^+/Y \fallingdotseq Tre^+/Tre^+ < Tre^+/Tre^+/Tre^+$. Here *0* means a deficiency for the gene. The number of wild type alleles induces higher sensitivity proportional to the gene dose in the female. In the male, on the other hand, a single gene dose of Tre^+ showed the same sensitivity to the female homozygous for Tre^+, but no hypersensitivity was observed in the male carrying an extra Tre^+ as a duplication.

That the intensity of a certain sensitivity leading to an animal behaviour can be modified by gene dose has not been reported earlier. The phenomenon as above clearly indicate that the gene Tre^+ produces a protein under quantitative control through dosage compensation mechanisms and the amount of the gene product is a determinant for sugar sensitivity and feeding behaviour.

The Effects of the Variation in Heterochromatin Content on Recombination Frequencies in *Drosophila melanogaster*

Masatoshi YAMAMOTO

Heterochromatin has long been an enigma when it comes to the nature of its function. Heterochromatin is a commonly found nuclear entity in almost all eukaryotic organisms. An interesting common finding among vast variety of organisms is "variation". The most of eukaryotes so far studied showed extensive variation not only in the amount of heterochromatin but also in the nucleotide sequences of heterochromatic DNA (satellite DNA) between species as well as within a species. Cytologically too, the variation of constitutive heterochromatin was observed more prominently. The positions where heterochromatic blocks existed in a genome were so variable in both between and within species. Constitutive heterochromatin can be found at centromeric, telomeric, interstitial regions or as free heterochromatic chromosomes, or every possible combinations of the above.

Recently it has been demonstrated that the alteration of the centromeric

heterochromatin content in *D. melanogaster* females causes decrease or increase in the amount of recombination *intra-* as well as *inter*-chromosomally. The degree of alteration in recombination frequency was strongly correlated with the changes of centromeric heterochromatin content. The more was the centromeric heterochromatin of the X deleted, the less occured the recombination on the X. On the contrary, the frequency of cross-overs on the 3rd chromosomes was higher if the X chromosome had larger deficiencies in the heterochromatin. Similar heterochromatic effect on chiasmata has been argued to be a general function of heterochromatin based on mainly cytological studies of meiosis in grasshoppers and several plants.

Many observations obtained from a variety of eukaryotes can strongly suggest a general rule or function of heterochromatin, but no more than that. They have totally lacking experimental tests for the speculations. The most eukaryotic organisms but *Drosophila melanogaster* are troublesome to handle for genetic studies and hardly meets this demand. *D. melanogaster* is only an organism we can use to study extensively the relationship between the variation of heterochromatin content at different chromosomal positions and the changes in cross-over frequencies. This is so we have constructed every class of heterochromatic chromosomes.

Interstitially heterochromatic chromosomes are a novel chromosomal rearrangement in *D. melanogaster*. This rearrangement was constructed from males of *T(X ; Y)B146* of which chromosomal breakpoints are 12F9 on the X and on the long arm of the Y. The males were irradiated with 3600R of X-rays and then allowed to mate with *C(1)DX, ywf* females. F_1 males were checked for phenotypic characteristic of yellow. The yellow males were likely to have a fragment of heterochromatic Y chromosome at 12F9 of the X. The chromosomes were then cytologically examined and confirmed that they were interstitially heterochromatic (Y origin) chromosomes.

Recombination on such interstitially heterochromatic chromosomes has not been studied in any organisms, although the ones having heterochromatin inserted were found in widely defferent species. In our experiment recombination was measured in the females either heterozygous or homozygous for the experimental chromosomes whose genetic backgrounds were all the same and the X chromosome euchromatin was replaced with the same origin. Under such stringent conditions, it has been demonstrated that the inserted heterochromatin has a drastic effect on cross-over frequencies. The females

heterozygous for an X chromosome containing comparatively large heterochromatin showed a big reduction in the amount of recombination, from 58% of control value to the mere 14% as a total map distance. In a small interval the effect was more prominent as from 24% to only 1%. The level of reduction in recombination frequency was clearly correlated with the size of heterochromatin inserted. The females homozygous for the heterochromatically duplicated X, however, hardly showed any alteration even though the heterochromatic size was altered.

VI. MUTATION AND MUTAGENESIS IN ANIMALS

The Mutagenic Activity of N-Ethyl-N-Nitrosourea in Various Germ-Line Cells of the Silkworm

Akio MURAKAMI

The mutagenic activity of N-ethyl-N-nitrosourea (ENU) has been well demonstrated in many species including mice. In mice, this carcinogenic chemical has been reported to be supramutagenic in spermatogonia (Russell and his co-workers, Proc. Natl. Acad. Sci. (USA), 76: 5818–5819 (1979), but the result of mutagenicity tests in female germ-cells is not yet reported. This gap may be attributed to some technical limitations: though it can be easily obtained a large number of offspring from small numbers of treated male mice, but not from the females. In the assessment of chemically-induced genetic hazards in man, it is an accepted practice to consider spermatogonia in males and oocytes in females as the germ-cell stages most at risk. In this respect, the silkworm specific-locus test method using egg-colour gene markers has an advantage to easily collect a large number of F_1 offspring from mating of the treated wild-type females as well as males with the tester stock. In addition, there is possible to test the mutagenicity of chemicals to whole germ-cells in gametogenesis for both sexes. The purpose of the present experiment is to find out whether or not ENU is mutagenic for induction of the recessive visible mutations by the specific-locus method in oocytes and post-spermatogonial germ-cells of the silk moth.

The silk moths used in this experiment were (C108 × Aojuku) F_1 hybrids homozygous for the wild-type at two egg-colour gene loci marked in the test stock. Freshly prepared ENU solution (0.1, 0.5, 1.0 and 10.0 μg/mg body weight) in pH 6.0 were administered by the injection method into the body-cavity of the wild-type males at the mid-pupal stage to detect the mutagenic activity of this chemical for both oocytes and spermatozoa. For the 4th- and 5th-instar wild-type F_1 male larvae ENU solutions in two different doses in 84.9 and 170.0 μg/mg body-weight were administered by the injection method to detect the mutagenic activity in growing spermatocytes and the early phase of meiotic spermatocytes, respectively. The mulberry leaves spread with ENU solutions were given by the feeding method to the 2nd and 3rd instar

male larvae in doses of 100 and 200 μg/larva. The corresponding germ-cell stages are the late spermatogonia and very early phase of spermatocytes, respectively. In this experiment, actual fed doses were calculated to be 69.0 and 138.7 μg/larva or 2.1 and 4.3 μg/mg body-weight. Controls were applied with 0.85% solution alone. After emergence, the treated silkworms were mated with the tester stocks to detect for the egg-colour specific-locus mutations at the two loci.

ENU treatments by the injection method produced significant increases in mutation frequencies compared to controls for spermatocytes, meiotic spermatocytes, spermatids in larvae and spermatozoa and oocytes in pupae. A striking difference between spermatocytes and spermatozoa in the frequency of induced mutations was observed: spermatozoa were more sensitive to ENU than spermatocytes, by a factor of 50–60. It is of inereest to note that the mutagenic activity of ENU in spermatozoa was simalar to that in oocytes. In the feeding experiments for young larvae, no significant increase of mutations was detected in the corresponding germ-cell stages, spermatogonia and immature spermatocytes. This finding may indicate that ENU is unstable in digestive juice and/or in body-fluid of the larvae. It can not exclude a possibility, however, that the incorporated dose might be not sufficient to induce the specific-locus mutations. Additional research will be necessary to clarify the mechanism(s) involved. From this silk moth specific-locus test of ENU, it is clear that this carcinogenic mutagen ENU was very mutagenic in oocytes, spermatocytes, spermatids, and spermatozoa, but not in spermatogonia contrary to the observation in mouse spermatogonia shown the most-potent mutagenicity among several mutagens detected in mice (Russell *et al.*, 1979). The mechanism(s) responsible for this differential sensitivity in interspecies is not clear, but it is likely that differential metabolic activity may be concerned in this difference.

Difference in Mutability by the Different Administration Routes of Chemical Mutagens in the Silkworm

Akio MURAKAMI

The intensity of chemically-induced mutagenicity is markeldy influenced by a number of factors. The metabolic activity in the body and in the target cell seems to be one of important factors regardless of directly- or indirectly-acting chemicals. This factor would be mainly concerned in the administra-

tion routes (and/or manners) of the chemical. From the standpoint of risk evaluations of the possible hazards to man of many environmental chemicals with mutagenic potential, it should take into consideration on the exposure pattern to the chemicals.

Silkworm larvae on the day 1 of the 5th instar were used in this experiment because the stage larvae are suitable for the administration of chemicals by either the feeding method or the injection method. Accordingly, it is possible to compare the mutagenic activity of the chemical mutagens to the germ-cells of the last instar larvae treated by both methods. In the male, the corresponding germ-cell stage is the early phase of meiotic spermatocytes. In this experiment, Mitomycin C (MC) was chosen for the model chemical agent. This antibiotic agent is an indirectly-acting mutagen which required metabolic activation to mutagenic derivatives and that is a strong mutagen followed by Aflatoxin B_1 in pupal oocytes and spermatozoa of the silkworm. The egg-colour specific-locus test was used as the method for detecting and quantifying the induction of recessive visible mutations. Freshly prepared MC in 0.85% NaCl solution was administered by the injection method into the body-cavity of the 5th instar wild-type (C108×Aojuku) F_1 hybrid male larvae in doses of 5, 10, and 20 μg/larva corresponding to 7.9, 15.7, and 31.5×10^{-3} μg/mg body-weight. The mulberry leaves with the MC solution were applied by the feeding method to the larvae in nominal applied doses of 20, 40, and 60 μg/larva or in effective fed doses of 2.13, 4.15, and 6.28×10^{-2} μg/mg body-weight, respectively. Control larvae were treated with 0.85% NaCl solution alone.

The results demonstrated that the incidence of specific-locus mutations in the spermatocytes of the last instar larvae was induced with the administration of MC by both injection and feeding methods. The shape of dose-response curves obtained by either parenternally or perorally administered routes was a linear fashion, but the slope was remarkably different from each other. The mutagenic activity of MC when given parenternally was about 130 times as active as when given perorally. This weak mutagenicity of MC by the feeding method may be the result of a detoxic activity for the antibiotic agent in digestive organs and/or in hemolymph. From these findings, it can be confirmed that the mutagenic activity of the chemical agent may be remarkably under the influence of the manner of administrations.

By the way, growing spermatocytes in the 4th instar larvae were more

sensitive to MC than meiotic spermatocytes in the last instar larvae. However, MC-induced mutational events in pupal spermatozoa were slightly but significantly less frequency than those in the spermatocytes. The present finding seems to be different from the well-accepted general view that post-meiotic germ-cells are more mutagenic to alkylating agents including MC than pre-meiotic ones. A similar observation has been reported in mice that post-spermatogonial stages are less sensitive to MC than spermatotonial stages [Russell and his co-workers, Mut. Res. 86: 329–354 (1980)]. In the silkworm, contrary to those observations, Ethylmethanesulphonate was quite active for the induction of the specific-locus mutations in spermatozoa, but did not induce a high incidence of the mutational events in spermatocytes. Such being the case, further studies with a variety of alkylating agents are necessary to draw the final conclusion for this topics.

Relationship between Chemical-Dose and Specific-Locus Mutations by a Mycotoxin Aflatoxin B_1 in Pupal Oocytes of the Silkworm

Akio MURAKAMI

To evaluate the genetic risk with chemical as well as physical agents the investigation of the dose-response curve is of dominant importance. The major object of the analysis of dose-response kinetics is to answer the point at issue whether or not the threshold dose for the induction of mutations is exist. For this purpose, clear dose-response curves should be obtained in the range of low dose levels. It is well known by our experiences, however, that the frequency of mutations induced with low dose levels tends to be covered by the spontaneous mutation frequency. Consequently, the sample size in an experiment point at low-dose levels has to be extremely large to establish significant differences between induced and spontaneous mutation frequencies. From this point of view, we considered that the silkworm specific-locus mutation test system using the egg-colour loci seems to be content with such the requirement: it can easily handle a large number of F_1 offspring and rapidly score the mutational incidence.

The present communication deals with the experimental results on the effect of low-dose levels, 0.01, 0.5, and 0.1 μg/capita on specific-locus mutations inducing from Aflatoxin B_1 ($AFTB_1$) applied to the oocytes of mid-stage pupal females which have been previously been demonstrated to be the

most potent mutagen in the silkworm and shown to be a linear dose-response kinetics at relatively high dose ranges between 0.1 and 5.0 μg/capita. In the incorporation experiment with [^{3}H] $AFTB_1$, it could be estimated that an oocyte (or ovium) took in about 0.037% of the total injected dose of $AFTB_1$ per female pupa: 0.01, 0.5 and 0.1 μg/pupa were corresponded to *ca.* 3.7, 37.0 and 370 pg/oocyte, respectively. (C108×Aojuku) F_1 wild-type females at the mid-stage pupa were injected with a single dose of the three different doses of $AFTB_1$ dissolved in 0.85% NaCl solution. After emergence they were then mated with untreated marker strain male moths homozygous for egg-colour genes, *pe* and *re* and the offspring were submitted to score the recessive visible mutations at the two loci, pe^+ and re^+. For each dose point, 500,000 or more of F_1 offspring were used to estimate the frequency of mutations.

The dose-response curves between 0.01 and 5 μg/pupa of $AFTB_1$ were shown in Figure 1 based on the present finding in the low-dose range together with the previous one in the relatively high-dose range from 0.1 to 5.0 μg/

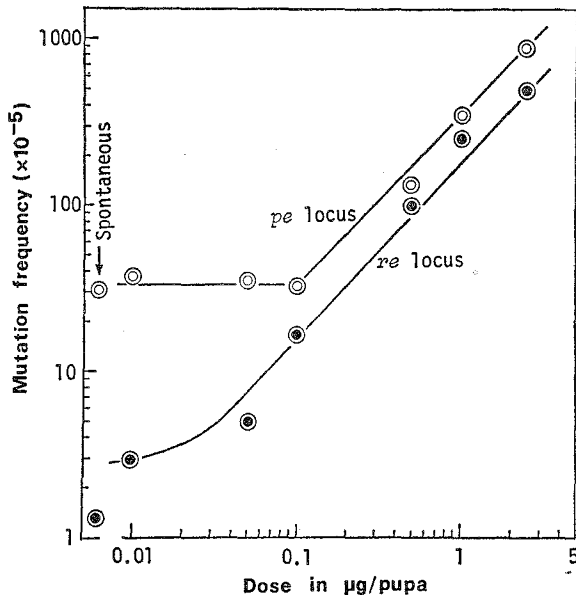


Fig. 1. The dose-response curves for mutagenicity of Aflatoxin B_1 in silkworm oocytes. Vertical lines indicate the 95% confidence intervals for each mutation frequency.

pupa. As can be seen from the figure, the fashion of a dose-response curve at the *pe*$^+$ locus is observed no difference between the induced mutation frequency obtained with a moderate dose at 0.1 μg/pupa and the spontaneous mutation frequency within the 95% confidence intervals. Whereas at the *re*$^+$ locus, the dose-response curve goes linearly down to by a dose at 0.01 μg/pupa or 3.7 pg/oocyte and the frequency of mutations induced with this dose is significantly above the control level. This finding indicates that a threshold dose will be at a dose less than 0.01 μg/pupa. This difference in sensitivity between the loci will be responsible for the frequency of spontaneous mutations for both loci: the spontaneous mutation frequency at the *pe*$^+$ locus was about one order as high as the *re*$^+$ locus.

Specific-Locus Mutations Induced by β-Emitter [Methyl-^{3}H] Thymidine in Primordial Germ-Cells of Dormant Silkworm Embryos

Akio MURAKAMI

Environmental contamination from internal β-emitters arises in various nuclear operations, such as fuel processing, nuclear power stations, nuclear fusion devices, *et cetera.* Consequently, there is increasing concerns over the possible mutagnicity as well as other biological effects of these internal radioemitters. In general, human exposure to β-radiation derived from various internal radiation sources in which were naturally or accidentally accumulated in the body or in the tissues will be low dose and low dose-rate. In this connection, silk moth (*Bombyx mori*) may have a biological advantage for detecting some genetical effects in germ-cells by incorporated radioactive substances at the above-mentioned exposure condition. The embryos of silkworm eggs usually take diapause in the early phase of embryogenesis for the maximum over one year. The duration of diapause state can be regulated easily by the control of incubation temperatures for the dormant eggs. The gonad in this embryonic stage consist of primordial germ-cells and the number of the cell is known to be at least 20 per embryo. In the silkworm, the mid-stage female pupae are suitable for the administration of radioisotopes because of the oocytes in this pupal stage can actively take in various nutritional materials as well as radioisotopes from hemolymph for vitellogenesis. In this investigation, [Methyl-^{3}H] thymidine was used as a model radionucleide for the internal β-emitters.

Wild-type C108 strain mid-stage female pupae treated with one of DNA precursors of ^{3}H-thymidine of 25 μCi/capita were mated to an another wild-type *Aojuku* strain males to allow deposition of the radioactive eggs. The duration for diapause period of the eggs was 263 days from Oct. 10th 1980 to July 1st 1981. After hatching, offspring were raised and then mated to the corresponding an opposite sex of marker stock moths homozygous at the two egg-colour genes (*pe* and *re*) scored for the recessive visible mutations. Five eggs sampled randomly from each batch were determined radioactivities (counts per minute) by the liquid scintillation counter. An average radioactivity per egg for a given batch was calculated to be ranging from 2500 to 3800 cpm. Each egg used in this experiment was about 0.45 mg in weight.

As has been anticipated from a nature of the test system adopted in this experiment, a number of mutant clusters with a large size *e.g.*, 10 to 20 mutants per batch (or 400 to 500 F_1 offspring) were often detected, indicating that an accurate determination of β-ray-induced mutation frequencies is difficult as the result of the marked cluster effect. However, data obtained clearly indicated that β-ray from [^{3}H] thymidine deposited in the dormant egg were highly mutagenic to embryonic primordial germ-cells. For β-ray-induced mutational events in these germ-cell stages, males were 2-3 times as high sensitive compared with females and *pe* locus was more sensitive than *re* locus. These finding are quite the same to external radiations, γ- or X-rays. The incidnece of mutations was markedly increased up to $100\text{–}300 \times 10^{-5}$ per locus when the embryos which would have more actively incorporated the isotopes were used. This suggests that mutational incidence induced with β-rays from [^{3}H] thymidine seemed to be proportional to the isotope doses deposited in the egg. The incidence of mutations in the highly radioactive group with *ca.* 3800 cpm/egg produced approximately the same frequency in dormant eggs irradiated with around 2500 R (or more) of γ-rays at a dose-rate of 300 R/min. The dominant lethal test by the reduction of egg-hatchability revealed, however, that in the 25 μCi/pupa of [^{3}H] thymidine treated groups the embryonic lethality was not significantly affected, whereas in 2500 R of γ-rayed dormant eggs the lethality was remarkable. This observation indicates that β-ray-induced cell-killing effect was clearly different from γ-rays. The differential sensitivity in genetic end-points may be due to the dose-rate effects of β- and γ-rays on the germ-cells in the dormant stage eggs. Not yet determined what quantities of [^{3}H] thymidine were actually deposited in the nucleus of the germ-cells. In a prelimianry

experiment, however, tritium water (THO), which is not a precursor of DNA, induced almost the same mutational incidence in an equivalent dose level of [^{3}H] thymidine. This finding and the others suggest that [^{3}H] thymidine might be predominantly deposited in the cytoplasm rather than in the nucleus of the germ-cells.

The experiment in this series is not yet completed, but it is likely to say that the method described here is a relevant test system to evaluate the genetic risk from the internal β-emitters at the low dose-rate and at the low dose level.

VII. RADIATION GENETICS AND CHEMICAL MUTAGENESIS IN MICROORGANISMS AND PLANTS

Enzymatic Comparison of DNA Damages Induced by Beta-Rays from Tritiated Water and by Gamma-Rays

Tadashi INOUE and Tsuneo KADA

Quantitation of the biological effects of tritium is of practical interest because this nuclide will be released into environment when nuclear fission reactor is peacefully developed in near future. Reactor-generated tritium enters the biosphere as tritiated water and the main concern for purposes of radiation safety is the effectiveness of the beta particle produced when it decays.

In order to assess the effectiveness, we compared the nature of the DNA lesions produced by internal beta-irradiation by tritiated water with those produced by external irradiation by ^{137}Cs-gamma-rays using several enzymes specific for DNA lesions. First of all, we examined effectiveness to introduce single strand scissions in covalently closed circular DNA of colicin E1. From the results of anlyses by neutral sucrose density gradient centrifugation, the RBE of tritium beta particles relative to the gamma-rays was estimed to be approximately 3. Terminals of DNA strand scissions induced by the irradiation were analyzed with two kinds of exonuclease, namely venom phosphodiesterase and spleen phosphodiesterase. The former enzyme initiates hydrolysis of oligonucleotides at a 3′-OH terminal and yields 5′-P mononucleotides, while the latter enzyme requires 3′-P terminal yielding 3′-P mononucleotides. When the DNA with avarage 25 single strand scissions per molecule was subjected to hydrolysis by venom phosphodiesterase, no difference was detected between two types of radiation which create the strand scissions in substrate DNA, suggesting that both types of radiation produce 3′-OH terminals in comparable efficiency. On the other hand, neither type of radiation produced lesion susceptible to spleen phosphodiesterase, indicating that very few number of 3′-P terminals were produced by both types of radiation.

In addition to strand scessions, ionizing radiation introduces apurinic sites (AP-sites) in DNA. Number of AP-sites in irradiated DNA was measured

by using AP-endonuclease of *B. subtilis*. The experiments yielded the results that while gamma-irradiation introduced 0.8 AP-site per one strand scission, only 0.3 AP-site per one strand scission was made by beta-irradiation.

These data suggest that lesions produced by beta-irradiation are both quantitatively and qualitatively different from those produced by gamma-irradiation.

Mechanisms of Environmental Antimutagenesis

Tsuneo KADA

In these several years, our knowledges on environmental mutagens increased greatly. We know that mutagens showing potent activities *in vitro* are often modulated in the body of mammals, indicating presence of *in vivo* factors antagonizing with the mutagens. We defined desmutagens as agents inactivating directly mutagens *in vitro* (T. Kada, 1982, Proc. 3rd Intern. Conf. Env. Mutagen). On the other hand, we call antimutagens those agents working as antagonists in cellular mutagenesis *in vivo*.

Systematic trials have been made to detect antimutagens in our environment. For this purpose, indicator bacteria were first mutagenicaed by one of chemical mutagens or radiations and exposed to test agents for several generations. Those agents reducing remarkably induced mutabilities without affecting considerably the cellular lethality were studied further in details.

Certain antimutagens are working probably by reducing errors involved in SOS repair of DNA damage induced by UV or 4NQO in *E. coli* strains. They include cobaltous chloride (Kada, Proc. 6th Int. Congr. Rad. Res, 1979), cinamldehyde (Ohta *et al.*, Mutation Res., in press), enmein (Kakinuma *et al.*, Ann. Meeting Abstr. Agr. Chem. Soc. 3B18, 1982) or protoanemonin (Komura *et al.*, Mutation Res., in press). Such a mechanism may explain part of antimutagenic actions of the human placenta factor (Kada and Mochizuki, J. Rad. Res. **22**: 297, 1981; Mochizuki and Kada, Mutatoin Res. **95**, 457, 1982). On the other hand, some of other antimutagens are working in mutageneis induced by ionizing radiation, MNNG or Trp-P-1. They inculde cobaltous chloride (Kada and Kanematsu, Proc. Japan Acad. **54B**, 234, 1978, Mochizuki and Kada, Mutation Res. **95**, 145, 1982) and carboxy-ethylgermanium sesquioxide (Mochizuki and Kada, Int. J. Radiat. Biol. in press). Studies are under way to elucidate mechanisms involved

in them.

The Effect of Septum-Initiation Mutations on Sporulation and Competent Cell Formation in *Bacillus subtilis*

Yoshito SADAIE and Tsuneo KADA

Sporulation of many gram-positive bacilli begins with the formation of an asymmetric forespore septum upon starvation for nutrients, followed by differential expression of genes on the chromosomes segregated into two cytoplasms, indicating the most simple system of differentiation. As the process of forespore septum formation may share common steps with vegetative cell division (Hitchin and Slepecky 1969), the elucidation of forespore septum formation in relation to vegetative division is required to study how nutritional starvation leads cells to divide asymmetrically. *Bacillus subtilis* is genetically well characterized and offers the best organism to study microbial differentiation. We studied the effect of septum-initiation mutations on sporulation with four *Bacillus subtilis* mutants carrying septum-initiation mutations of different loci, *div-31*, *div-341*, *div-12*, and *div-355* which showed filamentous growth at 45°C (Miyakawa and Komano 1981).

Cells were grown to T_1 stage before forespore septum formation at 30°C and transferred to 37°C or 42°C. These four strains sporulated well at permissive temperature (30°C) and showed poor sporulation at higher temperatures (37°C and/or 42°C). Thus the initial steps of septation mediated by *div-31*$^+$, *div-341*$^+$, *div-12*$^+$, and *div-355*$^+$ alleles are required for sporulation, although we do not know whether they are required for forespore septum formation or for some later processes in sporulation.

On the other hand, it was suggested that common steps were involve both in competent cell formation and in forespore septum formation (Sadaie and Kada 1982, IV Genetics of Industrial Microorganisms, Kyoto). Therefore, it is possible that vegetative septation is involved in competent cell formation as well. A competent culture was induced by diluting early stationary phase culture grown at 30°C with fresh medium and incubating for 90 min at 37°C and 40°C. The strain carrying the *div-31* mutation lost competence at any temperatures examined while the *div-341* and *div-355* strains showed less competence at higher temperatures (37°C and/or 40°C). Vegetative growth of these strains was not affected at these temperatures. The mutation *div-12* made cells slightly poor for competence at any temperatures examined.

Therefore, the mutations *div-31*, *div-341*, and *div-355* may affect forespore septum formation because these mutations affected competent cell formation which seemed to share common steps with forespore septum formation. The *div-12* mutation may affect sporulation in the stage later than forespore septum formation.

Div mutations employed in the present study affected competent cell formation and sporulation at intermidiate temperatures where vegetative growth was not affected. This fact suggests a qualitative difference which discriminates vegetative septation from asymmetric sporulation specific septation and a "division" in competent cell formation. (J. Bacteriol., Mol. gen. Genet. in press)

Purification and Characterization of a Human Placental Factor which Enhances the Priming Activity of γ-Irradiated DNA

Tadashi INOUE and Tsuneo KADA

A human placental factor which specifically enhances the priming activity or γ-irradiated DNA was purified near homogeniety by successive application of the extracts to γ-irradiated DNA cellulose, Sephacryl S200, and Phenyl-Sepharose column chromatographies. The factor was protein in nature and had a molecular weight of arround 48,500. In addition to the γ-irradiated DNA, the priming activity of neocartinostatin-treated DNA was also enhanced by the factor. Niether exonuclease nor endonuclease activity was detected toward intact DNA while γ-irradiated DNA was rendered acid-soluble to some extent. These results suggest that the factor is a γ-exonuclease acting on damages in DNA treated with γ-rays or certain chemicals to make the DNA suitable primer for repair synthesis by DNA polymerase.

Inhibition of DNA-Repair and Radiosensitization

Tsuneo KADA, Hajime MOCHIZUKI, and Akio YOKOIYAMA

Damage induced by gamma-ray-irradiation from a ^{137}Cs source in DNA of *Bacillus subtilis* was repaired to certain extent by *in vitro* treatment with the highly diluted extract obtained from human placenta tissues, as estimated from the increase in the transforming activity. This repair activity was

sensitive to heating (100°C) or to treatment with EDTA (5 mM), suggesting some enzymes involved (Mochizuki and Kada, in preparation). Nakatsugawa and Sugahara (Rad. Res. 84, 265, 1980) showed that cordycepin inhibits potentially lethal damage (PLR) of X-irradiated Chinese hamster *hai* cells and can be used as a radiosensitizing agent. That cordycepin is really acting as a repair inhibitior was shown using our *in vitro* reapir system where gamma-ray (10 kr) exposed transforming DNA of *Bacillus subtilis* was incubated with extracts of human placenta and the effects of cordycepin were observed. The placental extracts remarkably recovered the transforming capacity. Such a recovery was gradually lost by presence of the radiosensitizer of increasing concentrations. This system may offer a good one for screening radiosensitizers working in human (Kada *et al.*, in preparation).

Cordycepin was confirmed to be a real inhibitor working in the PLD repair. Results of radiosensitizing experiments using the DNA-repair inhibitors in tumor-bearing animals are so far promising and further developments in this field are expected.

Mutagenic Effect of Ekatin (thiometon) in Soybean Test System

Taro Fujii and Tadashi Inoue

Agricultural chemicals which are used commonly in our farming were applied to soybean test system. No increase of spotting frequency was observed with five chemicals (Antio, Baycid, Diazinon, EPN and Karphos) indicating that they may not have any mutagenic effects, at least at the concentrations examined (500–2000 times dilution). However, increase of mutational spots were observed by Ekatin treatment even at 2000 times dilution which is the usual concentration in practical use for pest control. Ekatin is a mixture of 25% thiometon and 75% solvent; the former is the essential component of this pesticide. Because Ekatin showed some mutagenic activity, effects of thiometon were examined with the same treatment procedure. An increase of mutational spots per leaf was evidently observed at 1000 and 500 times dilutions. From these results, it is apparent that thiometon, essence of Ekatin, has a mutagenic activity.

The activation of chemicals into mutagens by higher plants is a recently discovered hazard. Chemicals that are themselves not mutagenic but got convereted into mutagens through plants metabolism have been noticed in several plant materials such as maize and sybean. Since Ekatin and its

essence, thiometon, exhibited an unequivocal mutagenicity in the soybean test system, we examined the mutagenicity of this sampel on *Salmonella* TA98 and TA100. Thiometon has no mutagenic activity toward Salmonella in the absence or presence of rat liver S9 fraction. We also examined whether thiometon was activated by the soybean drug metabolizing system. However, the S9 fraction obtained from the soybean seedlings did not activate the pesticide. Furthermore, we could not detect any mutagenicity of the material extracted from the soybean seeds which had been incubated with thiometon for 24 hr. These results suggest that the mutagenic activity of Ekatin and thiometon is specific for plant cells.

Use of pesticide is a common procedure for crop production in modern farming. Therefore, if pesticides possess mutagenicity, even though the apparent hazard is minor, retention and accumulation of induced damage may eventually have detrimental effect upon the natural ecosystem. Additionally, residues or derivatives of these substances in the edible plants may affect human health. Systematic mutagenicity studies of agricultural chemicals using the plant systems are, therfeore, necessary.

VIII. POPULATION GENETICS (THEORETICAL)

Was Globin Evolution Very Rapid in Its Early Stages?: A Dubious Case Against the Rate-Constancy Hypothesis

Motoo KIMURA

Goodman *et al.*'s (1975) claim of accelerated evolution in the early stages of globin evolution (see *Nature* 253, 603–608) is based on an erroneous assignment of the time of divergence of vertebrate myoglobin and hemoglobin. When this is corrected, there is no basis for their claim. The data are much more consistent with the nearly constant rate expected on the neutral mutation-random drift hypothesis than with the uneven rates expected if most amino acid changes were caused by substitution of favorable mutants through Darwinian selection. In addition, the majority of the codons determined by their maximum parsimony method have turned out to be wrong when compared to the actual nucleotide sequences of rabbit α and human β hemoglobins determined by direct sequencing. For details, see *J. Mol. Evol.* **17**, 110–113.

Possibility of Extensive Neutral Evolution under Stabilizing Selection with Special Reference to Nonrandom Usage of Synonymous Codons

Motoo KIMURA

The rate of evolution in terms of the number of mutant substitutions in a finite population is investigated assuming a quantitative character subject to stabilizing selection, which is known to be the most prevalent type of natural selection. It is shown that, if a large number of segregating loci (or sites) are involved, the average selection coefficient per mutant under stabilizing selection may be exceedingly small. These mutants are very slightly deleterious but nearly neutral, so that mutant substitutions are mainly controlled by random drift, although the rate of evolution may be lower as compared with the situation in which all the mutations are strictly neutral. This is treated quantitatively by using the diffusion equation method in population genetics. A model of random drift under stabilizing

selection is then applied to the problem of "nonrandom" or unequal usage of synonymous codons, and it is shown that such nonrandomness can readily be understood within the framework of the neutral mutation-random drift hypothesis (the neutral theory, for short) of molecular evolution. For details, see *Proc. Natl. Acad. Sci. USA* 78, 5773–5777.

Estimation of Evolutionary Distances between Homologous Nucleotide Sequences

Motoo KIMURA

By using two models of evolutionary base substitutions—"three-substitution-type" and "two-frequency-class" models—some formulae are derived which permit a simple estimation of the evolutionary distances (and also the evolutionary rates when the divergence times are known) through comparative studies of DNA (and RNA) sequences. These formulae are applied to estimate the base substitution rates at the first, second, and third positions of codons in genes for presomatotropins, preproinsulins, and α- and β-globins (using comparisons involving mammals). Also, formulae for estimating the synonymous component (at the third codon position) and the standard errors are obtained. It is pointed out that the rates of synonymous base substitutions not only are very high but also are roughly equal to each other between genes even when amino acid-altering substitution rates are quite different and that this is consistent with the neutral theory of molecular evolution. For details, see *Proc. Natl. Acad. Sci. USA* 78, 454–458.

Some Calculations on the Amount of Selfish DNA

Tomoko OHTA and Motoo KIMURA

A population genetical theory is developed to treat the amount of selfish DNA in a genome. We assume that the selfish DNA consists of replicating units and that it evolves by multiplication within a genome, exchange between genomes, and random genetic drift at reproduction. Special reference is made to the mean and variance of the number of replicating units per genome in the population. Under the assumption of no systematic evolutionary pressure, the number of units changes randomly with time, and its variance increases by replication process. Although under certain cir-

cumstances the variance increases also by exchange process, under ordinary circumstances this process tends to decrease the variance. Random genetic drift also reduces the variance. The relationship between the mean and variance at equilibrium of the number of replicating units per genome in the population was derived. The analysis is extended to derive equations and simulate distributions to show how the numbers of tandem and dispersed repeat units of selfish DNA in a population change with time. It was found that whereas the mean number of clustered and dispersed units remains constant, the distribution of the population mean becomes increasingly dispersed with time. For details, see *Proc. Natl. Acad. Sci. USA* **78**, 1129–1132 and *Nature* **292**, 648–649.

Two-Locus Problems in Transmission Genetics of Mitochondria and Chloroplasts

Tomoko OHTA

A problem in transmission genetics was studied theoretically by regarding a cell as a population of organelle chromosomes. A hybrid cell of the two strains is assumed to consist initially of equal numbers of the two types of nucleoids, each containing an equal number of chromosomes. The two parent strains have different alleles at the two loci, and the two-locus dynamics of the hybrid cell line was analyzed. Within each nucleoid, stochastic matching and gene conversion are assumed to occur between the chromosomes, and at a certain rate, fusion and division are assumed to take place between the uncleoids within the cell. Under this model, the changes of average genotype frequencies at the two loci within a nucleoid and within a cell were formulated, and the final frequencies of recombinant chromosomes were obtained. Random segregation of nucleoids at cell division was incorporated into the analyses; in addition, the increase of homozygosity in the cell was formulated. The results may be useful for interpreting the observed data on organelle genetics of some species of algae and yeast. For details, see *Genetics* **96**, 543–555.

Genetic Variation in Small Multigene Families

Tomoko OHTA

In order to understand the evolution of genetic systems in which two genes

are tandemly repeated (small multigene family) such as has been recently found in the haemoglobin α loci of primates, haemoglobin β loci of mouse and rabbit and other proteins, a population genetics approach was used. Special reference was made to the probability of gene identity (identity coefficient), when unequal crossing-over is continuously occurring as well as random genetic drift, inter-chromosomal recombination and mutation. Two models were studied, cycle and selection models. The former assumes that unequal crossing-over occurs in cycles of duplication and deletion, and that the equilibrium identity coefficients were obtained. The latter is based on more realistic biological phenomena, and in this model it is assumed that natural selection is responsible for eliminating chromosomes with extra or deficient gene dose. Unequal crossing-over, inter-chromosomal recombination and natural selection lead to a duplication-deletion balance, which can then be treated as though it were a cycle model. The basic parameter is the rate of duplication-deletion which is shown to be approximately equal to $2(u+2\beta)X$, where u is the unequal crossing-over rate, 2β is the inter-chromosomal recombination rate and X is the frequency of chromosomes with three genes or of that with one gene. Genetic variation of the globin gene family, of which gene organization is known in most detail, is discussed in the light of the present analyses. For details, see *Genet. Res.* **37**, 133–149.

Genetic Variability and Rate of Gene Substitution in a Finite Population under Mutation and Fluctuating Selection

Naoyuki TAKAHATA

By using numerical method of solving stochastic difference equations, the level of gnetic variability maintained in a finite population and the rate of gene substitution under several models of fluctuating selection intensities were studied. It is shown that mutation and random genetic drift both play an important role in determining genetic variability and the rate of gene substitution. Compared with the case of neutral mutations, the fluctuation of selection intensity caused by temporal and spatial heterogeneity of environments generally increases the rate of gene substitution, but the level of genetic variability may be increased or decreased, depending upon the model and the parameters used. Although such a type of selection *per se* can not

be ruled out, when mutation is taken into account, it is difficult to explain both the observed amount of genetic variability and the rough constancy of evolutionary rate within a framework of fluctuating selection models. For details, see *Genetics* **98**, 427–440.

A Model of Evolutionary Base Substitutions and Its Application with Special Reference to Rapid Change of Pseudogenes

Naoyuki TAKAHATA and Motoo KIMURA

A model of evolutionary base substitutions that can incorporate different substitutional rates between the four bases and that takes into account unequal composition of bases in DNA sequences is proposed. Using this model, we derived formulae that enable us to estimate the evolutionary distances in terms of the number of nucleotide substitutions through comparative studies of nucleotide sequences. In order to check the validity of various formulae, Monte Carlo experiments were performed. These formulae were applied to analyze data on DNA sequences from diverse organisms. Particular attention was paid to problems concerning a globin pseudogene in the mouse and the time of its origin through duplication. We obtained a result suggesting that the evolutionary rates of substitution in the first and second codon positions of the pseudogene were roughly 10 times faster than those in the normal globin genes; whereas, the rate in the third position remained almost unchanged. Application of our formulae to histone genes H2B and H3 of the sea urchin showed that, in each of these genes, the rate in the third codon position is tremendously higher than that in the second position. All of these observations can easily and consistently be interpreted by the neutral theory of molecular evolution. For details, see *Genetics* **98**, 641–657.

A Mathematical Model of Extranuclear Genes and the Genetic Variability Maintained in a Finite Population

Naoyuki TAKAHATA and Takeo MARUYAMA

A mathematical theory of population genetics accounting for the genes transmitted through mitochondria or chloroplasts has been studied. In the model it is assumed that a population consists of N_m males and N_f females,

the genetic contribution from a male is β and that from a female $1-\beta$, and each cell line contains n effective copies of a gene in its cytoplasm. Assuming selective neutrality and an infinite alleles model, it is shown that the sum (H) of squares of allelic frequencies within an individual and the corresponding sum (Q) for the entire population are, at equilibrium, given by

$$\hat{H} \simeq \frac{1}{\left\{1+\rho \frac{(1-1/n)^{\lambda}}{1-(1-1/n)^{\lambda}} \frac{2N_e v\lambda}{1+2N_e v\lambda}\right\}(1+2nv)}$$

and

$$\hat{Q} \simeq \hat{H}/(1+2N_e v\lambda)$$

where $\rho=2\beta(1-\beta)$, $N_e=\{\beta^2/N_m+(1-\beta)^2/N_f\}^{-1}$, λ is the number of somatic cell divisions in one generation, and v is the mutation rate per cell division. If the genes are transmitted entirely through the female the formulae reduce to $\hat{H} \simeq 1/(1+2nv)$ and $\hat{Q} \simeq 1/\{1+(2N_e\lambda+2n)v\}$. Nonequilibrium behaviours of H and Q are also studied in the case of a panmictic population. These results are extended to geographically structured models, and applied to existing experimental data. For details, see *Genet. Res.* **37**, 291–302.

A Mathematical Study on the Distribution of the Number of Repeated Genes per Chromosome

Naoyuki TAKAHATA

I develop a mathematical model which can account for a distribution of the number of repeated genes per chromosome under the joint effects of sister chromatid exchange (*SCE*), inter-chromosomal crossing-over (*ICC*), and selection. The model can be applied not only to the cases of small gene clusters but also to multigene families. Based on this model, an appropriate mathematical formula is derived and used to obtain the equilibrium distribution. Assuming stabilizing selection and two simple schemes concerning *SCE* and *ICC*, I numerically calculate the equilibrium distribution and compare the result with observations on frequencies of single and triple α-haemoglobin genes in primates. It is also shown that if *SCE* and *ICC* occur according to the same probabilistic law, the distinction between them does not make much sense in the equilibrium distribution. For details, see *Genet. Res.* **38**, 97–102.

Algebra of Inclusive Fitness

Kenichi AOKI

Using the Cotterman k-coefficients in a one-locus two allele model, I show that under inbreeding Hamilton's rule ($br>c$) for the increase of an altruistic allele does not generally hold unless dominance is absent. If selection is weak in a random mating population, Hamilton's rule holds for altruistic interactions among any relatives of the same generation, not only for full sibs or other particular relationships as has been shown by previous authors. See *Evolution* **35**, 659–663 for particulars.

Estimating Evolutionary Distance from Restriction Maps of Mitochondrial DNA with Arbitrary G+C Content

Kenichi AOKI, Yoshio TATENO, and Naoyuki TAKAHATA

We develop a mathematical model for estimating evolutionary distance from restriction enzyme maps, which incorporates back and parallel mutation and non-uniformity of the rate of base substitution into the theory and allows for an arbitrary G+C content at equilibrium. When the G+C content differs significantly from 1/2, the traditional model of base changes can introduce a systematic bias even for phylogenetically close comparisons which depends upon the base composition of the restriction site. In addition, the accuracy of estimated evolutionary distance depends heavily upon the choice of restriction enzyme in that the expected number of sites is also affected. Monte Carlo experiments are conducted to check the validity of the present theoretical treatment and from which we draw several cautionary notes on estimation. An application is made to the available data on restriction enzyme maps of human mitochondrial DNA, and we suggest that Brown's estimate of racial differentiation may be an overestimate. See *J. Mol. Evol.* **18**, 1–8 for particulars.

One- and Two-Locus Models of the Origin of Worker Behavior in Hymenoptera

Kenichi AOKI and Michael MOODY

Hamilton suggested that, if females are singly inseminated, the asymmetries in intra-familial relationships inherent in haplo-diploidy should predispose

the Hymenoptera to evolve female sex-limited worker behavior, if either a worker raises sibs as reproductives but raises a bias of sisters (pathway 1), or she raises a combination of sibs and sons as reproductives but substitutes sons for brothers preferentially (pathway 2). To investigate these hypotheses when mating is random, we present mathematical and numerical analyses of the one-locus two-allele model of Charnov which we generalize. Furthermore, we extend the Charnov model to a more realistic two-locus situation where we postulate that the ability of a worker to raise a bias of sisters or to substitute sons for brothers preferentially is controlled by a locus separate from the locus controlling worker behavior, rather than being a pleiotropic effect of that locus. Considerations of the relative stringencies of the conditions for substitution of the allele for worker behavior and the constraints on linkage suggest that pathway 2 is more likely than pathway 1 for the origin of worker behavior. For pathway 1, we compare the Charnov model with its diplo-diploid analogue. This comparison reveals that, depending on the penetrance of the allele for worker behavior in the heterozygous state, both models permit the evolution of worker behavior even when workers raise the ratio of sisters to brothers laid by the queens, and that an advantage to haplo-diploidy becomes apparent only when workers raise a bias of sisters. For pathway 1, we also consider a generalization of the Craig model which is quite similar to the Charnov model. See *J. Theor. Biol.* **89**, 449–474 for particulars.

IX. POPULATION GENETICS (EXPERIMENTAL) AND EVOLUTIONARY GENETICS

Association of Chromosome- and Enzyme-Polymorphism in Natural Populations of *Drosophila melanogaster*

Yutaka INOUE, Kendo TSUNO and Takao K. WATANABE

The second chromosome of this species carries a lot of variations in nature. A chromosome inversion, *In(2L)t*, is frequently found as well as the standard sequence chromosome, *ST*. Enzyme loci, *Adh* and *α-Gpdh*, carry F and S alleles which are fairly frequent in nature. The frequencies of *In(2L)t*, Adh^S and $\alpha\text{-}Gpdh^F$ were surveyed in three Japanese populations; Katsunuma (Yamanashi), Akayu (Yamagata), Ishigaki (Okinawa) as shown in Table 1. Frequencies of these variations were yearly stable in each population. However, they were different from population to population. *Adh* and *α-Gpdh* loci are located in the left arm and two alleles (F, S) of these two loci are in linkage equilibrium. But these allozymes show significantly nonrandom association with *In(2L)t*. An almost complete linkage (97～98%) of *In(2L)t* with Adh^S and *In(2L)t* with $\alpha\text{-}Gpdh^F$ have often been confirmed by several natural populations.

The correlation coefficients between latitude and the frequencies of *In(2L)t*, Adh^S, $ST\text{-}Adh^S$ (=Standard chromosome with Adh^S), $\alpha\text{-}Gpdh^F$, $ST\text{-}\alpha\text{-}Gpdh^F$ were calculated by the data of Voelker *et al.* 1978, Knibb *et al.* 1981, Oakeshott *et al.* 1982, and the present study. A significantly negative correlation was observed in the cases for *In(2L)t* and Adh^S, but not for $ST\text{-}Adh^S$. On the other hand, it was significantly positive in the case for $ST\text{-}\alpha\text{-}Gpdh^F$, but not for $\alpha\text{-}Gpdh^F$. These results suggest that the two allozyme themselves may respond to the natural selection of each latitudinal population in different manner.

Table 1. The frequencies of *In(2L)t*, Adh^S and α-$Gpdh^F$ in three Japanese natural populations of *Drosophila melanogaster*. Sample size is given in parenthesis

Population		*In(2L)t*	Adh^S	α-$Gpdh^F$	Reference
Katsunuma	1969	.236 (123)	.466 (1050)	.847 (396)	Kojima, Gillespie and Tobari 1970
	1970	.180 (233)	.395 (263)	.833 (264)	Langley, Tobari and Kojima 1974
	1972	.125 (200)	.421 (197)	.777 (197)	Watanabe and Watanabe 1977
	1976	.115 (200)	.374 (240)	.749 (240)	The present study
	1979	.134 (232)	.341 (232)	.759 (232)	The present study
	1980	.147 (238)	.382 (238)	.761 (238)	The present study
	1981	.160 (244)	.361 (244)	.803 (244)	The present study
Akayu	1974	.263 (300)	.493 (215)	.874 (215)	Watanabe and Watanabe 1977
	1977	.225 (200)	.407 (240)	.916 (240)	The present study
Ishigaki-Jima	1973	.531 (81)	.744 (78)	.859 (78)	Watanabe and Watanabe 1977
	1974	.579 (252)	.784 (292)	.928 (292)	Watanabe and Watanabe 1977
	1976	.545 (200)	.841 (240)	.893 (240)	The present study

Typical Hybrid Dysgenesis is Absent from the Natural Population of *Drosophila melanogaster* in Japan

Akihiko YAMAMOTO and Takao K. WATANABE

Hybrid dysgenesis was investigated in a natural population of Japanese *Drosophila melanogaster*. Hybrid females with rudimentary ovaries emerge from some interstrain crosses, when they are developed under high temperature, e. g., 29°C. These gonadal dysgenesis are observed in one of the two reciprocal crosses, in which paternal strains are called "P" strains and maternal ones are referred to as "M" strains. Most strains from natural populations in U. S. A. and Australia were judged P by Kidwell *et al.* (1977), since males of them caused dysgenic daughters when they were mated with females from the M strain.

However, 46 isofemale strains from Katsunuma, Japan (strains kept in our laboratory for 6 months at 18°C), did not cause dysgenesis both when males of them were mated with the M strain and females with the P strain. Then, all of these strains were judged "Q" strains (Engels and Preston, 1981) and the nature of the wild population at Katsunuma differred apparently from the populations in U. S. A. and Australia.

Among 11 long-established laboratory strains (8 to 30 years) derived from Japan, 6 were M strains and others were Q or possibly in the intermediate state between Q and M. Therefore, Q strains discovered at Katsunuma will turn into M when they are kept in the laboratory.

Biochemical Phylogeny of the *Drosophila montium* Species Subgroup

Seido OHNISHI, Ki Won KIM and Takao K. WATANABE

The *D. montium* species subgroup to which 61 species belong is the biggest subgroup in the *D. melanogaster* species group (Tsacas, 1979). Recently, Baimai (1980) surveyed karyotypes of twenty species including new species such as *D. leontia* and *D. bocki*, and found much variation in the fourth and Y chromosomes, showing that most of the species can be distinguished by the karyotypes. Although morphological characters (sexcombs and genitalia of males) as well as karyotypes are useful tools for the qualitative identification of these species, quantitative relationships (e. g. genetic distances) between these species have never been shown in this subgroup.

In this study, a phylogenic tree among 21 members (14 different species) of the subgroup was constructed, based on the data set obtanied by two-dimensional electrophoresis (2DE). The results are as follows; The 21 members were classified into four groups. The first group is the *D. kikkawai* complex to which six species belong (*D. bocki*, *D. pennae*, *D. kikkawai*, *D. leontia*, *D. lini* and *D. barbarae*). The second one includes three species, *D. jambulina*, *D. punjabiensis-like* and *D. punjabiensis*, whose systematics was reported in Jap. J. Genet. (in press) in more details. The third one is the *D. auraria* complex including four species (*D. auraria*, *D. biauraria*, *D. triauraria* and *D. quadraria*). The last one is *D. rufa* which is distantly related to the other species. However, an analysis of starch gel electrophoresis showed that *D. rufa* was more closely related to the *D. kikkawai* complex, and that only *D. jambulina* was classified into a separate group. In the 2DE analysis, it was found that *D. quadraria* was very similar to *D. triauraria*, although these two species are different in geographical distribution. It suggests that *D. quadraria* seems to be a variation of *D. triauraria*. In addition, an unclassified species which had been collected on Iriomote Island (Japan) in 1979 was found to be the same as *D. bocki* in the 2DE analysis. This was confirmed by an interspecific hybridization between them. Thus, it was evidenced that these electrophoretic techniques are useful as adjunctive tools in the study of systematics.

X. HUMAN GENETICS

Retinoblastoma: Mutational Mosaicism or Host Resistance?

Ei MATSUNAGA

Carlson and Desnick's proposals (Am. J. Med. Genet. 4: 365–381, 1979) of multiple allelism and mutational mosaicism to account for the observed variability in penetrance and expressivity in retinoblastoma do not seem to give a clear-cut explanation for the facts that 1.) a bilaterally affected patient could have an unaffected carrier child who in turn produced bilaterally affected offspring, and 2.) carriers of the retinoblastoma gene are prone to non-radiogenic osteosarcoma irrespective of their phenotypes. However, these observations are readily explained by assuming that inherited host resistance plays an important rôle in the manifestation of a single major gene for retinoblastoma. Based on their model, Carlson and Desnick offer recurrence risk figures for genetic counseling, but their figures concerning the transmissible types of retinoblastoma do not seem more accurate, as assumed by them, than those previously estimated from the family data.

We present evidence that *host resistance* may be regarded as a multifactorial threshold character with high heritability, and we propose that tissue-specific genes, which we presume to be involved in normal differentiation of retinoblasts and osteoblasts, play a crucial rôle in malignant transformation. Two lines of suggestions are made for future studies: one, to search for polymorphic genes associated with retinoblastoma, and the other, to see whether the principle of almost synchronous appearance of multiple tumors in persons at high risk applies to other cancers as well. For details, see Am. J. Med. Genet. 8: 375–387, 1981.

Genetics of Wilms' tumor

Ei MATSUNAGA

The analyses of published data for Wilms' tumor show that the genetics and genesis of this tumor is essentially similar to that of retinoblastoma. Main results obtained are as follows:

1) The mode of inheritance of familial cases, which constitute less than 1% of all Wilms' tumors, is autosomal dominant with variable penetrance and expressivity. The familial pattern reveals no evidence that this tumor is associated with a vertically transmitting tumor virus.

2) There is a significant correlation between the first-degree relatives affected with Wilms' tumor in regard to expressivity as measured by the proportion of bilaterally affected or by the age of the patients at diagnosis. In particular, expressivity in the children affected varies consistently with expressivity in the carrier parents, indicating that inherited host resistance plays an important role in the manifestation of the major gene. The average risk of developing Wilms' tumor for offspring of unaffected carriers, who may be regarded as inherently resistant to tumor formation, is estimated at about 0.30. Although the data are limited, the risk for offspring of survivors of hereditary unilateral or bilateral cases may be as high as 0.40 or more. The data from seven pairs of monozygotic twins, including the discordant one associated with del (11p) and aniridia, are consistent with the host resistance model.

3) In sporadic cases, the proportion of bilateral involvement is about 3%, in contrast with 20% in the familial cases. For bilateral cases, however, the distribution of ages of the patients at diagnosis is virtually the same whether sporadic or familial, and in two-thirds of the cases the disease is already bilateral when the first tumor is noted. These findings suggest that bilateral cases are probably always hereditary. Ages of the patients at diagnosis of sporadic unilateral cases tend to be much higher than in the patients with familial unilateral cases, indicating that most sporadic unilateral cases are nonhereditary. The overall risk for survivors of sporadic unilateral Wilms' tumor to transmit the disease to children may be as low as 2~4%. For details, see Hum. Genet. **57**: 231–246, 1981.

The "loss" of Kinetochores from Chromosomes of Aged Individuals

Yasuo NAKAGOME and Tatsuo ABE[1)]

An increase in the proportion of aneuploid cells in aged women is well known (Jacobs, P. A. *et al.* Ann. Hum. Genet. **27**: 353, 1964). A phenomenon called premature centromere division (PCD) also increases with ad-

1) Dept. of Internal Med., Kyoto Prefectural Univ. Kyoto.

vancing age (Fitzgerald, P. H. *et al.* Ann. Hum. Genet. **38**: 417, 1975). It is also widely known that the frequency of meiotic non-disjunction increases with advancing maternal age resulting in both autosomal trisomies and a few numerical sex chromosome abnormalities (Trimble, B. K. and Baird, P. A. Amer. J. Med. Genet. **2**: 1, 1978). Causes of these phenomena have not been known.

In 1974, Eiberg (Nature **248**: 55, 1974) described a technique called Cd banding which stains two small dark spots on both sides of the kinetochore. Cd denotes a centric dot; however, there was a controversy as to whether it, in fact, represented the kinetochore (Roos, U. P. Nature **254**: 463, 1975). Later, it was established that a positive Cd reaction represented the presence of an active kinetochore and a negative reaction either its inactivation (supression) or loss (Nakagome, Y. *et al.* Clin. Genet. **9**: 621, 1976; Daniel, A. Hum. Genet. **48**: 85, 1979; Nakagome Y. this report, **30**: 97, 1980; Maraschio, P. *et al.* Hum. Genet. **54**: 265, 1980).

In the present study, the Cd-band technique was applied on mitotic spreads obtained from aged women. Blood samples were obtained from 14 females over 60 years of age (66 to 87y, average=78y4m). Two aged males, 84 and 90 years, were also examined for comparison. In Cd-stained preparations, each chromosome showed small dots on both sides of the kinetochore. To avoid effects of possible technical variables, only cells in which all 6 members of A-group chromosomes showed positive Cd spots were included in the study. The remaining 40 chromosomes, B through G groups, were scored except for those overlapping at the kinetochore. For comparison, some of the preparations were treated by either the C-band technique or the distamycin-DAPI (dD) method (Schweizer, D. *et al.* Exp. Cell Res. **111**: 327, 1978). Only cells in which all 6 members of the A group showed a distinct constriction at the kinetochore were included in the study. The rest of the chromosomes were examined to determine whether both chromatids were separated or attached at the kinetochore.

In aged individuals, 56 were Cd negative out of a total of 6476 examined chromosomes. In controls, 11 were Cd negative out of a total of 3057. The difference was highly significant ($X^2=7.6$, d. f.$=1$, $p<0.01$). Fourty seven of the former and nine of the latter assumed parallel-bar appearance. The rest showed constriction at the kinetochore although they were Cd negative. Thirty one of the Cd-negative chromosomes belonged to the C group which was higher than the expected 22.4. Six of the 14 aged females

were examined by either the C or the dD method. Thirteen out of a total of 2080 chromosomes showed separation of their kinetochores. In 11 controls, only 7 showed separation out of 5080 chromosomes examined. The difference was highly significant. In two aged males, 8 Cd-negative chromosomes were detected out of 1426 examined. The frequency (0.0056) was between the levels of aged females (0.0087) and control females (0.0036).

It was concluded that the kinetochore tended to deteriorate and lose Cd-positive material in aged individuals implicating its causative relationship with the increase of both aneuploid cells in mitoses and possibly non-disjunction in meioses.

XI. BEHAVIORAL GENETICS AND ECOLOGICAL GENETICS

The Behavioral Changes in Mice Continuously Reared under Noise Condition*

Tohru FUJISHIMA

Mice continuously reared under noise conditions have been examined for the behavioral changes in comparison with those reared under normal, or non-noise conditions as a control. The mice of the two groups were originally derived from the same full sib families. They were kept at 25°C with 12 hour light and 12 hour darkness. The mice in the noise treated group were exposed six times to noise (pink, 100 phon) for one hour each at one hour interval during the dark period (6 p. m. to 6 a. m.). The avoidance and discriminatory learning performances of mice approximately 70 days old were measured with an automated Y-type maze apparatus. Their activity and body weight were also recorded.

The result of the fourth generation obtained this year showed significant differences in avtivities and body weights between the treated and the control groups, coinciding with the result of the previous generation. This suggested that the effect of noise might arouse the emotionality of mice. Then, to examine whether mice recover from the effect of noise by their removal from noise to normal conditions, a portion of the mice of the noise treated group was removed to the normal condition at birth. The result showed that no behavioral change occurred by the removal, indicating no recovery from noise effects. These results suggested that the behavioral changes occurred in the noise treated group might have resulted from the effect of noise given to mice before birth.

Weed-Mediated Natural Selection in a Segregating Population of Rice

Hiroko MORISHIMA

The response of rice to weeding or weed tolerance is known to differ according to varieties, hence it is genetically controlled. In an attempt to look

* This work was supported by a grant from the Ministry of Environment.

into the effect of natural selection imposed by weeds, a segregating population derived from a cross between Indica and Japonica rice varieties was divided into three parts, and one was grown in a weed-free condition while the other two were reared with weeds (*Echinochloa crus-galli*, barnyard grass, and *Cyperus difformis*, a sedge, were respectively sown in the first year). After two successive generations of natural selection, twenty successful plants were selected from each plot, and their progeny lines were tested in the above three conditions, respectively. The tolerance to weed was evaluated by the difference in log-transformed plant weight of rice between weed-free and sedge-sown plots, or between weed-free and barnyard grass-sown plots. The results showed that lines from weedy plots had higher tolerance to weed those from weed-free plots. Although the lines varied in a wide range, the analysis of variance of line-group means proved that the differences among the three treatments were highly significant. Three lines from the sedge plot gave a higher performance with sedge than in weed-free condition. Although this experiment was incomplete, the data proved that plants were rapidly selected for the ability to coexist with their neighboring plants. This may promote niche differentiation between coexisting species in a community, resulting in genotypic differences in niche dimension within species populations.

Demography and Populational Variability of *Rumex obtusifolius* Conditioned by Biotic Environment

Kenji TERAI[1] and Hiroko MORISHIMA

To gain an understanding of the effect of biotic environment on population dynamics of plant species, five populations of a perennial weed, *Rumex obtusifolius*, were observed with regard to emergence, mortality, density, growth performance, and some other traits. Composition of coexisting species in the community was also observed. The biotic environment was represented by the diversity of life-form in the coexisting species. Life-form of each species was categorized based on its reproductive system and plant form. Each study-site was taken so as to cross over zones differing in the intensity of habitat disturbance and other conditions, for instance, covering dike of arable land, grassland and roadside. Various demographic parameters as well as measurements of community diversity were compared

1) Faculty of Education, Akita University.

among sub-sites within site. We found that mortality and recruitment rates, standing density, distributional pattern of *R. obtusifolius* differed within a site according to the community diversity. Inter-plant variability of plant size of *R. obtusifolius* tended to be larger in the community with medium degree of diversity than in either of the community with high and low diversity. It was also found that *R. obtusifolius* and its two relatives, *R. acetosa* and *R. acetosera*, had differential zones of distribution along the variation in community diversity.

Further, germination behavior under different temperatures, characteristics of seed morphology and various traits of seedlings grown in a uniform condition were studied, using seed samples of 30 populations collected on an individual basis from various habitats, such as waste places or roadside in town, dikes of crop fields, and semi-natural grassland. Variation in each trait was partitioned into between- and within-populations, as well as into between- and within- (between progeny plants derived from a mother) individuals in a population. Low-temperature response of germination behavior varied between and within populations and its variation pattern seemed to reflect proportion of bare-land in the collection site (degree of vegetational saturation). Populations collected in town showed larger inter-populational and smaller intra-populational variation in many characters than those collected in semi-natural sites outside town, suggesting that the populations distributed in strongly disturbed habitats have higher selfing rate than those in less-disturbed habitats.

The above observations indicate that population dynamics is, at least partly, conditioned by the intensity of habitat disturbance and by consequently resulted changes in biotic environment. To what extent genetic variation is related to interaction with biotic environment remains unsolved.

Isozyme Variation in *Altingia* Forest at Ciwidey, West Java

Toru Endo

Nature of tropical natural forests has been characterized with high species diversity and low genetic diversity per unit area from the view point of plant ecology. The genetic diversity per unit number of any plant species, however, must not always be similar or low, because of much more favorable environmental conditions for various mutants in tropical zone than those in temperate or arctic zones. Such a problem has not yet been examined

in tropical forests.

From February 3 to 5, 1982, a teem of five Japanese members and three staffs of Tropical Forest Biology Section of BIOTROP, Bogor, conducted a study in natural forests of *Altingia excelsa* at the foot of Mt Patuha, Ciwidey. Thirty eight trees of *Altingia* at Plot 2 (50 × 50 m) were mapped and characterized, and leaves of all *Altingia* trees, including three poles and one sapling were collected for estimation of morphological characters as well as for the zymographic analysis of peroxidase isozymes.

Using the zymograms from 38 trees totally 703 combinations were carried out, and disagreement number counts (DNC) were obtained in each tree. The DNC ranged from 2.53 to 5.79, and the mean was 3.44. Although the locus number of the isozymes is not estimated, the value of *Altingia* was unexpectedly low, being compared with the value 12.99 from a sample plot of *Thujopsis dolabrata* at Aomori (Sakai and Miyazaki 1972, Silv. Genet. **21**: 5).

Cross Resistance to Organophosphorous Insecticides in *Drosophila*

Yutaka Inoue

From the Tochigi population of *D. melanogaster*, a Diazinon resistant strain (TG20) and a Sumithion resistant strain (TG44) were obtained. Adult survival tests against Diazinon (40 ppm, 24 hrs), Sumithion (80 ppm, 48 hrs) and DDT (0.25%, 2 hrs) were carried out using the TG20 and TG44 strains and additional DDT-resistant (Hikone-R), Ether-resistant (Eth-29) and Oregon-R (OR) strains. The results are shown in Table 1. OR and Eth-29 were not resistant to any insecticide. TG44 and Hikone-R were resistant to both Sumithion and DDT. TG20 showed moderate resistance to Sumithion and DDT. However, there was no strain showing strong resistance to all three insecticides. The tendency of cross resistance was observed between different insecticide-resistant strains. Hikone-R has never been exposed to Sumithion, but it was resistant to Sumithion as well as to DDT.

Flies were cultured in the food containing Diazinon (30 ppm) or Sumithion (45 ppm) for long generations. Diazinon-treated flies showed significant Diazinon-resistance at the 12th generation, and they also showed Sumithion-resistance at the 24th generation. Sumithion-treated flies were significantly Sumithion-resistant at the 24th generation and their Diazinon-resistance

Table 1. The comparison of cross-resistances of the several strains to DDT, Diazinon and Sumithion. Number of tested females in each experiment were 80 for DDT, and 60 for Diazinon and Sumithion

Treatment	Mortality				
	TG20	TG44	Hikone-R	OR	Eth-29
DDT	0.26	0.16	0.16	0.69	0.84
Diazinon	0.50	0.75	1.00	1.00	1.00
Sumithion	0.46	0.08	0.12	1.00	1.00

was observed at the 36th generation. Thus, the corss resistances seemed to be grown up in a few generations after the occurrence of the drug specific resistance. The amount of deleterious genes maintained in the resistant populations was compared. Diazinon(30 ppm)-treated and Sumithion (45 ppm)-treated populations did not show any difference from a control population in the frequency of lethal, semilethal and sterile genes on the X and the second chromosomes.

XII. APPLIED GENETICS

Numerical Evaluation of the Indica-Japonica Differentiation in Rice Varieties

Hiroko MORISHIMA and Hiko-ichi OKA[1)]

Multivariate analysis of the data for 11 characters of 89 cultivars of *Oryza sativa* sampled at random from a large collection of Asian origin has reconfirmed the previous assertion by the junior author that the Indica-Japonica differentiation of rice cultivars is recognizable on the basis of character-association patterns, although there is no key character discriminating between the two types. The probability of misclassification by a single character ranged from about 3 percent by the potassium chlorate resistance of seedling to about 40 percent by the length/width ratio of spikelets. Discriminant functions combining 3 or 4 character values gave lower probabilities of misclassification, although there still remained a few intermediate forms having recombined characteristics. The F_1 sterility relationships were almost useless for the purpose of classification. About a half of the total character variance among the cultivars was attributable to Indica-Japonica differentiation. The normalized genetic identity between the two types was estimated to be 0.62 from data for 28 alleles at 12 loci. A great number of genic differences seem to be involved in the Indica-Japonica differentiation. For details, see Japan. J. Breed. **31**: 402–413.

Associative Nitrogen Fixation of Rice Plants with Isolated Bacteria

Taro FUJII and Koki OHTA

In our previous experiment, bacterial inoculation onto germ-free seedlings growing in the test tube filled with sterilized soil has been carried out to investigate the interaction of rice and bacterial strains in associative nitrogen fixation. Two strains of nitrogen fixing bacteria isolated from rhizosphere soil, COC-8 (*Azospirillum lipoferum*) and NG-13 (*Klebsiella oxytoca*) showed appreciable acetylene reduction activity (ARA). In the present experiment,

[1)] Chung Hsing University, Taichung, Taiwan

synthesized medium was used for systematic study of nitrogen fixation by association of rice plants with particular bacterial species. Medium used is basically the same as White's but nitrogen and carbon sources have been omitted since nitrogen source inhibit nitrogenase activity and bacterial growth is promoted by carbon source.

Germ-free seedlings growing in the medium for one week after germination were inoculated with COC-8 and NG-13. ARA was measured weekly intervals during one to 4 weeks after inoculation. ARA was increased up to 3 weeks after inoculation and decreased the 4th week. Number of inoculated bacteria was also measured. Sudden increase in number was observed one week after inoculation, namely about 10^2 inoculum was reached to order of 10^7, and almost same number was maintained up to 4th week. On the other hand, seedling growth was arrested about 4 weeks after germination due to lack of nitrogen and carbon sources in the medium. The results indicated that the inoculated bacteria propagated by supply of carbon source(s) excreated from roots, exhibited nitrogenase activity. However, when plant growth stopped ARA decrease because of the lost of the supply of carbon source.

To sustain the seedling growth, a small amount of KNO_3 and $Ca(NO_3)_2$, equivalnt to one-tenth of White's basal medium was added to the medium and same experiment was again carried out. When a small amount of nitrogen is added to the medium slightly better seedling growth occurs and there was very little change in the ARA. Exploitation of modified medium for much better plant growth is now being studied.

Diallel Analysis of Nitrogen Fixation in the Rhizosphere of Rice

Shinya IYAMA, Yoshio SANO and Taro FUJII

The genetics of nitrogen fixing ability in the rhizosphere of rice was investigated by diallel analysis. Diallelic crosses were made among 11 strains of rice chosen from the collection which was kept at the Genetic Stock Center of the Institute. They were chosen to cover a wide range of origins from South Asia to Japan. F_1 plants and their parent strains were grown individually in a small plastic pot containing 180 g of soil. Nitrogen fixing activity in the rhizosphere of them were measured by the acetylene reduction method with the aid of gas chromatography. The measurement was done

at the heading time of each plant.

In an 11×11 diallel set, presence of non-allelic interaction was revealed. Excluding the interacting parents, a 7×7 diallel set which fitted to the additive-dominance model indicated the average degree of dominace being partial to complete dominance and the excess of dominant alleles over recessive alleles. Relationship between parental performance and (Wr+Vr) value calculated from the diallel table suggested that there was no difinite relation between nitrogen fixing activity in the rhizosphere and direction of dominance but that of the highest parent was likely governed by recessive alleles. Positive correlation between nitrogen fixing activity of parent and that of F_1 was statistically significant ($r=0.88$). It was concluded from the above results that the nitrogen fixing activity in the rhizosphere are inherited to the progeny through hybridization and can be improved by selection among the progeny of hybridization.

Genetic Variation of Herbicide Tolerance among F_5 Lines of Barnyard Grass

Shinya IYAMA

Crosses between barnyard grass (*Echinochloa crus-galli*) strans of different tolerance to propanil were made and kept by single seed decent procedure from F_2 to F_4. Their F_5 lines were examined for the tolerance to propanil. Seedlings of 2 weeks old were sprayed by a given amount of propanil solution and the number of plants survived in each line was counted after 2 weeks. The result indicated that the segregation regarding the tolerance was found among the F_5 lines. It was observed in most cross combinations that tolerance of some lines exceeded the range of the parents, suggesting that more tolerant genotypes may appear through recombination of responsible genes among progenies of natural hybridization in a field.

BOOKS AND PAPERS PUBLISHED IN 1981 BY STAFF MEMBERS

AMANO, E. 1981: Genetic and biochemical characterization of waxy mutants in cereals. Environmental Health Perspectives **37**: 35–41.

AMANO. E. 1981: Flow system for automated analysis of maize pollen. Environmental Health Perspectives **37**: 165–168.

AOKI, K. and M. MOODY* 1981: One- and two-locus models of the origin of worker behavior in Hymenoptera. J. Theor. Biol. **89**: 449–474.

AOKI, K. 1981: Algebra of inclusive fitness. Evolution **35**: 659–663.

AOKI, K., Y. TATENO* and N. TAKAHATA 1981: Estimating evolutionary distance from restriction maps of mitochondrial DNA with arbitrary G+C content. J. Mol. Evol. **18**: 1–8.

ENDO, T. 1981: Differential regulation of peroxidase isozymes coded by *Px-1* locus in rice. Jap. J. Genet. **56**: 175–183.

ENDO, T. 1981: Developmental modification and hybridization of allelic acid phosphatase isozymes in homo- and heterozygotes for the *Acp*-1 locus in rice. Biochem. Genet. **19**: 373–384.

FUJII, T. 1981: Mutagenic effect of L-ethionine in soybean and maize. Exp. Environ. Botany **21**: 127–131.

FUJISAWA, T. and C. N. David* 1981: Commitment during nematocyte differentiation in *Hydra*. J. Cell Sci. **48**: 207–222.

HIROTA, Y., M. YAMADA, A. NISHIMURA, A. OKA*, K. SUGIMOTO*, K. ASADA* and M. TAKANAMI* 1981: The DNA replication origin of *Escherichia coli*: Structure and function of the *ori*-containing DNA fragment. Progress Nucleic Acid Res. Mol. Biol. **26**: 33–48.

SATO, C*., K. KOJIMA*, K. NISHIZAWA* and Y. HIROTA 1981: Cell surface charge and cell division in *Escherichia coli* after X irradiation. Radiation Research **87**: 646–656.

UTSUMI, R*., H. TANABE*, Y. NAKAMOTO*, M. KAWAMUKAI*, Y. SAKAI*, M. HIMENO*, T. KOMANO* and Y. HIROTA 1981: Inhibitory effect of adenosine 3′ 5′-phosphate on cell division of *Escherichia coli* mutant derivatives. J. Bacteriol. **147**: 1105–1109.

IMAI, H., Y. MATSUDA*, T. SHIROISHI and K. MORIWAKI 1981: High frequency of X-Y chromosome dissociation in primary spermatocytes of F_1 hybrids between Japanese wild mice (*Mus musculus molossinus*) and inbred laboratory mice. Cytogenet. Cell Genet. **29**: 166–175.

HIRAI, H*., Y. SAKAGUCHI* and H. IMAI 1981: C-band polymorphism in a Japanese lung fluke *Paragonimus ohirai* (Trematoda; Platyhelmintes). Heredity **47**: 249–252.

INOUE, T. 1981: Ataxia-telangiectasia. Jap. J. Genet. **56**: 315–334.

INOUE, T., K. MORITA* and T. KADA 1981: Purification and properties of a plant desmutagenic factor for the mutagenic principle of tryptophan pyrolysate. Agric. Biol. Chem. **45**: 345–353.

INOUE, T., S. OTA*, Y. SADAIE and T. KADA 1981: Effect of cobaltous chloride on spontaneous mutation induction in a *Bacillus subtilis* mutator strain. Mutation Res. **91**: 41–45.

INOUE, T., A. YOKOIYAMA and T. KADA 1981: DNA repair enzyme deficiency and *in vitro* complementation of the enzyme activity in cell-free extracts from ataxia telangiectasia fibroblasts. Biochimica et Biophysica Acta **655**: 49–53.

KADA, T. and H. MOCHIZUKI 1981: Antimutagenic activities of human placental extract on ultraviolet light- and gamma-ray-induced mutation in *Escherichia coli* WP 2B/r *trp*. J. Radiat. Res. **22**: 297–302.

LEIFER, Z*., T. KADA, M. MENDEL*, E. ZEIGER*, R. STAFFORD* and H. S. ROSENKRANZ* 1981: An evaluation of tests using DNA repair-deficient bacteria for predicting genotoxicity and carcinogenicity. Mutation Res. **87**: 211–297.

KARUBE, M*., T. MATSUNAGA*, T. NAKAHARA*, S. SUZUKI* and T. KADA 1981: Preliminary screening of mutagens with a microbial sensor. Anal. Chem. **53**: 1024–1026.

KAWANISHI, M. and T. K. WATANABE 1981: Genes affecting courtship song and mating preference in *Drosophila melanogaster*, *Drosophila simulans* and their hybrids. Evolution **35**: 1128–1133.

OHNISHI, S. and M. KAWANISHI 1981: The sexcomb of *Drosophila simulans*: Geographical variation and genetic analysis. Kontyû **49**: 37–44.

KIMURA, M. 1981: Estimation of evolutionary distances between homologous nucleotide sequences. Proc. Natl. Acad. Sci. USA **78**: 454–458.

KIMURA, M. 1981: Was globin evolution very rapid in its early stages?: a dubious case against the rate-constancy hypothesis. J. Mol. Evol. **17**: 110–113.

KIMURA, M. 1981: Doubt about studies of globin evolution based on maximum parsimony codons and the augmentation procedure. J. Mol. Evol. **17**: 121–122.

KIMURA, M. 1981: Possibility of extensive neutral evolution under stabilizing selection with special reference to nonrandom usage of synonymous codons. Proc. Natl. Acad. Sci. USA **78**: 5773–5777.

SUZUKI, T*. and Y. KOMEDA 1981: Incomplete flagellar structures in *Escherichia coli* mutants. J. Bacteriol. **145**: 1036–1041.

KURODA, Y. (ed.) 1981: Genetic Experiments Using Cultured Cells. (in Japanese) Kyoritsu-Shuppan (Tokyo), 380p.

KURODA, Y. 1981: *In vitro* cells. In "Ageing of Cells" (ed. by Tauchi & Kuroda). (in Japanese) Kyoritsu-Shuppan (Tokyo), pp. 121–217.

MARUYAMA, T. and E. SOEDA 1980: Molecular evolution in papova viruses and their host species, and in bacteriophages. Proc. Stadler Genetics Symp. **12**: 83–96.

MARUYAMA, T. and N. TAKAHATA 1981: Numerical studies of the frequency trajectories in the process of fixation of null genes at duplicated loci. Heredity **46**: 49–57.

MARUYAMA, T. and H. IMAI 1981: Evolutionary rate of the mammalian karyotype. J. Theor. Biol. **90**: 111–121.

MARUYAMA, T. 1981: Stochastic problems in population genetics: Applications of Itô's stochastic integrals. In "Stochastic Nonlinear Systems in Physics, Chemistry, and Biology" (eds. L. Arnold and R. Lefever), 154–161. Springer-Verlag, Berlin.

MARUYAMA, T. and M. NEI 1981: Genetic variability maintained by mutation and over-

dominant selection in finite populations. Genetics **98**: 441–459.

MATSUNAGA, E. 1981: Genetics of Wilms' tumor. Hum Genet. **57**: 231–246.

MATSUNAGA, E. 1981: Retinoblastoma: Mutational mosaicism or host resistance? Am. J. Med. Genet. **8**: 375–387.

URUSHIBARA, T*., C. NISHIMURA* and **K. MIURA** 1981: Process of cap formation of messenger RNA by vaccinia virus particles carrying an organized enzyme system. J. gen. Virol. **52**: 49–59.

URUSHIBARA, T*., K. OKAMOTO*, C. NISHIMURA, T. KAMIMURA*, Y. OZAKI*, K. YAMAGUCHI*, T. HATA* and **K. MIURA** 1981: Methylation of the 5′-5′ confronting dinucleotides by vaccinia associated enzyme system. Nucleic Acids Res. Symp. Ser. No. **10**: 237–240.

INOUE, A*., M. NAKAMURA, S. NAKANISHI*, S. HIDAKA*, **K. MIURA** and S. NUMA* 1981: 5′-Terminal nucleotide sequence of the messenger RNA coding for bovine corticotropin/β-lipotropin precursor. Europ. J. Biochem. **113**: 531–539.

MORISHIMA, H. and H. I. OKA 1981: Phylogenetic differentiation of cultivated rice, XXII. Numerical evaluation of the Indica-Japonica differentiation. Jap. J. Breed. **31**(4): 402–413.

ASSEMAT, L*., **H. MORISHIMA** and H. I. OKA 1981: Neighbor effects between rice (*Oryza sativa* L.) and barnyard grass (*Echinochloa crus-galli* Beauv.) strains, II. Some experiments on the mechanisms of interaction between plants. OEcol. Plant. **2**(16), No. **1**: 63–78.

MORIWAKI, K., H. YONEKAWA*, M. MINEZAWA*, Y. MATSUDA, T. SHIROISHI and N. MIYASHITA 1981: Genetic status of Japanese wild mouse, Mus musculus molossinus. Hereditas **94**: 15.

MIZUOCHI, T*., Y. YAMASHITA*, T. HAMAOKA* and **K. MORIWAKI** 1981: The role of Ia antigens in the activation of T cells to concanavalin A. Cell. Immunol. **57**: 28–41.

SUZUKI, S*., K. TSUJI* and **K. MORIWAKI** 1981: Friend murine leukemia virus resistance in Japanese wild mice: Possible allelism with Fv-4 in FRG mice. J. Nat. Cancer Inst. **66**: 729–731.

ODAKA, T*., H. IKEDA*, H. YOSHIKURA*, **K. MORIWAKI** and S. SUZUKI* 1981: Fv-4: Gene controlling resistance to NB-tropic murine leukemia virus. Distribution in wild mice, introduction into genetic background of BALB. C mice and mapping of chromosomes. J. Nat. Cancer Inst. **67**: 1123–1127.

MINEZAWA, M*., **K. MORIWAKI** and K. KONDO* 1981: Geographical survey of protein variations in wild populations of Japanese house mouse, Mus musculus molossinus. Jap. J. Genet. **56**: 27–39.

IKAWA, Y*., G. KIYAMA*, K. SUDO*, K. SUZUKI* and **K. MORIWAKI** 1981: Strain-specific appearance of a possible fetal a-globin polypeptide chain in mice. Develop., Growth and Differ. **23**: 1–8.

YONEKAWA, H*., **K. MORIWAKI**, O. GOTOH,* J. HAYASHI*, J. WATANABE*, N. MIYASHITA, M. L. PETRAS* and Y. TAGASHIRA* 1981: Evolutionary relationships among five subspecies of *Mus musculus* based on restriction enzyme cleavage patterns of mitochondrial DNA. Genetics **98**: 801–816.

SHIROISHI, T., T. SAGAI and **K. MORIWAKI** 1981: A simplified micro-method for cyto-

xicity testing using a flat-type titration plate for the detection of H--2 antigens. Microbiol. Immunol. **25**: 1327–1334.

MATSUBARA, T.*, K. IINUMA*, Y. NAKAGOME and T. YOKOCHI* 1981: Familial case of Down's syndrome. A psu dic (21) (q 22) and a rob (14q21q) in cousins. Jpn. J. Hum. Genet. **26**: 55–59.

TAKEDA, Y.*, A. NISHIMURA, Y. NISHIMURA, M. YAMADA, S. YASUDA, H. SUZUKI* and Y. HIROTA 1981: Synthetic ColEl plasmids carrying genes for penicillin-binding proteins in *Escherichia coli*. Plasmid. **6**: 86–98.

STERNGLANZ, R.*, S. DINARDO*. K.A. VOELKEL*, Y. NISHIMURA, Y. HIROTA, K. BECHERER*, L. ZUMSTEIN* and J. C. WANG* 1981: Mutations in the gene coding for *Escherichia coli* DNA topoisomerase I affect transcription and transposition. Proc. Natl. Acad. Sci. USA **78**: 2747–2751.

NOGUCHI, T. 1981: Mouse teratomas. (in Japanese) Gan to kagakuryoho **8**: 979–988.

OZAWA, M*., J. FUJIYAMA*, T. FURUKAWA*, K. SATO*, T. NOGUCHI and T. MURAMATSU* 1981: Glycoproteins from teratocarcinomas and organs of adult mice: Analysis by affinity chromatography on lectin-conjugated agarose. Develop. Growth and Differ. **23**: 515–522.

OHTA, T. 1980: Two-locus problems in transmission genetics of mitochondria and chloroplasts. Genetics **96**: 543–555.

OHTA, T. 1981: Genetic variation in small multigene families. Genet. Res. **37**: 133–149.

OHTA, T. 1981: Population genetics of selfish DNA. Nature **292**: 648–649.

OHTA, T. and M. KIMURA 1981: Some calculations on the amount of selfish DNA. Proc. Natl. Acad. Sci. USA **78**: 1129–1132.

SADAIE, Y., T. INOUE, H. MOCHIZUKI and T. KADA 1981: Efficiencies of DNA Inactivation and mutation induction by tritiated glycerol in bacterial systems. J. Radiat. Res. **22**: 387–394.

SANO, Y., T. FUJII, S. IYAMA, Y. HIROTA and K. KOMAGATA* 1981: Nitrogen fixation in the rhizosphere of cultivated and wild rice strains. Crop Science **21**: 758–761.

TODOH, N., K. SHINOZAKI and M. SUGIURA 1981: Sequence of a putative promoter region for the rRNA genes of tobacco chloroplast DNA. Nucleic Acids Research **9**: 5399–5406.

TOMIOKA, N., K. SHINOZAKI and M. SUGIURA 1981: Molecular cloning and characterization of ribosomal RNA genes from a blue-green alga, *Anacystis nidulans*. Mol. Gen. Genet. **184**: 359–363.

SUGIURA M. 1981: An *Escherichia coli* acid phosphatase which hydrolyzes preferentially nucleoside 3′, 5′-diphosphates. FEBS Lett. **123**: 285–286.

TAKAIWA, F. and M. SUGIURA 1981: Heterogeneity of 5S RNA species in tobacco chloroplasts. Mol. Gen. Genet. **182**: 385–389.

KATO, A., H. SHIMADA, M. KUSUDA and M. SUGIURA 1981: The nucleotide sequences of two $tRNA^{Asn}$ genes from tobacco chloroplasts. Nucleic Acids Research **9**: 5601–5607.

TAKAHATA, N. 1981: A mathematical study on the distribution of the number of repeated genes per chromosome. Genet. Res. **38**: 97–102.

TAKAHATA, N. 1981: Genetic variability and rate of gene substitution in a finite population

under mutation and fluctuating selection. Genetics **98**: 427–440.

TAKAHATA, N. and T. MARUYAMA 1981: A mathematical model of extranuclear genes and the genetic variability maintained in a finite population. Genet. Res. **37**: 291–302.

TAKAHATA, N. and M. KIMURA 1981:: A model of evolutionary base substitutions and its application with special reference to rapid change of pseudogenes. Genetics **98**: 641–657.

YAGI, Y*., K. NAKAMURA*, I. TOMITA*, K. TUTIKAWA and N. SHIMOI* 1980: Teratogenic potential of di- and mono-(2-ethylhexyl) phthalate in mice. J. Environ. Pathol. Toxicol. 4–2, **3**: 533–544.

WATANABE, T. K. and M. KAWANISHI 1981: Asymmetrical mating success and the phylogeny of *Drosophila*. Zool. Mag. **90**: 317–324.

NAKAMURA, M., M. YAMADA, Y. HIROTA, K. SUGIMOTO*, A. OKA* and M. TAKANAMI* 1981: Nucleotide sequence of the *asn*A-gene coding for asparagin synthetase of *E. coli* K-12. Nucleic Acids Res. **9**: 4669–4679.

YAMAMOTO, M. 1981: Mechanism of meiotic chromosome pairing in the *D. melanagaster*. (in Japanese) Jap. J. Genet. **56**: 79–96.

YOSIDA, T. H. 1981: Studies on the karyotype differentiation of the Norway rat, V. Hypotrichotic mutant rats appeared in the inversion stock (LEM). Proc. Jap. Acad. **57**(B): 29–34.

YOSIDA, T. H. 1981: Chromosome alteration and the development of tumors, XXIII. Banding karyotype analysis of methylcholanthrene-induced tumors in the Indian spiny mouse, *Mus platythrix*, with special ragard to the anomalies of chromosomes with nucleolar organizer regions. Cancer Genet. Cytogenet. **3**: 211–220.

YOSIDA, T. H. 1981: Chromosome alteration and development of tumors, XXIV. Hypopentaploid chromosome constitution of a spontaneous tumors developed in the Oceanian type black rat, with particular reference to centromeric fission. Proc. Jap. Acad. **57**(B): 260–265.

YOSIDA, T. H. 1981: Chromosome polymorphism of the large naked-soled gerbil, *Tatera indica* (Rodentia, Muridae). Jap. J. Genet. **56**: 241–248.

YOSIDA, T. H. and T. INOUE* 1981: Cytogenetic studies on the Indian spiny mouse, I. Frequency of sister chromatid exchanges in lung primary culture. Proc. Jap. Acad. **57**(B): 13–17.

YOSIDA, T. H. 1981: Population cytogenetics of the black rat, *Rattus rattus*. Proc. XIV Intern. Cong. Genet. (Symposium), Vol. II: 96–117.

TAKATSUKI, K*., H. MASAI*, A. UBUKATA*, J. KAMAMATSU* and T. H. YOSIDA 1981: Quantitative variation in cytoarchitectures of the retine and the striate cortex among some species of Myomorpha (suborder) in relation to their habits. Zool. Anz. Jena **206**(198): 263–272.

---

* Author not on NIG staff.

ABSTRACTS OF DIARY FOR 1981

January	29	270th Meeting of Misima Geneticists' Club
March	7	271st Meeting of Misima Geneticists' Club
	17	175th Biological Symposium
	20	176th Biological Symposium
	26	272nd Meeting of Misima Geneticists' Club
April	4	177th Biological Symposium
	20	178th Biological Symposium
May	12	273rd Meeting of Misima Geneticists' Club
July	16	274th Meeting of Misima Geneticists' Club
	24	275th Meeting of Misima Geneticists' Club
August	28	179th Biological Symposium
September	12	180th Biological Symposium
October	5	181st Biological Symposium
	9	182nd Biological Symposium
	15	276th Meeting of Misima Geneticists' Club
	23	183rd Biological Symposium
	24	184th Meeting of Misima Geneticists' Club
	28	185th Meeting of Misima Geneticists' Club
November	12	186th Meeting of Misima Geneticists' Club
	20	187th Meeting of Misima Geneticists' Club
	20	188th Meeting of Misima Geneticists' Club
December	19	277th Meeting of Misima Geneticists' Club

FOREIGN VISITORS IN 1981

February	16–Sept. 30, '82	SUN, Chong-ron, Futan University, China
March	16–20	Fujimura, Robert K., Oak Ridge National Laboratory, U. S. A.
	17	Yamazaki, H., International Agency for Research on Cancer, World Health Organization, France
	20	PETROV, Spetrov P., Institute for Wheat and Sunflower, Bulgaria
	21–23	CROW, James F., University of Wisconsin, U. S. A.
	30–Apr. 23	DYER, Tristan A., Plant Breeding Institute England
April	1–Jul. 29	KIM, K. W., Chonnam Nat. University, Korea
	2	XU, Y. and Others, Inst. Developm. Biol., China
	10–11	JEFFERSON, Roland M., U. S. National Arboretum, U. S. A.
April	17–20	WHITFELD, Paul R., Commonwealth Scientific and Industrial Research Organization, Australia
	20	HIRTH, Léon, Université de Strasbourg, France
	24–May 15	SCHWARZ, Uli, Max-Planck-Institüt, West Germany
May	2	WALKER, D. A., University of Sheffield, England
	13–16	STEVENS, L. C., The Jackson Laboratory, U. S. A.
	27	Nasoetion, Andi H., Institute Pertanian Bogor, Indonesia
	29	TONEGAWA, S., Basel Institute for Immunology, Switzerland

June	19	PEACOCKE, A. Robert, Clare College, Cambridge, England
August	19	KUO, Chun-Yen, South China Inst. of Botany, Academia Sinica, China
	20–Sept. 4	NEI, M., University of Texas, U. S. A.
	28	GARTLAND, W. J., Jr., National Institutes of Health, U. S. A.
September	10–26	SHARMA, A. K., University of Calcutta, India
	16–Oct. 31	SHANKEL, Delbert M., University of Kansas, U. S. A.
	17–Dec. 7	WANEK, Nancy Lynn, University of California, U. S. A.
	24–Oct. 28	CROW, James F., University of Wisconsin, U. S. A.
	24	KIHLMAN, B. A., University of Uppsala, Sweden
	26	ROH, J. K., Korea Advanced Institute of Science and Technology, Korea
		OKAMOTO, K., University of New South Wales, Australia
		SMUTKUPT, S., Kasetsart University, Thailand
		BHUNYA, S. P., Utkal University, India
		MCKENNA, P. G., The University of Ulster, Northern Ireland
		BOGAJEWSKI, J., Institute of Human Genetics, Polish Academy of Sciences, Poland
		CHOUROULINKOV, I., Institut de Recherches Scientifiques sur la Cancer, France
		AUERBACH, C., The University of Edinburgh, England
		OMENN, G. S., University of Washington, U. S. A.
		KIHLMAN, B. A., University of Uppsala, Sweden
		SCHULL, W. J., University of Texas Health

		Science Center, U. S. A.
		RUSSELL, W. L., RUSSELL, L. B., Selby, P. B., Oak Ridge National Laboratory, U. S. A.
		LIM-SYLIANCO, C. Y., University of the Philippines, Philippines
		HASHEM, N., Ain-Shams University, Egypt
		WOOD, R. D., University of California, U. S. A.
		FISCHMAN, H. K., New York State Psychiatric Institute, U. S. A.
	28–Oct. 1	TAN, C. C., Futan University, China
October	15	RANFT, Dietrich and NICKEL, Dietmar; Max-Palnck Gesellschaft, West Germany
	15	KAJI, A., University of Pennsylvania, U. S. A.
	19	FREY, Kenneth J., Iowa State University, U. S. A.
	20–21	STRONINGER, Jack, Harvard University, U. S. A.
	21	JOLLY, M. S. and others, Central Sericultural Research and Training Institute, India
	23	OHNO, S., City of Hope Research Institute, U. S. A.
	23–24	Gross, Hans J., Würtzburg Universität, West Germany
	31–Nov. 1	ARBER, Werner, Basel Universität, Switzerland
	11	RIEGER, R., Institute of Genetics and Research in Cultivated PLANTS, Academy of Sciences, German Democratic Republic
	17–18	ALBERTS, Bruce, University of California, U. S. A.
	20–21	FRAENKEL-Conrat, Heinz, University of California, U. S. A.
	20	BODMER, Walter F., Imperial Cancer Research Fund, England

20	FIERS, Walter, Rijks Universiteit-Gent, Belgium
28	BEAKWITH, Jon, Harvard University, U. S. A.

AUTHOR INDEX

ABE, T. 110
ACHERMANN, J. 56
AOKI, K. 103
ASADA, K. 26, 27
ASAKURA, M. 52
BONHOMME, F. 69
CHAN, H. W. 21
DE ZOLESSI, L. C. 69
EGAMI, N. 46
ENDO, T. 48, 115
FUJII, T. 95, 118, 119
FUJISAWA, T. 55
FUJISHIMA, T. 113
FURUICHI, M. 21
GOÑI, B. 69, 71
GOTOH, O. 42
GROPP, A. 42
HANAKATA, A. 73, 74
HANAOKA, K. 57
HATA, T. 12, 13
HATTORI, M. 13
HIMENO, M. 24
HIROTA, Y. 24, 25, 26, 27, 28, 31, 34
IMAI, H. T. 68, 69, 71
INOUE, T. 91, 94, 95
INOUE, Y. 105, 116
ISONO, K. 79
IYAMA, S. 119, 120
JIKUYA, H. 20, 21
KADA, T. 91, 92, 93, 94
KAMIMURA, T. 12
KATO, J. I. 28
KAWAMUKAI, M. 24
KIM, K. W. 107
KIMURA, M. 97, 98, 101
KOJIMA, K. 25
KOMANO, T. 24
KOMEDA, Y. 29, 34
KONDO, K. 69, 71
KONDO, M. 71
KUBOTA, M. 71
KURODA, Y. 49, 50, 51, 52, 53, 71
KUSANO, T. 67
KUSUDA, J. 22
MAKI, Y. 20
MARTIN, M. A. 21
MARUYAMA, I. 28
MARUYAMA, T. 101
MATSUDA, Y. 69
MATSUNAGA, E. 109
MATSUTANI, E. 50
MIGITA, S. 45
MIKAMI, H. 53
MINATO, K. 59
MIURA, K. I. 12, 13
MIYAMURA, T. 21
MIYASHITA, N. 38, 39, 40, 45
MOCHIZUKI, H. 94
MOODY, M. 103
MORISHIMA, H. 113, 114, 118
MORIWAKI, K. 36, 38, 39, 40, 42, 44, 45, 46, 68, 69
MURAKAMI, A. 83, 84, 86, 88
NAKAGOME, Y. 110
NAKAMOTO, Y. 24
NAKAMURA, M. 28
NAKANO, Y. 20, 21
NISHIMIYA, C. 55
NISHIMURA, A. 30, 33
NISHIMURA, Y. 13
NISHIZAWA, K. 25, 68
NOGUCHI, T. 58
OHNISHI, S. 107
OHTA, K. 118
OHTA, T. 98, 99
OISHI, K. 53
OKA, A. 26, 27
OKA, H. I. 118

Okamoto, K. 24
Sadaie, Y. 93
Sakai, H. 24
Sagai, T. 36
Sakaizumi, M. 45, 46
Sano, Y. 119
Sasaki, H. 27
Sato, C. 25, 59
Sato, M. 58
Shinozaki, K. 14, 17
Shiroishi, T. 36
Soeda, E. 20, 21
Soma, M. 28
Sugimoto, K. 26, 27
Sugiura, M. 14, 17
Sugiyama, T. 55, 56
Sun, C, R. 48
Suzuki, H. 28, 67
Takagi, T. 28
Takahata, N. 100, 101, 102, 103
Takaiwa, F. 14, 17
Takanami, M. 26, 27
Takano, J. 56
Takeda, Y. 24
Takemoto, K. K. 20
Tanabe, H. 24
Tashiro, H. 20
Tateno, Y. 103
Taya, C. 44
Terai, K. 114
Thow, Y. P. 71
Tomioka, N. 17
Tsuboi, M. 13
Tsuno, K. 105
Urushibara, T. 12
Utsumi, R. 24
Wang, C. H. 45
Watanabe, T. K. 53, 105, 107
Winking, H. 42
Yamada, C. 17
Yamada, M. 24, 26
Yamagata, T. 50
Yamaguchi, K. 12, 13
Yamamoto, A. 107
Yamamoto, M. 73, 74, 76, 77, 79, 80
Yasuda, S. 28
Yokoiyama, A. 94
Yonekawa, H. 42
Yong, H. S. 71
Yoshiike, K. 21
Yosida, T. H. 60, 61, 62, 63, 64, 65, 66, 67

国立遺伝学研究所年報　第32号
昭和57年12月20日　印刷
昭和57年12月25日　発行

発行者　田　島　弥　太　郎
国立遺伝学研究所内
編集者　広　田　幸　敬
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
電　話　代表 (0559) (75) 0771

to Gotenba
Nihon Univ.
to Hakone
to Shizuoka
Mishima Station
Rakujuen
Izuhakone Line
Mishima Shrine
New Tōkaido Line
Tōkaido Line
National Highway 1
to Tokyo
Hirokozi Station
City office
Daiba River
National Institute of Genetics
Official Residences
Official Residences
Tamachi Station
N
Hutukamachi Station
1:25,000