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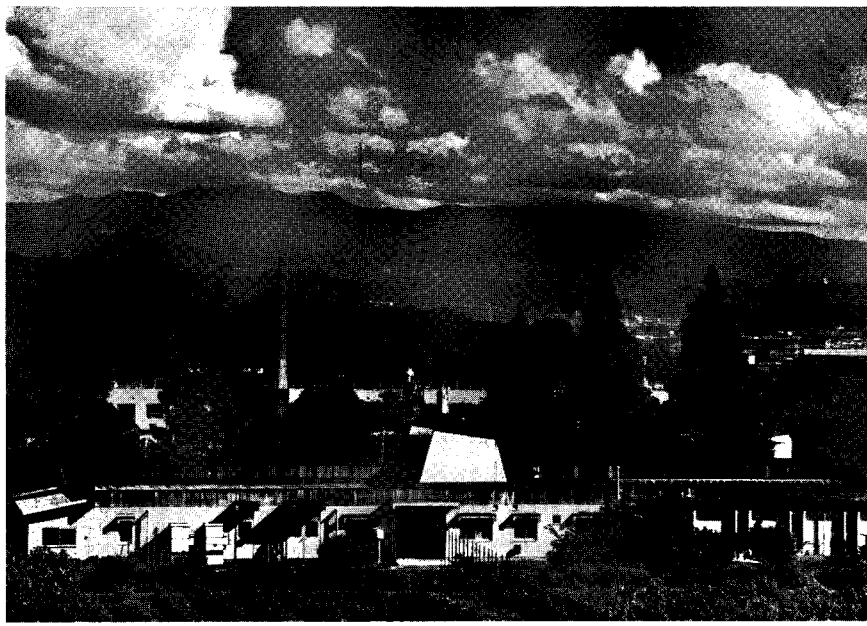

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GENERAL STATEMENT

A prospect for switching over the administration category of the institute from the present system to that for joint use by the universities has turned bright. The Ministry of Education, Science and Culture has organized a special committee to investigate the reorganization plan of this institute.

The institute was founded in 1949 as one of the national institutes under a jurisdiction of the Ministry. The original idea for characterizing the institute as a national institute was to have it independent from the universities. This resulted in much success in selecting qualified researchers from various universities, but caused several inconveniences to the personal activities of researchers because of tight service regulations. Even under such circumstances innumerable ingenious contributions have been achieved in this institute.

In the meantime, institutes of a new category have been established by the same Ministry, so as to be suitable especially for the promotion of research. Those institutes are independent from a specific university but open for university staffs to carry out cooperative works and are classified into "the institute for joint use by the universities." The institute of such category has several merits, but has to assume, on the other hand, a responsibility for planning and conducting cooperative researches, in compliance with the proposal of university researchers. This may add some trouble to the staff members, but I believe that the new system may provide them with far more convenience to their research and increased chances to exchange the views with several experts. Another merit may be the adoption of guest professor's system. Under the new system a guest professor will be available for each group of departments. This new system will be especially useful for the refreshing the idea and introducing new methodology. Standing at a very crucial turning point of the institute, it is very important that we must bear in mind not to stray from the right direction.

Regarding the personnel change in the Department of Molecular Genetics, Dr. M. Sugiura, head of the 2nd laboratory, left his position early September, 1982 and joined with Faculty of Science, Nagoya University, and Dr. K. Miura, head of the Department, will be appointed a professor of the Faculty of Technology of the University of Tokyo next April. Further-

more, two other staff members of the Department will be transferred to their new posts. After 13 years since the birth of the Department it will be reorganized comprehensively.

Dr. H. Kihara, who had devoted himself to the development of the institute as the second director for 13 years, attained his 90th birthday on Octover 21st and still remains hale and hearty. We planned to construct his relief, congratulating his long life and admiring his great achievements in the field of genetics. It was engraved by Mr. Shimoyama, a reputed sculptor, and has been installed on the wall of the landing to the second floor in the main building. It reminds us of him and encourages us in the morning and evening.

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.

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IMAI, HIROTAMI, D. Sc.

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The 2nd Laboratory

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The 3rd Laboratory

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The 2nd Laboratory

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

The 3rd Laboratory

KADA, Tsuneo, D. Sc., Head of the Laboratory (the first half year)
SADAIE, Yoshito, D. Sc., Head of the Laboratory (the second half year)
TEZUKA, Hideo

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The 1st Laboratory

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The 2nd Laboratory

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

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The 2nd Laboratory

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AOKI, Kenichi, Ph. D.

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SHIMOTOHNO, Kunitada, D. Pha.

The 2nd Laboratory

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KUSUDA, Jun, D. Ag.

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Microbial Section

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NISHIMURA, Akiko

12. ***Experimental Farm***

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MIYAZAWA, Akira

13. ***Department of Administration***

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OOIDE, Yukio, Chief of the Finance Section

Honorary Members

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OSHIMA, Chozo, D. Sc.
OKA, Hiko-Ichi, D. Ag.

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YOSIDA, Tosihide H., Managing Director, Head of the Cytogenetics Department
SINOTO, Yosito, Manager
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 1982

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)
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

Behavioral genetic studies in animals (FUJISHIMA)
Theoretical studies on breeding techniques (IYAMA)
Genetic studies of trees in natural forest (IYAMA)
Evolutionary studies on 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, INOUE 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 and cytogenetic studies on retinoblastoma and Wilms' tumor (MATSUNAGA and NAKAGOME)
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)
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)
Transformation of human lung and rat fibroblast cells with viral promoter DNA (SOEDA)

Biogenesis of papovaviruses (SOEDA)

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)
Establishment of new experimental animals from wild rodents (YOSIDA and MORIWAKI)
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 1982

I. MOLECULAR GENETICS

Genomic Information of Murine Papovavirus K Genome

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

Murine papovavirus K (KV) was first isolated by Kilham in 1953 and has been classified as a member of papovavirus family. Inoculation of this virus to new born mice induces a fetal pneumonia, one of the well characterized pathogenesis, serological studies have indicated that KV was ubiquitous to a mouse population. KV is different from polyomavirus (PyV) and other papovaviruses in respect to its ability to induce tumour in animals, but the cultured cells from the mouse lung infected with KV exhibited transformed phenotypes as well. One the other hand, KV shares permissive host with PyV which can code for middle T antigen. PyV middle T antigen is thought to be implicated in cellular transformation and tumorigenesis. Gene comparison of PyV with other papovaviruses including simian virus 40 (SV40), human BKV and JCV has revealed that PyV middle T antigen is unique among other papovaviruses, arising a question how other papovaviruses induce tumours without viral coded middle T antigen. We are interested in the different pattern of tumorigenicity between PyV and KV and determined the nucleotide sequence of KV DNA in order to see whether KV genome code for the relevant of PyV middle T antigen or not.

A total nucleotide sequence of KV DNA has been deduced by the method of Maxam and Gilbert. It consists of 4994 uncleotides in length. Examination of the nucleotide sequence indicated that the genomic organization of KV genome was very similar to that of PyV genome except that KV genome could not code for the relevant of Py middle T antigen. These evidences suggest that papovaviruses have diverged from a common origin and enforce further that PyV middle T antigen is important for induction of tumour formation in animals.

1) Lab. of Viral Diseases, NIAID. NIH, Bethesda, Maryland 20205, USA.

Structure of Murine Papovavirus K Genome: Heterogeneity of Nucleotide Sequence in the Region Containing the Origin of DNA Replication

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

Murine papovavirus K (KV) DNA extracted from a viral stock was cloned molecularly in pBR322. Four clones were obtained, of which inserts were different in sizes to each other. Restriction enzyme mapping indicated that the variation in sizes resides in the Eco RI/Pst I fragments. So, these fragments were submitted to sequence DNA and compared with those of other papovaviruses which were sequenced.

Nucleotide sequences of these fragments were determined by the method of Maxam and Gilbert. When compared with those of papovaviruses, GC rich sequences flanked by consecutive AT sequences were found in common among these fragments and highly homologous to the sequences of papovaviruses spanning the origin of DNA replication. Downstream of these regions, one long reading frame starting with ATG initiation coding remained open to the ends. The predicted amino acid sequences are identical to that from the N-terminus of VP2/3 of polyomavirus. These evidences indicated that these fragments span predominantly regulatory region containing the origin of DNA replication and predicted that the early coding region might be present at the opposite site of the VP-2/-3 coding region. Heterogeneity of the nucleotide sequences was predominantly present between the origin of DNA replication and the early coding region which may be characterized by the presence of tandem repeat sequences.

1) Lab. of Viral Diseases, NIAID, NIH, Bethesda, Maryland 20205, USA.

Automatic DNA Sequencer: Computer Programmed Microchemical Manipulator for Maxam-Gilbert Sequencing Method

Akiyoshi WADA[1], Masahiro YAMAMOTO[2] and Eiichi SOEDA

Recent advances in microprocessors make it possible to provide an automated tool which is able to operate a fairly long series of micro-chemical

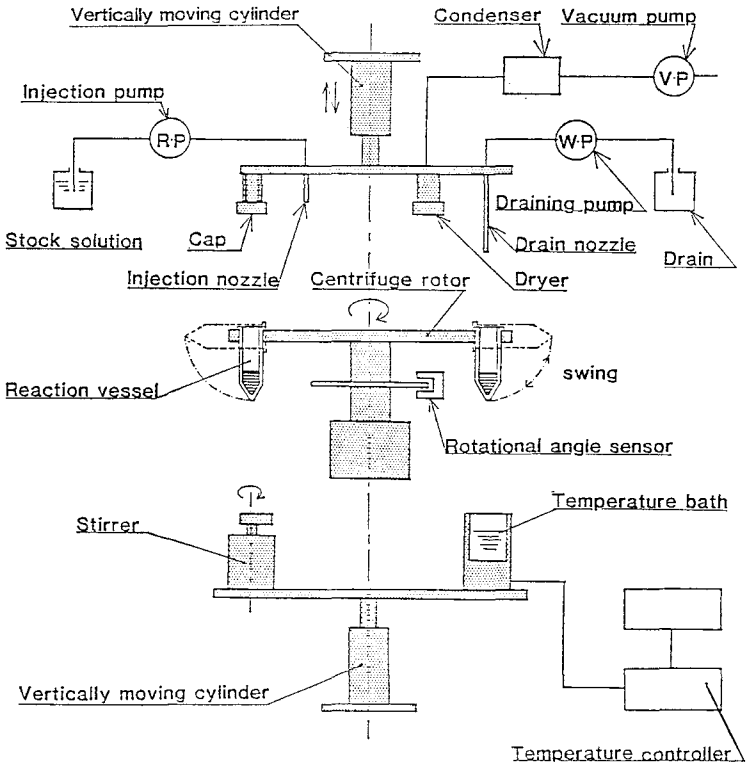

Fig. 1. Block diagram of the system. The system consists of a centrifuge rotor (radius: 15 cm) which holds reaction vessels. The last two tabes support the solution manipulation units.

[1] Department of Physics, Faculyt of Science, The University of Tokyo, Hongo, Tokyo, Japan.

[2] Daini Seikosha Co. Ltd., Takenoshita, Oyama-cho, Sunto-gun, Shizuoka Pref. 410-13, Japan.

manipulations for the analysis or the synthesis of biological materials. Routin and tedious laboratory operations can be replcaed by a button operation for the input of computer commands.

This work is to aim the development of a micro-chemical robot which carries out a series of chemical reaction steps in DNA sequence analysis using Maxam and Gilbert's scheme. The performance of the apparatus includes the quantitative addition of solution, mixing, centrifugation, draining, drying under vacuum, and cooling and heating of sub-milliliter solution. Within a two-hour period of operation of this apparatus, one of the four types of solutions for G, A+C, C+T, or C analysis for a DNA sample (restriction fragment), is prepared and is ready for subsequent electrophoretic analysis.

In addition to the DNA sequencing, the instrument is applicable treatments for analysis and synthesis of biochemical materials; the steps required for the reaction sequence are performed one after another, according to a computer programme that is designed just prior to the operation.

It is proven that the chemical robot operated by a computer command is capable of speedily accomplishing the fragmentation reaction for DNA sequencing with accuracy similar to that of a well trained technician.

Cloning of a Fibroin Gene from Wild Mulberry Silkworm *Bombyx mandarina*

Jun KUSUDA, Kimiji ONIMARU, Osamu NINAKI,† Yoshiaki SUZUKI* and Yataro TAZIMA

Morphological and chromosomal studies showed that *Bombyx mandarina* is one of the possible ancestor of domesticated silkworm *Bombyx mori.*

In order to elucidate the phylogenic relationship between two silkworm species, we attempt to compare the structure of the fibroin gene of *B. mandarina* to that of *B. mori.*

The fibroin gene of *B. mori* was already cloned into *E. coli* plasmid by Suzuki and the sequence around the 5′ end of the gene was determined. At first, we have isolated the fibroin gene of *B. mandarina* by cloning techniques using cloned DNA (pFb29) contained the fibroin gene of *B. mori* as a hybridization probe.

* National Institute of Basic Biology.
† Sericultural Experimental Station.

The genomic DNA of *B. mandarina* was digested with Bam HI and separated by agarose gel electrophoresis. The DNA fragments transfered to a nitrocellulose filter were hybridized with labeled pFb29. The radioactive probe hybridized to single fragment with molecular weigh of 14.5 Kb. The fragments recovered from the gel fraction indicated a positive signal were cloned into Bam HI site of Charon 30.

Twelve positive clones were detected by plaque hybridization to the probe. Recombinant DNAs prepared from each clones were analyzed on agarose gels after digestion with Bam HI and hybridized to the probe. It was found that 7 clones containe 14.5 Kb fragment homologous to the fibroin gene sequence of *B. mori*.

II. MICROBIAL GENETICS

The 245 Base-pair *ori*C Sequence of the *E. coli* Chromosome Directs Bidirectional Replication at an Adjacent Region

Satoshi TABATA*, Atsuhiro OKA*, Kazunori SUGIMOTO*, Mituru TAKANAMI*, Seiichi YAUDA and Yukinori HIROTA

The replication origin of the *E. coli* K-12 chromosome has been isolated as autonomously replicating molecules (*ori*C plasmid), and the DNA region essential for replicating function (*ori*C) has been localized to a sequence of 232–245 base-pairs (bp) by deletion analysis. In this report, the functional role of *ori*C was analyzed by using an *in vitro* replication system and various OriC$^+$ and OriC$^-$ plasmids previously constructed. The results obtained were summarized as follows: (1) The *ori*C sequence contained information enough to direct bidirectional replication. (2) The actual DNA replication began at a region near, but outside, *ori*C and progressed bidirectionally. (3) Initiation of DNA synthesis at the specific region required the *dna*A-complementing fraction from cells harboring a *dna*A-carrying plasmid. (For detail, see Nucleic Acids Research, *11*, 2617–2626, 1983).

Suppressor Genes of a *dna*A Temperature Sensitive Mutation in *Escherichia coli*

Yutaka TAKEDA** and Yukinori HIROTA

Recombinant plasmids were constructed from *Eco*RI digests of *Escherichia coli* chromosomal DNA and pMB9 DNA by selecting for suppression of a *dna*A-T46 temperature-sensitive mutation. Two types of plasmid capable of suppressing the *dna*A mutation were isolated. They did not carry any genetic markers around *dna*A and physical mapping with various restriction enzymes showed that neither of the plasmids contained the *dna*A gene. One plasmid, pYT47, was characterized further and the protein responsible for the suppression was identified by two-dimensional gel electrophoresis.

* Institute for Chemical Research, Kyoto University, Uji, Kyoto 611.
** Faculty of Agriculture, Nagoya University, Chikusa, Nagoya.

The molecular weight of the suppressor protein was about 68 Kdal and this is clearly different from the *dna*A gene product. (For detail, see Mol. Gen. Genet *187*, 67–71, 1982).

The Fine Architecture and Function of the Gene Coding for PBP-3 of *Escherichia coli*

Ichiro N. MARUYAMA, Akihiko YAMAMOTO, Takeo MARUYAMA and Yukinori HIROTA

A synthetic ColE1 plasmid, pLC26–6, found in a gene bank was shown to carry the gene (*fts*I or *pbp*B) coding for PBP-3. Using this DNA fragment, we determined the entire nucleotide sequence of the *fts*I, consisting of 1,764 base-pairs which code a polypeptide of 588 amino acid residues and of a molecular weight 63,850. In this symposium, we present new findings on the gene's fine structure and mechanism responsible for regulating the function of PBP-3. (For detail, see "The Target of Penicillin, International FEMS-Symposium on the Murein Sacculus of Bacterial Cell Walls-Architecture and Growth" (1983). Publ. Walter De Gruyter, Berlin-New-York).

N-Acetylmuramoyl-L-alanine Amidase of *Escherichia coli* K12: Possible Physiological Functions

Claudine PARQUET*, Bernard FLOURET*, Mireille LEDUC*, Yukinori HIROTA and Jean Van HEIJENOORT*

Various experiments were carried out in an attempt to determine the possible physiological function of the N-acetylmuramoyl-L-alanine amidase purified from *Escherichia coli* K12 on the basis of its activity on N-acetylmuramoyl-L-alanyl-D-γ-glutamyl-*meso*-diaminopimelic acid [MurNAc-LA1a-DGlu(msA_2pm)]. A Km value of 0.04 mM was determined with this substrate. Specificity studies revealed that compounds with a MurNAc-LA1a linkage are the most probable substrates of this enzyme *in vivo*. Purified amidase had no effect on purified peptidoglycan and only low levels (1–2.5%) of cleaved MurNAc-LA1a linkages were detected in peptidoglycan isolated from normally growing cells. However, the action of the amidase *in vivo* on peptidoglycan was clearly detectable during autolysis. The amidase activity of cells treated by osmotic shock, ether or toluene, as

* Centre National de la Recherche Scientifique, Institut de Biochimie, Orsay.

well as that of mutants with altered outer membrane composition was investigated. Attempts to reveal a transfer reaction catalysed by amidase were unsuccessful. Furthermore, by its location and specificity, amidase was clearly not involved in the formation of UDP-MurNAc. The possibility that it might be functioning *in vivo* as a hydrolase degrading exogeneous peptidoglycan fragments in the periplasma was substantiated by the fact that MurNAc itself and MurNAc-peptides could sustain growth of *E. coli* as sole carbon and nitrogen sources. Finally, out of 200 thermosensitive mutants examined for altered amidase activity, only two strains had less than 50% of the normal level of activity, whereas ten strains were found to possess more than 50%. In fact, two of the overproducers encountered present a 4-5-fold increase in activity. (For detail, see Eur. J. Biochem *133*: 371–377, 1983).

Isolation and Characterization of an *Escherichia coli* Mutant Lacking tRNA-Guanine Transglycosylase Function and Biosynthesis of Queuosine in tRNA

Shigeru NOGUCHI*, Yukinobu NISHIMURA, Yukinori HIROTA, and Susumu NISHIMURA*

An *E. coli* mutant that lacks tRNA-guanine transglycosylase was isolated by random screening from a collection of *Escherichia coli* mutants obtained with N-methyl-N′-nitro-N-nitrosoguanidine. The defective gene, named *tgt*, was mapped at about 9 min on the *E. coli* chromosome, and the gene order was shown to be *pho*B-*tgt-tsx*. *tgt* was transferred to an *E. coli* strain with a defined genetic background by P1 transduction to investigate its function. The mutant thus obtained lacked queuosine (2-amino-5-[(3, 4R, 5S)-4, 5-dehydroxycyclopent-1-en-3-ylaminomethyl]-7-(β-D-ribofuranosyl)-pyrrolo-[2, 3-D]-pyrimidin-4-one) in tRNA, indicating that the enzyme is actually involved in the biosynthesis of queuosine in tRNA. No clear biological defect was observed in the mutant, and, in fact, it grew slightly faster than the control isogenic strain. $tRNA^{Tyr}$ lacking queuosine, isolated from the mutant, showed no significant biological difference from normal queuosine-containing tRNA in amino acid acceptor activity or amino acid transfer in a cell-free protein synthesizing system directed by synthetic polynucleotide. The only phenotypic change observed in the

* The Biology Division, National Cancer Center Research Institute, Chuo-ku, Tokyo.

mutant thus far is marked reduction of viability when the cells are kept under unsuitable conditions for growth, suggesting that the presence of queuosine in tRNA is important to *E. coli* for survival in the natural environment. (For detail, see The Journal of Biological Chemistry, **257**, 6544–6550, 1982)

Synthesis of Flagellin and Hook Subunit Protein in Flagellar Mutants of *Escherichia coli* K-12

Yoshibumi KOMEDA

I have examined *Escherichia coli* K-12 flagellar mutants for existence of flagellin and hook subunit protein. Immune precipitation experiments were employed. Flagellin was detected in mutants defective in the gene—*flaS*, *flaT*, *flaU* and *flbC*. The *flaE or flaZ* mutants made a little flagellin. The other mutants did not have flagellin.

The hook subunit protein was found in mutants defective in the genes—*flaA*, *flaB*, *flaC*, *flaD*, *flaE*, *flaG*, *flaH*, *flaL*, *flaM*, *flaN*, *flaO*, *flaP*, *flaQ*, *flaS*, *flaT*, *flaU*, *flaV*, *flaW*, *flaX*, *flaZ*, *flaZ*, *flaA*, *flbC*, *flbD*, and *hag*. The *flaK*, *flaI* and *flbB* mutants did not carry the protein. The results conformed to the prediction by indrect gene fusion study (Komeda 1982).

Localization of *E. coli* Flagellar Gene Products Using Gene Fusion Technique

Yoshibumi KOMEDA

It has been known that a fused gene, into which MudII301 (Apr, *lac*) phage is inserted, codes for a fused enzyme carrying beta-galactosidase as C-end. The MudII301 (Apr, *lac*) phage was inserted into each of the *E. coli* flagellar genes (*fla*, *flb*, *hag* and *mot*). The insertion mutants were expected to carry fused proteins consisted of N-terminal flagellar gene products and promoter distal side of beta-galactosidase.

Localization was analyzed in these mutants. Since the beta-galactosidase is known to be located in cytoplasm, then N-terminal portion is responsible for the alteration of location if the fused enzyme is located in other than cytoplasm.

At first, *motA*: : MudII301 (Apr, *lac*), *hag*: : MudII301 (Apr, *lac*), and *flaK*: : MudII301 (Apr, *lac*) mutants were studied. These genes code for

motility protein, flagellin, and hook subunit protein, respectively. The products are known to be located in inner membrane, outside, and outside, respectively. It was found that these fusion products located as follows.

	fusion protein	intact protein
mot product	inner membrane	inner membrane
hag product	cytoplasm	outside
flaK product	cytoplasm	outside

The *motA* product was shown to be inner membrane protein by lambda *mot* infection study. The fused protein, *motA-lacZ* product, was also shown to be located in inner membrane in this study. Therefore, it was expected that *motA* protein has a signal to orient itself into inner membrane. The *hag* and *flaK* products are located outside, but fusion proteins were shown to be cytoplasmic. This fusion did not have the signal to define location or original products did not have location signal.

Next, a whole set of flagellar mutants were collected, which carried MudII301 (Apr, *lac*) insertions and protein fusion. They were examined for the location of fused beta-galactosidase activity. Among them, *flaU*, *flaA*, and *flaB* fusions were found to be located in membrane fraction. These gene products were imagined to have signal sequence that altered cellular locations of the fusions.

III. BIOCHEMICAL GENETICS AND IMMUNOGENETICS

Urethan-Induced Lung Tumor Development in (Susceptible × Resistant)F_1 Hybrids of H-2 Congenic Strains of Mice

Nobumoto MIYASHITA and Kazuo MORIWAKI

It has demonstrated that the I-A and I-E regions in the H-2 complex are related to the regulation of the lung tumor development (Moriwaki and Miyashita, A.R.N.I.G. **31**, 38–39, 1981). In the present study, we investigated the urethan-induced lung tumor development in F_1 hybrids between the susceptible strain which is expressing the E^k molecules on the antigen-presenting cell controlled by H-2^a haplotype and the resistant strains which are not expressing the E molecules. Average number of lung tumor foci per mouse in (susceptible × resistant)F_1 hybrids were intermediate between the parental strains of the congenic mice on the A background. These response patterns are remarkably similar to H-2 linked Ir gene response to lactate dehydrogenase B (LDH-B) and IgG2a myeloma protein. It can be postulated that the T-cell dependent immunity plays a role in the regulation of the susceptibility to the lung tumor development.

Table 1. Urethan-induced lung tumor development in (susceptible × resistant) F1 mice

Strain	H-2 haplotype	Alleles of H-2 complex						Type of E molecule	Lung tumor foci per mouse
		K	A_β	A_α	E_β	E_α	D		
A	a	k	k	k	k	k	d	$E_\alpha^k E_\beta^k$	30.6±1.3 (24)
A.BY	b	b	b	b	b	b	b	—	13.8±0.6 (58)
A.SW	s	s	s	s	s	s	s	—	12.3±0.7 (51)
A.CA	f	f	f	f	f	f	f	—	17.0±0.6 (58)
(A × A.BY)F_1	a/b	k/b	k/b	k/b	k/b	k/b	d/b	$E_\alpha^k E_\beta^k$, $E_\alpha^k E_\beta^b$	23.9±1.3 (25)
(A × A.SW)F_1	a/s	k/s	k/s	k/s	k/s	k/s	d/s	$E_\alpha^k E_\beta^k$, $E_\alpha^k E_\beta^s$	18.9±1.1 (13)
(A × A.CA)F_1	a/f	k/f	k/f	k/f	k/f	k/f	d/f	$E_\alpha^k E_\beta^k$, $E_\alpha^k E_\beta^f$	21.7±1.1 (17)

( ): Number of mice observed.

Production of Monoclonal Antibodies against H-2 Complex in B10. MOL-SGR Mouse Strain

Tomoko SAGAI, Toshihiko SHIROISHI and Kazuo MORIWAKI

Previously, we reported that the intra-H-2 recombination frequency of B10. MOL-SGR (H-2^{wm7}) which carries the H-2 complex of Japanese wild mouse extremely high (about 3%). In this congenic strain, most of the crossing over have occured between H-2K and IA region (Nature **300**, 370–372). We tried to obtain some hybridomas producing monoclonal antibodies for H-2 complex of B10. MOL-SGR, because those antibodies should be useful for analysis of the high recombination mechanism.

P3U1 myeloma cells were fused with spleen cells prepared from three B10 congenic strains immunized with B10. MOL-SGR lymphocytes. Activities and specificities of the monoclonal antibodies were roughly analysed by the complement dependent microcytotoxicity test, reported by us pre-

Table 1. Hybridoma cell lines produced against B10. MOL-SGR lymphocytes

Immunized recipients	Established hybridomas
B10	Hd1, Hd2
B10.A	Hd10, Hd12, Hd15, Hd17
(B10×B10.A)F1	Hd24, Hd25, Hd28

Table 2. Characterizations of monoclonal antibodies against B10. MOL-SGR

H-2 recombinants	H-2 complex					Type I	Type II	Type III	Type IV
	K	A	E	S	D	Hd24, 25	Hd2, 12, 15 17, 28	Hd1	Hd10
R201	k |	w	w	w	w	—	+	+	+
R203	k	k	k	| w	w	−	−	+	−
R202	k	k	k	d	| w	−	−	+	−
R204	w |	k	k	d	d	+	−	+	−
R212	w	w	w	| d	d	+	+	+	+
R406	b |	w	w	w	w	−	+	+	+
R407	w |	b	b	b	b	+	−	+	+
B10	b	b	b	b	b	−	−	−	+
B10.BR	k	k	k	k	k	−	−	+	−
B10.MOL-SGR	w	w	w	w	w	+	+	+	+

viously (Microbiol. Immunol. **25**, 1327–1334).

Table 1 summarizes nine hybridomas established. These hybridomas were classified into four types by the range of reactions (Table 2). Type I and II produced antibody for H-2K and I regions of B10. MOL-SGR respectively. Type III exhibited cytotoxic reaction for H-2 public antigen on the both K and D regions. It was also crossreactive with B10. BR. Type IV antibody was reactive with the I regions, but crossreactive with B10.

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 immunological factors governing the *in vivo* growth of embryo-derived teratoma, 7 day-old embryos of several A. H-2 congenic strains were transplanted under the kidney capsule of each dault recipients. After 40 days, the grown tumors were weighed to compare the effect of MHC in the host.

Among five A. H-2 congenic strains, A. TH and A. TL strains were significantly greater in the tumor weight than A. BY and A. SW strains. A strain was the intermediate size of tumor (Table 1). A. TH, A. TL and A strains have d haplotype in H-2D region, but A. BY and A. SW strains have b and s haplotypes respectively. On the other hand, A. TH and A. SW strains have the same haplotypes in the proximal parts of H-2 com-

Table 1. Syngeneic transplantation of 7 day-old embryo under the kidney capsule: Effect of H-2 complex on the growth of embryo-derived treatoma

Strains	H-2 haplotype				No. of mice observed	Weight of teratoma
	K	A	E	D		
A. TH	s	s	s	d	9	1998.9±476.0 mg
A. TL	s	k	k	d	23	1752.0±316.7
A	k	k	k	d	33	1421.5±219.2
A. BY	b	b	b	b	47	1004.6±167.1
A. SW	s	s	s	s	23	923.6±206.1
B10. A	k	k	k	d	12	606.8±180.0

Table 2. Allogeneic transplantation of 7 day-old embryo under the kidney capsule: Effect of H-2 complex on the growth of embryo-derived treatoma

Strains	H-2 haplotype	H-2 complex difference	No. of mice observed	Weight of teratoma
A. BY→B10	b→b	(Identical)	11	No tumor
B10→A. BY	b→b	(Identical)	4	No tumor
A→B10. A	a→a	(Identical)	3	No tumor
B10. A→A	a→a	(Identical)	2	No tumor
A. TL→A. TH	t1→t2	A E	3	668.0±132.4 mg
A. TH→A. TL	t2→t1	A E	8	459.5±84.3
A. SW→A. TH	s→t2	D	2	302.0
A. TH→A. SW	t2→s	D	2	266.5

plex to H-2D region. Thus, these results suggest that H-2D region or distal part to H-2D affect the tumor growth in A. H-2 congenic strains. Teratoma produced in B10.A strain was smaller than any other A. H-2 congenic strains, suggesting that the genetic background of A strain is favorable to the tumor growth.

In the allogeneic combinations, no tumor was developed from the transplant of A. BY to B10, B10 to A. BY, A to B10. A and B10. A to A, whereas the definite tumor growth was observed by the introduction of A. TL to A. TH, A. TH to A. TL, A. SW to A. TH and A. TH to A. SW (Table 2). These results suggest that the locus/loci controlling the growth of embryonic tissues are located out of MHC as well.

Southern Blot Analysis of H-2K Genes of Japanese Wild Mice

Hitoshi SUZUKI, Toshihiko SHIROISHI, Tomoko SAGAI, Gabriel GACHELIN*, Takeharu KANEHISA** and Kazuo MORIWAKI

The classical transplanation antigens (H-2 K, D and L) have extensive polymorphism but the details of their evolutional process remain unknown. We started the survey of the H-2 gene complex among several subspecies of *Mus Musculus* based on restriction enzyme cleavage patterns comparing to the serological data. Southern blot analysis using class I cDNA probe (pH-2^d-4) revealed the following results.

* Institut Pasteur.
** The Graduate School of Science and Technology, Kobe University.

(1) B10 H-2 recombinant mice with H-2 haplotype d, k and b exhibited H-2 K-specific 3.7 Kb, 3. 2Kb and 2.5 Kb bands, respectively, in Bgl II digestion as shown by Xin *et al.* (EMBO Jour. **4**, 467–471, 1982).

(2) DNA from B10. MOL-SGR showed a H-2K^{k} like 3.2 Kb band in Bgl II digestion. Using a recombinant line, R214 (K^{wm7}, D^{d}) (see Nature **300**. 370–372, 1982), we could map the Bgl II band on K locus. In this connection, it may be noteworthy that the antigenicities of H-2K molecules were relatively similar each other between B10. BR (H-2 k haplotype) and B10. MOL-SGR.

(3) B10. MOL-OKB which carries H-2 K31 (H-2K^{d} private) antigenic specificity had a 3.7 Kb band in Bgl II digestion.

These results suggest that H-2K region of each haplotype has its characteristic segment which can be detected by Bgl II digestion of genomic DNA. During the subspecies differentiation, these haplotype specific Bgl II sites might have been well conserved.

Southern Blot Analysis of Ribosomal DNA in *Mus Musculus*

Hitoshi SUZUKI, Ryo KOMINAMI*, Masami MURAMATSU*,
Takeharu KANEHISA** and Kazuo MORIWAKI

Restriction patterns of ribosomal DNA (rDNA) were examined in five inbred strains and several subspecies of *Mus Musculus* by Southern blot analysis using two restriction enzymes (Bam HI and Eco RI) and a DNA probe of 0.7 Kb cloned fragment extending from 3′ end of the 28S ribosome gene to the following spacer region. European subspecies, *M. m. domesticus*, *M. m. musculus* and *M. m. brevirostris* and Asian subspecies, *M. m. bactrianus*, *M. m. castaneus* and *M. m. molossinus* were examined, each of which showed the characteristic cleavage patterns (Table 1). Intrasubspecific heterogenity in rDNA was found in either of *M. m. domesticus*, *M. m. musculus*, *M. m. castaneus* or *M. m. molossinus*. In five inbred strains, B10, BALB/c, DBA/1, DBA/2 and CBA, the cleavage patterns of rDNA in Bam HI and Eco RI were relatively similar to those of *M. m. domesticus*.

* Tokyo University, School of Medicine.
** The Graduate School of Science and Technology, Kobe University.

Table 1. Restriction fragment of rDNA in various mouse subspecies

	Restriction enzymes						
	Bam HI				Eco RI		
Length of fragments (Kb)	2.3	6.6	5.5	6.6	7	9	10
M. m. domesticus	+++			+++			
	++	++		+++			
	+	++		+++			
M. m. musculus	±	+++				+++	+
M. m. brevirostris	++	+	++	++			
M. m. bactrianus		+++		+	++		
M. m. castaneus		+++		+		++	
		+++			++	++	
M. m. molossinus		++				+++	

DNA probe; cloned 0.7 Kb segments from 3′ end of the 28S gene to the following spacer region.

Serological Survey of Thy-1 and Lyt-1,2,3 Antigens in Wild Mice

Yasuyuki KURIHARA and Kazuo MORIWAKI

Thy-1 and Lyt-1,2,3, thymocyte surface antigens of mice, are known as differentiation antigens. Each of them has two alleles (e.g. Thy-1^a and Thy-1^b). In Thy-1 locus, Thy-1^a and Thy-1^b alleles determine two antigenic molecules, Thy-1.1 and Thy-1.2. Distribution of those antigens in laboratory mice has already been known. We examined these antigens of wild mice maintained in our laboratory, using the microcytotoxicity test.

Although most laboratory mice express Thy-1.2 and only 15 strains express Thy-1.1, we found that almost all *M. m. subspecies* except *M. m. domesticus* expressed Thy-1.1. Such data agreement with the generally accepted assumption that the laboratory mice mainly derived from *M. m. domesticus*. In survey of Lyt-1 and Lyt-2, we could detect only Lyt-1.2 and Lyt-2.1, but not Lyt-1.1 and Lyt-2.2 (Table 1).

Thus, thymocyte antigens such as Thy-1, Lyt-1 and Lyt-2 seem to be monomorphic within each subspecies.

Table 1. Survey of differentiation antigens in *Mus musculus subspecies* and some laboratory mice

Strains	Thy-1	Lyt-1	Lyt-2	Lyt-3
Wild mice				
M. m. domesticus	2	2	1	2
molossinus	1	2	1	1
musculus	1	2	1	1
castaneus	1	2	ND	ND
bactrianus	1	2	ND	ND
M. m. subspecies				
BJN III	1	2	1	1
SHH I	1	ND	1	1
JYG I	1	2	1	ND
LZH II	1	2	1	ND
Inbred mice				
C57BL/6	2	2	2	2
BALB/c	2	2	2	2
129	2	2	2	2
DBA/2	2	1	1	2
AKR	1	2	1	1

ND: not detected.

IV. DEVELOPMENTAL GENETICS AND SOMATIC CELL GENETICS

In Vitro Formation of Adult Structures from Cells of Lethal Embryos of *Drosophila melanogaster*

Yukiaki KURODA

When cells from post-gastrula embryos of *Drosophila melanogaster* are cultured in ecdysterone-free medium, they differentiate into larval epithelial cells, muscle cells, cellular spheres and nerve cells having their respective characters and functions. On the other hand, when these embryonic cells are cultured in ecdysterone-containing medium, they form adult structures such as the leg, the wing and the compound eye. This indicates that larval or adult tissues and cells can be selectively differentiated from embryonic cells by adding or removing of ecdysterone in the medium.

Homozygous and hemizygous embryos of a sex-linked mutant, *deep orange* (*dor*, 1-0.3; salivary gland chromosome map, 1F1-2A2) died around the stages of gastrulation and mid-gut formation during embryogenesis. When cells from post-gastrula embryos of the *dor* mutant were cultured in ecdysterone-free medium, epithelial cells, muscle cells and nerve cells differentiated normally. However, *dor* cells had defects in the differentiation and function of some specific types of cells. The syncytium formation of muscle cells, the formation of cellular spheres and the droplet secretion on the nerve fibers were not observed in these cultures.

When embryonic *dor* cells were cultured in medium containing 1–10 μg/m*l* of ecdysterone, some structures of the adult legs developed. This suggests that the *dor* lethal gene may express in some specific types of cells which show defects in normal differentiation and function, but some other types of cells differentiate normally and form not only larval tissues and cells but also adult structures in culture.

Localization of Lectins and Their Changes in Development of Chick Embryos

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

Mesenchyme cells dissociated from the limb buds of chick embryos differentiate into cartilage cells under *in vitro* culture conditions. We have previously reported that lectins had stimulating effects on the *in vitro* chondrogenesis of these mesenchyme cells (Develop. Biol., **89**: 521–526, 1982).

In the present experiment, the localization of endogenous lectins and their quantitative changes in the limb bud tissue of developing chick embryos were examined by using the immunofluorescent antibody technique, to find the role of lectins in normal development of chick embryos. The localization of galactose which binds specifically to chick lectins was also examined by using a FITC-labeled plant lectin, RCA.

No lectins were detected in the limb bud of chick embryos before the stage 24. They appeared in muscle tissue at the stage 25 and increased in amount as the developmental stages proceeded. Lectins were also found in the prospective cartilage region at the center of the limb bud and increased further in the process of osteogenesis. In the muscle tissue, lectins were present in the cytoplasm of cells, whereas in cartilage tissue lectins were detected in the intercellular matrix.

No galactose was detected in muscle tissue, whereas it was detected in cartilage tissue after the stage 24. Mesenchyme cells in the limb bud are stabilized to express the differentiated character of the cartilage cells and obtain the autonomous differentiating activity after the stage 24. The time of quantitative changes in endogenous lectins and galactose in the limb bud coincided with the time when mesenchyme cells are stabilized their differentiating activity. The intimate relationship between these two events may be suggested. This was supported by an experiment in which cultured mesenchyme cells were stimulated to differentiate into cartilage cells by adding embryonic chick lectin to medium.

1) Mitsubishi-kasei Inst. of Life Science, Machida, Tokyo.

Mutagenic Activity of Environmental Aromatic Compounds in Cultured Chinese Hamster Cells

Yukiaki KURODA and Masumi ASAKURA

The mutagenic activity of most aromatic compounds has not been detected in microbial assay systems. Recently, however, some aromatic compounds like aniline were reported to be carcinogenic in experimental animals. In the previous report, it has been found that aniline had a strong mutagenic activity in cultured Chinese hamster V79 cells.

There have been no report on the mutagenic activity of other aromatic compounds such as chloroaniline, nitrobenzene and hexachlorobenzene. These compounds are produced in large amounts as the materials for dyestuff, mordants and medicines by the manufacturing industry and frequently found in natural water and substratum in our environment. In the present experiment, the activity of these compounds to induce 8-azaguanine (8AG)- and ouabain (OUA)-resistant mutations in V79 cells was examined.

O-, *m*- and *p*-chloroaniline had stronger cytotoxic effects than aniline on V79 cells. The values of LD_{50} for 4 hour treatment of these three compounds were 0.5–0.6 mg/m*l*. The frequency of 8AG-resistant mutations induced by these compounds were $1.3\text{–}2.5\times10^{-5}$ which were lower than that of aniline. No detectable OUA-resistant mutations were induced by these compounds.

Nitrobenzene showed almost the same cytotoxic effect as that of chloroaniline. The frequency of 8AG-resistant mutations induced by nitrobenzene was as low as 1.6×10^{-5}. The induced mutation frequency increased to 5.6×10^{-5}, by adding of S-9 Mix, showing a metabolic activation of nitrobenzene.

Hexachlorobenzene had a strongest cytotoxic effects among all aromatic compounds tested. Its LD_{50} value for 4 hour treatment was 0.04 mg/m*l*. However, hexachlorobenzene was weakly mutagenic for induction of 8AG-resistant mutations (1×10^{-5}). No OUA-resistnt mutations were detected by treatment with this compound. The tumor-forming activity of these aromatic compounds are unknown, but it is necessary to examine their carcinogenicity in experimental animals.

[1] Japan Bioassay Laboratory, Hadano, Kanagawa.

Effects of EGF and FGF on Growth Pattern of Cultured Human Diploid Cells in Clonal Culture

Yukiaki KURODA

Normal human diploid cells are extensively used as a model system of cell aging, since they have a definite life span of about 50 population doublings (PD). In the present study the mode of proliferation of individual cells was analyzed by clonal cultures of young cells (6PDL) and aged cells (18 PDL). The effects of epidermal growth factor (EGF) and fibroblast growth factor (FGF) on the mode of prliferation of both cells were also examined.

The mode of proliferation was analyzed by scoring the number of cells per colony in replicate cultures in many petri dishes which were inoculated with 3×10^3 single cells in 60 mm dishes and fixed every 24 hours in cultivation. In cultures of young cells, beside some synchronously dividing cells which increased in number as 2^n, other asynchronously dividing cells wree observed. In the latter cells, after one cell division one daughter cell proceeded to the next division, but another daughter cell did not proceed to the next division. Some cells remained as single cells in whole culture period of 7 days.

In cultures of aged cells, the mode of proliferation was almost the same as that of young cells. But the rate of proliferation was prolonged in aged cells. During cultivation for 72 hours a most actively dividing cell produced 42 cells after more than 5 divisions in young cell cultures, whereas a most actively dividing cell in aged cell cultures produced only 10 cells for the same culture period.

When EGF or FGF was added to medium, in young cell cultures the proliferation rate was markedly stimulated by these growth factors, although the mode of proliferation remained unchanged. The number of cells which remained as single cells started to divide again and the number of single cells decreased. This indicates that these growth factors had stimulatory effects on the proliferation of both dividing and non-dividing cells in young cell population.

On the contrary, the proliferation of aged cells were not or slightly affected by EGF and FGF. This suggests that human diploid cells may be reduced in their responsibility to these growth factors as their culture age increased.

Hypertrophic Growth of Neuroblasts in a *Notch* Lethal, *Df (1) Notch 8* of *Drosophila melanogaster*

Kiyoshi MINATO

The lethal alleles at the *Notch* locus of *Drosophila melanogaster* are known to be lethal in embryogenesis, producing an extensive hypertrophy of nervous tissue. To know whether this hypertrophy is due to either the formation of too many neuroblasts segregated from the ectodermal layer in the early period of embryonic development or an increased rate of cell divisions of neuroblasts, one of *Notch* lethals, *Df(1) Notch 8* was analyzed for the growth of nervous tissue in the histological preparations, by using a recently developed technique with more efficient fixation (Zalokar, 1976) and embedding in resins which made an easy orientation of fixed materials.

At the final stage of nerve differentiation 10 or 12 hours after fertilization, the fully condensed central nervous system was seen in normal embryos. At this stage, there were about twice as many ganglionic cells, though loosely condensed, in lethal embryos as those in normal embryos. At the earlier stage of 5 or 6 hours, neuroblasts or ascendants of ganglionic cells became firstly distinguishable from ectodermal cells. At this stage, there were already about twice as many neuroblasts in lethal embryos as those in normal embryos. Thereafter the mitotic activity of neuroblasts in lethal embryos was not different from that in normal embryos.

These results indicate that the hypertrophy of nervous tissue in this mutant embryo may be due to only the hypertrophy of neuroblasts at the earlier stage of embryogenesis.

Morphogenetic Potentials in Chimeric Hydra Strains

Tsutomu SUGIYAMA, Jun TAKANO and Chiemi NISHIMIYA

In order to determine which cell types in hydra are responsible for morphogenetic potentials, we have constructed chimeric hydra from the standard wild type (105) and the regeration-deficient strain (reg-16) or the slow budding strain (L4). Reg-16 has a greatly reduced head regenerative capacity and L4 has a very low budding rate and is large in size. Both strains have a very low head-activation potential and a high head-inhibition potential comparing to 105.

Hydra tissue consists of three self-renewing cell lineages: the ectodermal epithelial cell lineage (referred to as Ect), the endodermal epithelial cell lineage (referred to as End) and the interstitial cell lineage (referred to as I) which includes interstitial stem cells and their differentiation products, nerve cells and nematocytes. By combining these cell lineages between two strains it is possible to construct chimeric strains (Marcum and Campbell, 1978; Wanek, 1981). For example, from 105 and reg-16 the following 6 chimeric strains can be constructed: $Ect_{105}/End_{105}/I_{reg\text{-}16}$, $Ect_{reg\text{-}16}/End_{reg\text{-}16}/I_{105}$, $Ect_{105}/End_{reg\text{-}16}/I_{105}$, $Ect_{105}/End_{reg\text{-}16}/I_{reg\text{-}16}$, $Ect_{reg\text{-}16}/End_{105}/I_{105}$, $Ect_{reg\text{-}16}/End_{105}/I_{reg\text{-}16}$.

The head regenerative capacity of chimeric strains constructed from 105 and reg-16 and the budding rate and the size of chimeric strains constructed from 105 and L4 were examined in detail and compared to those of their respective parental strains.

The regenerative capacity was greatly reduced when the reg-16 epithelial cell lineages are present in chimeras, although it was slightly higher than that of reg-16. The influence of the reg-16 endodermal epithelial cells was larger than that of the reg-16 ectodermal epithelial cells on head regeneration. The influence of the reg-16 interstitial cells on head regeneration was slight but significant. These results indicate that the regenerative defect of reg-16 resides mainly in its epithelial cells and that the endodermal epithelial cells contribute more to head regeneration than the ectodermal epithelial cells.

The budding rates and the sizes of chimeras constructed from 105 and L4 resembled to their donor strain of the ectodermal epithelial cell lineage. The endodermal epithelial cells and the interstitial cells showed no influence on these traits. These results indicate that the ectodermal epithelial cells are the determinant of the budding rate and the size of hydra.

Position Dependent Nematocyte Differentiation in Hydra

Toshitaka FUJISAWA

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), all of which are differentiated from multipotent interstitial stem cells.

The spatial distribution of the differentiating nematocytes along the body

column is very characteristic. For example, stenoteles are differentiated predominantly in the lower budding region and their number decreases gradually in the more distal regions, while desmonemes and isorhizas are differentiated mainly in the distal half of the body column.

In order to know the mechanism by which the position dependent pattern of nematocyte differentiation is formed, stenotele differentiation was analysed in detail.

Among possibilities the following two are most likely for the position dependency of stenotele differentiation: (1) The inhibitor for stenotele differentiation is produced in the head and its concentration decreases in the more proximal regions. More stenoteles are differentiated in the regions where the inhibitor concentrations are lower. (2) The stimulator for stenotele differentiation is produced in the foot and its concentration decreases in the more distal regions. More stenoteles are differentiated in the regions where the stimulator concentrations are higher. The following two experiments were carried out to differentiate these possibilities.

1) The head tissue was grafted laterally into the lower budding region to see if the grafted tissue inhibits stenotele differentiation. The foot tissue was grafted laterally into the distal region to see if the grafted tissue induces stenotele differentiation. The results showed that only the head tissue inhibited stenotele differentiation.

2) The inhibitor for stenotele differentiation has been found in the hydra tissue. To examine if this factor is most abundant in the head and its concentration decreases gradually in the more proximal regions, the body column of hydra was divided into 5 regions (head, upper body region, middle body region, lower body region and foot) and the concentration of the factor was determined in each region. The results showed that the inhibitor concentration was the highest in the head and it gradually decreased in the more proximal regions.

These results favor the first possibility.

A Single Recessive Gene(ter) Causing Germ Cell Deficiency and an Extremely High Incidence of Testicular Teratomas in 129/Sv-ter Mice

Takehiko NOGUCHI

The genetic basis of a germ cell deficiency which is accompanied by an

extremely high incidence of testicular teratomas, and which is peculiar to the 129/Sv-ter strain of mice (J. Natl. Cancer Inst. **69**: 907–913, 1982) was studied.

In the males, germ cell deficiency became more severe as age advanced. The males having germ cell deficient testes were sterile whether they had bilateral teratomas or not. Of 147 males with abnormal testes 110 (74.9%) had tumors bilateraly, 28 (19.1%) had them unilateraly, and only 9 (6.1%) were tumor free. In the females, however, the deficiency did not progress, and most of the females were fertile.

Germ cell deficient males exhibited smaller testes except when the testes had teratomas. The detection of the abnormal males was possible by observing the appearance of the seminiferous tubules even when they had teratomas. In the females, the germ cell deficiency correlated with smaller ovaries. The establishment of the method of detecting the abnormal individuals made it possible to study the genetic basis of the abnormality. One hypothesis is that the germ cell deficiency may stem from the homozygous state of a recessive mutant gene(ter).

Matings between females with small ovaries and males with normal testes, but without the genetic factor, produced no F1 offspring having germ cell deficient gonads. However if these F1 offspring were mated together, individuals having germ cell deficient gonads appeared again in the F2 generation. This strongly suggests that the genetic factor is recessive. When females having small ovaries were mated with fertile males having the genetic factor, half of the F1 offspring (146/296) had abnormal gonads. If the F1 offspring with normal gonads were mated together, F2 animals with abnormal gonads appeared at a frequency very close to 1/4. These results strongly suggest that the genetic factor is a single recessive gene. This hypothesis implies that this mice colony is composed of animals with three different genotypes, +/+, +/ter and ter/ter.

Teratoma incidences estimated for the males with different genotypes were 1.3% for +/+, 22% for +/ter, and 94% for ter/ter.

V. CYTOGENETICS

Sequentiality of Chromosome Evolution

Tosihide H. YOSIDA

When we observe the karyotypes in some rodent species, the chromosome evolution seems to have occurred sequentially from one to others. For example, the chromosome polymorphism in pair nos. 1, 9 and 13 found in Asian type black rat ($2n=42$) should have occurred by the sequential inversion of these acrocentrics. The Ceylonese and Oceanian type black rats with 40 and 38 chromosomes also seemed to have occurred in southern India by sequential Robertsonian translocation. Another sequential karyotype evolution was observed in the black rats distributed in the Mauritius Island. This island was found by Portuguese in the 16th century. In the Oceanian type black rats migrated to this island the centric fission should have occurred sequentially in metacentric pair nos. 14 and 18 during a short time after the rats landed on this island. Possibilities of species differentiation through the sequential centric fusions were found in the other *Rattus* species, such as *R. fuscipes* ($2n=38$), *R. leucopus* ($2n=34$) and *R. conatus* ($2n=32$) distributed in Australia and New Guinea. Sequential centric fusions and fissions were also observed in *Rattus villosissimus* with 50 chromosomes in Australia.

Karyotype of the tabacco mouse, *Mus poschiavinus*, with 26 chromosomes has been derived from centric fusion of 14 acrocentric pairs (Gropp *et al.* 1970). A similar karyotype change by the centric fusion has been observed in *Mus* (*Leggada*) *minutoides* indigenous to Africa (Matthey 1963). These chromosome variations seemed to have occurred by sequential events of the centric fusion. The Indian spiny mouse, *Mus platythrix*, was characterized by having 26 acrocentric chromosomes due to sequential tandem fusion of each two in several acrocentric pairs (Yosida 1980a). Basic karyotype of the house shrew, *Suncus murinus*, had 40 chromosomes, but those inhaviting in southern India and Sri Lanka had $2n=32$ and 30 (Satya Parakash and Aswathanarayana 1976; Yosida 1982). Karyotypes of the latter specimens have been resulted from the sequential centric fusion of 5 and 6 acrocentric paris, respectively.

A good evidence of the sequential karyotype change was provided in the LEW-strain rat, *Rattus norvegicus*, breeding in the laboratory. In one female a translocation between pair nos. 1 and 12 was primarily observed (Yosida 1980b). In the next generation inversion of pair no. 1 was detected. This was explained that the 1/12 translocation should have spontaneously occurred and in the next generation inversion might have sequentially occurred in the normal chromosome partner in the early embryogenic stage. A new hairless mutant (*ba*) appeared after a few inbreeding generations in the inversion stock of this animal (Yosida 1981). The above findings provide a good evidence for the sequentiality of chromosomal and/or gene mutation.

Parallelism of Karyotype Evolution

Tosihide H. YOSIDA

Phenomena of parallelism of the karyotype evolution due to the pericentric inversion, Robertsonian fusion and fission has been found frequently in genus *Rattus*. Some examples of the parallelism will be described in *Rattus* and some other mammals and discussed on a mode of the karyotype evolution.

1) Examples in genus *Rattus*: Basic karyotype of the black rat (*R. rattus*) with $2n=42$ was characterized by 13 acrocentric (nos. 1 to 13) and 7 metacentric autosome pairs (nos. 14 to 20) and acrocentric X and Y (the Asian type). Acrocentric and subtelocentric polymorphism due to pericentric inversion was observed in pair nos. 1, 9 and 13 of this animal. Some other *Rattus* species, such as *R. annandalei*, *R. muelleli*, *R. exulans*, *R. losea* and *R. norvegicus* had $2n=42$ and their karyotype were similar to the black rat, but pair nos. 1, 9 and 13 were either one of the acrocentric or subtelocentric. It seems to have occurred by parallelism of karyotype evolution in these *Rattus* species.

Parallelism of the karyotype evolution by the Robertsonian fusion was found in genus *Rattus*. Both Ceylonese type black rat, *R. rattus kandianus*, and *R. bowersii* had $2n=40$ with one large metacentric pair derived from the Robertsonian fusion between acrocentric nos. 11 and 12. A similar karyotype to the Oceanian type black rat with ($2n=38$) having two large metacentric pairs due to the Robertsonian fusion was found in *R. fuscipes* living in Australia. Karyotype evolution by the other Robertsonian fusions was observed in *R. leucopus* ($2n=34$) and *R. conatus* ($2n=32$). They should

have developed by parallel karyotype evolution due to Robertsonian fusion. Mauritius type black rats were characterized by having 4 small acrocentric pairs due to the Robertsonian fission of two small metacentric pairs, nos. 14 and 18. A parallel karyotype evolution to the Mauritius black rat was found in *R. villosissimus* ($2n=50$), which was remarkable by having extra 12 small acrocentric pairs derived from the Robertsonian fission of 6 small metacentric pairs, nos. 14 to 19.

2) Examples in the other mammals: Evolution by inversion of chromosomes was often reported in the other genus in Rodentia, such as *Peromyscus*, *Neotoma*, *Thomomys*, *Ctenomys* and some others. A typical case of the parallel karyotype evolution through the Robertsonian fusion was found in two different species belong to genus *Mus*, *Mus minutoides* distributed in Africa and the tabacco mouse (*Mus poschiavinus*) distributed in Europe. Parallel karyotype evolution through the Robertsonian fusion was reported in the other taxon, such as Bovidae, Equidae, Felidae and Primates. Karyotypes in mammals would have evolved through such parallelism and sequentiality described in the previous report.

Cytogenetical Study on a Mammary Cancer of the Domestic Cat with a Note on an Increase of Cells with a Normal Karyotype after Deep Freezing

Tosihide H. YOSIDA and Kyoko KAWAHARA

Karyotypes of a mammary cancer spontaneously developed in the domestic cat were analysed in comparison with those of normal somatic cells ($2n=38$). Normal karyotype of the domestic cat was characterized by having $2n=38$, consisting of eighteen autosome pairs classified into A to F groups. Chromosome number in 100 cancer cells growing *in vitro* of the mammary cancer ranged from 26 to 110. Interesting is the fact that this tumor was characterized by having the bimodal distribution of chromosome numbers; hypodiploid and hypotetraploid. In hypodiploid cells several chromosomes were monosomic consisting of one mode at 31 chromosomes. Normal diploid chromosome number ($2n=38$), however, was found only in two cells (2%). Such chromosome constitution was observed for about 10 culture generations, so far the cultivation was continued by use of the same technique.

The primary culture of the tumor cells was stored into the deep freezer

at −80°C by use of 10% DMSO for 7 days. The cells thus frozen for 7 days were cultured again in the same culture median as above. Shape of cultured cells growing *in vitro* after deep freezing was not different from those before freezing. Chromosome numbers in tumor cells in one batch (A) were counted in 180 cells in the first culture generation after freezing. Among them 159 cells (88.3%) had a normal diploid number of 38 chromosomes. In the culture from the other batch (B), 50 cells were counted, among them 46 cells (92.0%) had 38 chromosomes. The karyotypes of all cells with 38 chromosomes obtained from these batches were normal. These complements were maintained for about 10 generations so far the cells cultured *in vitro*. It could not be examined by us whether the cells with the diploid karyotype in the feline tumor developed after deep freezing have still malignant characteristics or not, because the diploid cells could not be transplanted to the same host. If the tumor malignancy has reduced by increase of diploid cells after deep freezing, the evidence presented may be available for seeking any clue for medical treatment of the tumor.

Remarkable Difference on Frequency of Single and Twin Sister Chromatid Exchanges in Endoreduplicated Blood Syndrome Cells

Yukimasa SHIRAISHI[1] and Tosihide H. YOSIDA

The increase rate of sister chromatid exchanges (SCEs) in Bloom syndrome (BS) cells has been known, but its mechanism is unknown. Shiraishi *et al.* (1981) showed that when BS cells labeled with bromodeoxyuridine (BUdR) for one round of DNA replication were fused with nonlabeled normal cells, the hybrid cells had a normal level of SCE at the first mitosis after fusion. On the other hand, single and twin SCEs in endoreduplicated cells have been shown that an equal number of SCEs occurred in each of the two cell cycles. On the basis of these data, a 50% reduction in BS chromosome SCE frequency could theoretically be expected to be observed one cell cycle after fusion. But we observed a clearcut normalized level of SCE. Therefore, the theoretical half reduction is clearly inconsistent with that of our exact data (Shiraishi *et al.* 1981). In order to know, the frequency of SCE in the first and second cell cycles the frequencies of single and twin SCE were analysed in endoreduplicated cells induced by treatment

[1] Kochi Medical College, Nangoku, Kochi-ken.

with colcemid. Each SCE that arised during S1 appearred as a twin SCE at the second mitosis (M2), and an exchange that occurred during S2 gave rise to a single SCE in only one of the pair of daughter chromosomes at M2. In the present study we demonstrated that though a manyfold increase of single SCEs was detected, twin SCEs were very rare. Namely 141 single SCEs on the average were counted in endoreduplicated BS metaphase, while only 5.4 twin SCEs were countable. This finding strongly suggests that most of BS SCE occur during second cell cycle when BrdU-containing DNA is used as a template for replication.

Effect of BrdU on SCE in Welsh Onion and D-6 Chinese Hamster Cells by Treatment with 5-Fluorodeoxyuridine

Hitoshi SUZUKI, Michiharu SEGAWA,[1] Tosihide H. YOSIDA and Katsuhiro KONDO[1]

5-Fluorodeoxyuridine, an inhibitor of thymidilate synthetase catalyzed conversion of dUMP to dTMP, induced sister chromatid exchanges depending on the concentration of BrdU used for chromosome label during the two cell cycles to detect SCEs in D-6 Chinese hamster *in vitro* as well as Welsh onion *in vivo*. On the contrary, mitomicin C did not produce, at any concentration, any dependent-effect of 5-Bromodeoxyuridine. In D-6 cells, when BrdU label was carried out only during the first cell cycle and FdU was added to the culture medium in the absence of labelling BrdU during the second cell cycle, the SCE induction caused by FdU was completely suppressed by a synchronous addition of thymidine or BrdU, while the induction was not suppressed by deoxycytidine or uridine. Based on these studies, it was suggested that SCE induction by FdU is depended on the thymidylate starvation in the process of DNA synthesis.

[1] Faculty of Integrated Arts and Science, Hiroshima University.

Multiple Sex Chromosome Mechanism (XX-Y) Found in Two dragonet Fishes

Makoto MUROFUSHI,* Shohei NISHIKAWA** and Tosihide H. YOSIDA

Although it has been considered that the sex-chromosomes are not differentiated in fishes, XX-Y mechanism has been found by us in filefish (Murofushi *et al.* 1980). Very similar case of the multiple-sex-chromosome mechanism has been found to occur in two dragonet fish species, *Callionymus beniteguri* and *C. ornatipinnis* collected from the near coast of Yamaguchi and Shizuoka Prefecture. The diploid chromosome-number of the female specimens of these two dragonet species was thirty-eight ($2n=38$), all elements being subtelocentric. In contrast, the diploid chromosome-number of the male ones was thirty-seven ($2n=37$), among which one large metacentric chromosome was conspicuous in their karyotypes. The metacentric element occurring only in the male specimens was the Y chromosome. Based on the different chromosome-numbers in the male and the female in these species it is suggested that the male has the X_1X_2-Y, while the female has the X_1X_2-X_1X_2. To confirm the above findings the secondary spermatocytes were investigated in the male specimens of these two species. There were two cell types, one of them being $n=19$, whereas the other $n=18$. In the former cells, one metacentric chromosome was always present, whereas in the latter, no such element was found. The above findings have shown that the sex-chromosome mechanism of these two species is male heterogametic with an X_1X_2-Y mechanism.

The metacentric Y chromosome in the multiple sex-chromosome mechanism occurring in these fishes should have been derived from the Robertsonian fusion between an ordinarily acrocentric Y and one member of an autosome pair. These findings suggest that the Robertsonian fusion between the Y chromosome and an autosome is to be one of the mechanisms in morphological differentiation of the sex-chromosome in fishes.

* Mishima Junior College, Nihon University, Mishima.
** Shimonoseki University of Fisheries, Shimonoseki.

Effect of H-2 Complex on the Frequency of Urethan-induced Chromosome Aberrations in the Mouse Bone Marrow Cells

Kazuo MORIWAKI, Nobumoto MIYASHITA and Shinji NITO*

We reported that the susceptibility to urethan induced lung tumor is genetically controlled by H-2 complex, in addition to ptr gene. To pursue the mechanism of genetic regulation by H-2 complex for the induction of lung tumor, the frequency of chromosome aberrations after urethan treatment was surveyed in various H-2 congenic strains. To 6 weeks mice, 10% urethan was given at 0.5 mg/g B.W.. After 24 hrs, valid chromosome breaks were scored in the bone marrow cells. Percentage of aberrant cells was 22.3% in A strain ($H\text{-}2^a$), whereas 11.0% in A. BY ($H\text{-}2^b$), 10.5% in A. CA ($H\text{-}2^f$) and 6.8% in A. SW ($H\text{-}2^s$). This tendency was confirmed in B10 H-2 congenic mice as well. Micronucleus test done in the same combinations of the congenic mice exhibited parallel results with those in chromosome breaks. Further cytogenetical surveys in H-2 recombinant strains, A. TH and A. TL, suggested the possible relevance of a locus to the right of H-2IE region.

Implications of the Genetic Divergence between European Wild Mice with Robertsonian Translocations from the View Points of Mitochondrial DNA

Kazuo MORIWAKI, Hiromichi YONEKAWA**, Osamu GOTOH**, Mitsuru MINEZAWA***, Heinz WINKING**** and Alfred GROPP****

Genetic divergences between the wild mouse populations with various Robertsonian translocations from the Poschiavo Valley, Yougoslavia, Milan and the Apennines, were estimated based on the mitochondrial (mt) DNAs. The mtDNA isolated from the liver were analysed by agarose slab-gel electrophoresis after digestion with eight kinds of restriction endonucleases; BamHI, EcoRI, HindII, HindIII, PstI, HpaI, HpaII and BglI. These preparations were further used to make restriction maps. From which approximate divergence time between each Rb variation was calculated to be in the order of 10^5 years (Table 1). These data appear to be in con-

* Tanabe Seiyaku Co.
** Saitama Cancer Center Research Institute, Saitama.
*** Primate Research Center, Inuyama.
**** Medizinische Hochschule Lübeck, Lübeck.

Table 1. Effect of H-2 complex on the urethan-induced chromosome aberrations and micronuclei

Strain	Chromosome aberration test			Micronucleus test	
	Number of observed cell	Breaks per cell (%)	Percentage of aberrant cell	Number of observed cell	Micronucleus per cell (‰)
B10	800	19.1±4.0	14.3±2.3	4000	27.5±6.6
B10. A	700	39.3±4.9	22.9±3.4	4000	38.5±6.0
B10. BR	400	37.0±9.1	22.3±3.6	4000	21.0±4.2
B10. D2	600	30.7±4.4	21.2±1.5	4000	22.8±6.0
B10. S	300	31.3±1.5	20.0±1.0	4000	28.5±6.2
(B10×B10. A)F_1	800	35.9±8.9	20.4±3.3	4000	38.0±4.4
B10. A (2R)	300	22.7±1.2	15.7±4.0	Not Tested	
B10. A (3R)	300	37.7±11.1	25.7±5.8	6000	40.8±4.2
B10. A (4R)	700	20.0±6.7	14.3±4.4	6000	29.2±4.6
B10. A (5R)	400	42.3±4.2	27.3±1.5	6000	40.0±5.1
B10. S (9R)	400	46.0±6.7	27.0±2.2	Not Tested	
A/Wy	700	31.1±9.0	22.3±2.1	4000	30.3±5.4
A. AL	500	42.0±6.6	22.2±1.6	6000	23.8±4.0
A. TL	400	19.5±1.0	17.0±0.8	4000	25.3±5.9
A. SW	400	6.8±2.6	6.8±1.3	4000	18.8±9.3
A. TH	400	13.8±1.3	10.5±1.3	Not Tested	
A. CA	400	10.5±1.0	10.5±1.3	6000	30.3±4.2
A. BY	600	13.3±3.7	11.0±1.4	4000	28.0±4.9

Table 1. Sequence divergence and time of divergence estimated from mtDNA among five variant Robertsonian populations 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

flict with the present concept that the Rb variations occurred during the last several thousand years. Both, however, might be reconciled by assuming genetic introgression of the founder with a small number of Rb translocations into other subspecies populations genetically remote by the order of 10^5 years, and the subsequent rapid accumulation of Rb translocations unique to each population due to a hybrid dysgenesis-like mechanism. Occurrence of a new Rb (9, 15) translocation in the intersubspecies hybrid between *M. m. molossinus* and *M. m. domesticus* on the Ogasawara Islands in Japan may support this notion.

The Effects of H-2 Complex to the Mutagen-Induced Chromosome Aberrations and Micronuclei

Shinji NITO*, Nobumoto MIYASHITA and Kazuo MORIWAKI

Moriwaki *et al.* (1983) have recently reported that the H-2 complex affects the frequency of urethan-induced chromosome aberrations.

In the present study, the effects of H-2 complex was examined with the 6 chemical mutagens (cyclophosphamide: CP, mitomycin C: MMC, adria-

* Tanabe Seiyaku Co.

mycin: AD, potasium chromate, colchicin and colcemid) and γ-ray.

We used 6-weeks male mice of the B10 and B10. A congenic strains, and the sensitivity to the mutagens (in terms of chromosome aberrations and micronuclei) was compaired between the two strains, in which the haplotype of the B10 and B10. A is H-2^b and H-2^a respectively, and the latter is sensitive to urethan.

The results are summarized in Table 1. The table clearly indicates that the B10. A strain is more sensitive than the B10 strain to most of mutagens, except colchicin and colcemid which do not attack DNA molecule. Almost the same tendency was obtained by the two methods employed (i.e., chromosome aberration and micronucleus test).

The high sensitivity of the B10. A strain may be due to the DNA repair capacity, and is controled by genes linked to the H-2 complex.

Table 1. Comparison of effects of H-2 complex to the mutagen-induced chromosome aberrations and micronuclei between the B10 and B10. A congenic strains

Compound	Dose (mg/kg)	Number of breaks per cell (%)		Number of micronuclei (‰)	
		B10 (H-2^b)	B10. A (H-2^a)	B10 (H-2^b)	B10. A (H-2^a)
CP	25	22.5±10.6	50.0±4.2*	25.7±4.3	34.3±5.4*
MMC	1.5	18.0±0.0	40.5±7.8*	44.5±3.2	54.7±6.0*
AD	5	15.5±2.1	19.0±2.8	29.0±3.6	36.5±5.9*
Potasium cromate	50	NT	NT	5.0±0.6	12.1±2.1*
Colchicin	1	NT	NT	28.3±5.0	24.2±1.6
Colcemid	10	NT	NT	10.7±2.6	2.7±2.4
γ-ray	200R	48.5±7.8	70.5±5.0*	38.0±3.9	33.5±4.6
	200R	69.0±0.0	85.5±0.7*	(+)	(+)

N.B. Mean±SD. NT: Not tested. *: $P<0.05$. (+): Gross destruction of bone marrow.

VI. MUTATION AND MUTAGENESIS IN ANIMALS

Genetical Behaviour in the Silkworm (*Bombyx Mori* L.) Carrying an X-Autosome Translocation

Akio MURAKAMI

Translocations of the silkworm Y (previously W) to other chromosomes including X(Z), could be easily detected. Several cases of such type translocations have been published and utilized in agricultural industry to date. While no translocation of X to autosome has yet been published. Recently we found two X-autosome translocations in this insect which had been treated pupal oocytes with chemical mutagens, MNNG and Sterigmatocystine (STC). These two lines have a translocation between X chromosome and an autosome no. 5 linkage group. The present communication describes genetical behaviours of the firstly obtained X-5 translocation which has been derived from an MNNG-induced fractional-body mutant expressed both wild-type black (+) and yellowish-white (*pe*) serosa membrane cells in the egg. According to the genetical analysis of this line, a proximal piece of the 5th linkage group chromosome which contains the wild-type allele of the *pe* (pink eye and pink egg, 0.0), but not the ok^+ locus (5: 4.7) is translocated onto the X chromosome or $\widehat{X^{pe+}}$. This translocation line is designated to be as the T(X: 5)[1]. Another X-5 translocation [T(X: 5)[2]] is originated in STC-induced fractional-body mutant and is also not included at the ok^+ region, $\widehat{X^{pe+}}$.

When the females having the translocated X chromosome $\widehat{X^{pe+}}$ and heterozygous for egg-colour genes ++/*pere* or $\widehat{X^{pe+}}$/Y: ++/*pere* were crossed with the males homozygous for the normal X chromosome and the egg-colour genes, X/X: *pere*/*pere*, female black (X/Y: ++/*pere*) and yellowish-white (X/Y: *pere*/*pere*) eggs in their progeny would have been free from the translocated chromosome. In male progeny, however, both black ($\widehat{X^{pe+}}$/X: ++/*pere*) and red ($\widehat{X^{pe+}}$/X: *pere*/*pere*) eggs should be the translocation heterozygous for X chromosome. Consequently, the number of black and red eggs should be expected in a ratio of one to one. In other words, the number of black, yellow, and red eggs per batch should be segregated in a ratio of 2: 1: 1. For reference, as is well known, egg-colour

for the *pe* and *re* gene is due to the colour in somatic (serosa-membrane) cells and the *pe* gene is epistatic to the *re* gene, so that eggs homozygous for *pe* and *re* genes turn yellowish-white in their serosa cells.

Whereas the number of black eggs was clearly less than the expectation. In opposition, yellowish-white eggs distinctly exceeded the expectation and red eggs came short of the expectation. In addition to these unusual segregations, a significant number of fleckered eggs which are composed of black and yellowish-white serosa cells. The decrease of black or red eggs and the increased of yellowish-white eggs might be interpreted on the assumption that the pe^+ segment on the X chromosome did not at least partly affect on the *pe* gene on the autosome no. 5 in the heterogametic male. The selection for cells having the $\widehat{X^{pe+}}$ chromosome might have been influenced on the decrease of the black eggs in males. It is also not reasonable to fully exclude a possibility of a loss of the pe^+ segment on the X chromosome. The occurrence of the fleckered eggs might be caused by an incomplete affection of the pe^+ to the *pe* gene on the autosome no. 5. By the way, the cross between females having chromosome constitution, $X/\widehat{Y^{pe+}}$: ++/*pere* and males having X/X: *pere*/*pere*, it could not observed the unusual phenomenon. The abnormal observation in the $T(X/5)^1$ line seems to be analogous to findings in mice (Lyon, 1961; Russell *et al.*, 1961; etc.) interpreted as the X chromosome inactivation hypothesis. It is possible to suppose that mechanisms responsible for the X chromosome inactivation appear to be a universal phenomenon regardless of the heterogametic female or male. In any case, it required much more information to get a final conclusion whether or not the present finding with the $T(X: 5)^1$ line in the silkworm is due to the X chromosome inactivation.

The Induction of Gynogenesis and Androgenesis in the Silkworm and the Mechanism of Their Induction

Susumu TAMAZAWA* and Akio MURAKAMI

Gynogenesis and androgenesis are known to be induced in the silkworm relatively easily by low temperature treatment of the egg, but the mechanism of the induction has not been elucidated. Whether the ontogenesis is derived from fusion of the egg nucleus and polocyte, fusion of two sperm nuclei or from a single female or male nucleus can be theoretically assumed from

* Faculty of Agriculture, Hokkaido University, Sapporo.

the growth stage of the female and male nuclei in the eggs at the start of the treatment. Therefore, we analyzed ontogenetically the relationship between the mechanism of gynogenesis or androgenesis and the developmental stage of the sperm and egg nuclei. For the detection of androgenesis, we used the hybrid: w_2^+/w_2^+; ch^+/ch^+; p^s/p^s♀ × w_2/w_2; ch^+/ch; p^+/p^+♂ and for the detection of gynogenesis: w_2/w_2; ch^+/ch; p^+/p♀ × w_2^+/w_2^+; ch^+/ch^+; p^s/p^s♂. Thirty minutes was allowed for egg laying, and the eggs collected between 0 and 210th minute after being laid were kept at 0° or 10°C for 24 hours to induce abnormal ontogenesis. To identify the origin of the individuals thus obtained, they were backcrossed with a line having w_2, *ch* and *p* genes homozygously. Then, the segregation of the various characters in the BF_1 were obtained.

In the BF_1 of the individuals induced from the eggs laid during 0–60 minutes, in the experiment to induce androgenesis, ch^+ : *ch* and p^+ : *p* were segregated at the ratio of 1 : 1 which confirms that they had been derived from the fusion of 2 sperm nuclei. On the other hand, in the BF_1 of the individuals induced 60–120 minutes in the same experiment, only ch^+ and p^+ were segregated in most, which confirms that they had been derived from a single sperm nucleus. The mosaic individuals with ch^+ and *ch* detected simultaneously were presumed to have derived from 2 sperm nuclei with different genetic types.

In the experiment to induce gynogenesis, in contrast to the male nucleus ontogenesis, both male and female were induced from gynogenetic individuals. The females all showed dominant larval characters (ch^+; p^+), and the male individuals had either dominant (ch^+; p^+) or recessive (*ch*; *p*) character. In the BF_1 of the female individuals hatched from normal eggs, ch^+; *ch*, p^+; *p* and ♀ : ♂ segregated at the ratio of 1 : 1, which confirms that they had been derived from the fusion of egg nuclei and polar bodies with different genetic types. In the BF_1 of the male individuals, only the dominant characters (ch^+ or p^+) were segregated, and they had normal ability to fertilize. Thus, the males were confirmed to be 2n and had derived from the egg nucleus with X chromosomes. Simultaneously, triploids and tetraploids were frequently induced, but the mechanism of induction of these polyploids is under examination.

In the present experiments, it has been genetically confirmed that androgenesis and gynogenesis can be induced by low temperature treatment of the egg, and that as has been suggested previously, besides the individuals

derived from the fusion of 2 sperms or egg nucleus and polocyte individuals produced by androgenesis and gynogenesis derived from single gamates, *i.e.*, monogamy, can develop normally and they are capable of fertilization. Low temperature treatment was suggested to have some effect on the ploidy of chromosomes (DNA) of female and male (pro)nucleus and to promote the development of the individual just as if it was fertilized normally.

Cytological Analysis of Mutations Produced by Super-cooling of Silkworm Eggs at an Early Stage of Development
I. Polyploids

Susumu TAMAZAWA* and Akio MURAKAMI

Polyploids of silkworms are relatively easily induced by various physical and chemical treatments of the egg. The polyploids frequently induced are triploids. Although tetraploids were indirectly obtained by crossing $6n♀ \times 2n♂$, there were few cases of tetraploids induced directly by physical or chemical treatment. However, when the oocytes obtained by crossing between the stocks labeled by visible genes (p/p; re^+/re^+, w_2/w_2, $ch/ch \times p^s/p^s$, re/re, w_2^+/w_2^+, ch^+/ch^+) were exposed to $-10°C$ for 24 hours at the stage just after to 90 minutes after the egg was laid, *i.e.*, at first meiosis, and then incubated at 25°C for 20 hours, hatched and reared by standard methods, along with triploids which are presumed to have been produced by conjugation of a diploid synkaryon of oocytes produced by low temperature treatment and one sperm as has been reported, tetraploid females were found to be produced by syngamy of a triploid synkaryon produced during the meiosis of autosome constitution ($p/p/p/p^s$, $w_2/w_2/w_2/w_2^+$, $ch/ch/ch/ch^+$) and one sperm. But no tetraploid males have been detected to date. The autosome constitution of the egg given low temperature treatment during the period beginning at the 90th minute after being laid until the 210th minute (the first cleavage stage) via syngamy of egg (sperm)-pronucleus stage, was $p^s/p^sp/p$, $w_2^+/w_2^+w_2/w_2$, re^+/re, $ch^+/ch^+ch/ch$, and the sex chromosome constitution of the female and male tetraploids X/X Y/Y and X/X X/X were obtained at the same frequency. The explanation given is that although normal chromosome duplication of the normally fertilized nucleus can proceed at the first egg cleavage stage, the spindle fiber is inactivated by the low temperature treatment. Thus the two daughter nuclei cannot

* Faculty of Agriculture, Hokkaido University, Sapporo.

divide and male and female tetraploids with 112 chromosomes are induced.

Cytological Analysis of Mutations Produced by Super-Cooling of Silkworm Eggs at an Early Stage of Development

II. Deletion of Chromosomes

Susumu TAMAZAWA and Akio MURAKAMI

We previously reported that many polyploids, mosaics including gynandromorphs, abnormally developing individuals derived from mono (or di) sperm(s), and individuals induced by fertilization of single egg nucleus or egg nucleus and polocyte were produced when the eggs obtained by the above mentioned cross or its reverse cross were treated with low temperature, $-5°C$ (4 days) $\sim -10°C$ (1 day), during the early stage of development, that is, between the 90th minute after being laid which was immediately after completion of meiosis and the 210th minute after being laid. Besides these, many mottled silkworms with different degrees of deletion of fragmental chromosomes which include the p^s locus on chromosome 2, were detected. Mottled silkworm have been reported to be induced by ionizing radiation. In such cases it has been considered that ionizing radiation causes chromosome breakage and in the cells from which the chromosome fragment containing the dominant gene p^s factors has been released, the recessive character (p) is expressed, and thus both recessive and dominant characters are expressed in a complicated pattern. This suggests that chromosomal fragmentation is also induced by low temperature treatment, and the fragmented chromosome is of the diffuse kinetochore type. Among the F_1 of the treated individuals, normal (p) silkworms were frequently detected besides the expected type, black stripes (p^s/p). Genetical analysis of several of such exceptional silkworms showed that the spots of the larval character were detected only in p and not in p^s silkworms. This shows that mutants were induced in p^s (2–0.0), but this has been explained to be due to deficiency of chromosomes caused by chromosome breakage near p^s rather than to the induction of gene mutations by the low-temperature treatment. Furthermore, many chocolate newly hatched larvae (*ch*) were detected among the F_1 besides the expected black newly hatched larvae (ch^+/ch), and the chocolate newly hatched larvae were also considered to be due to deletion at the ch^+ locus of the allele of *ch* (13–9.6) because none of the BF_1 examined for their phenotypic expression had the ch^+ factor. Besides these mutants,

kidney shaped eggs (*ki*) and grey eggs (*gr*) were also found. By elucidating the genetic characters of these mutants, a conclusive decision can be made on whether the above mentioned exceptional silkworms were produced by breakage of chromosomes or by gene mutations.

Locus Differential Sensitivity for the Induction of the Specific-Locus Mutations after Irradiation of Primordial Germ-Cells with X-rays

Akio MURAKAMI

In the silkworm, various systems for measuring the mutagenicity of internal β-emitters at low dose-rates have been devised. Among them the experiments have extensively been carried out with the use of primordial germ-cells in diapausing eggs (or embryos). However, this system is lacking in several data on the mutagenic response of the primordial germ-cells to radiations, among other things, the dose-mutation frequency relationships. The present communication reports the results of experiments for the dose-response kinetics in the stage germ-cells induced with external X-irradiation by the egg-colour specific-locus method. Diapausing eggs of wild-type (*C108*$\times$*Aojuku*)F_1 hybrids were irradiated with six different doses (125, 250, 500, 750, 1000, and 1250R) of 180 kVp X-rays (25 mA, 1.0 mm Al filter and a dose-rate at 1R/min). Non-treated materials were set up for the control. After completion of diapause, both treated and non-treated eggs were incubated at 25°C and hatched larvae were bred in the usual way. The resultant moths were mated with marker stocks homozygous for egg-colour genes, *pe* and *re*, on the 5th chromosome for detecting the recessive visible mutations.

The dose-mutation frequency curves obtained were non-linear (or multi-hit) fashion regardless of sex and clearly varied with the locus. Mutagenicity of males was significantly higher than that of females as well as other stage germ-cells. The mechanism responsible for this sex-differential sensitivity is not yet clear, but it may be caused by different repairability and germinal selection for the cells having lesion between the sexes. Also, the difference in mitotic division times and in time for the duration of one cell cycle in primordial germ-cells (and gonial cells) may have influence on the sex differential radiosensitivity.

The dose-response curves for both loci showed the same fashion to 750R,

but mutagenicity of the pe^+ locus was higher than that of the re^+ locus. The curve at the pe^+ locus was exponentially increased up to the highest dose at 1250R. At the re^+ locus the curve was slightly increased from 750R to 1000 R and declined to some extent at 1250 R. This difference in the dose-response curve between the loci might be due to a gene site (locus) on the chromosome: the pe^+ gene locus is locating on the proximal site (5: 0.0), whereas the re^+ locus on the middle portion (5: 31.7). According to an well accepted opinion, most of the specific-locus mutations in higher organisms are considered to be either small deletion or deficiency type rather than gene mutations at the molecular level. If this view is correct, the system used in this experiment, a single-break event at the pe^+ locus, would be sufficient to induce the deficiency and two-break events at the re^+ locus required for induction of the deletion. Such the difference in mutational events between the loci would be affected a viability of mutants detected. Actually, the viability of mutants at the pe^+ locus was clearly higher than that at the re^+ locus. The cluster size for the pe^+ locus mutants per batch basis was also significantly larger than that for the re^+ locus.

It should be noted that hatchability for the mutants after treatment of primordial germ-cells with X-rays was markedly lower (less than 10%) when compared with other stage germ-cells irrespective of locus. In addition, almost of all larvae hatched were lost before progeny tests. The marked reduction of hatchability in F_1 and of viability in F_2 appeared to be analogous to either dominant or inherited lethality, suggesting that X-rays could induce a fairly large number of chromosomal rearrengements including translocations and many other things in the primordial germ-cells of silkworms.

A Comparison of Mutagenic Activity of Ethylmethanesulphonate (EMS) and Methylmethanesulphonate (MMS) in Oocytes of the Silkworm

Akio MURAKAMI

In the silkworm, EMS is more highly mutagenic to pupal spermatozoa by the egg-colour specific-locus method than MMS. According to the recent studies on the dose-mutation frequency relationship in the spermatozoa with EMS and MMS, it showed a linear fashion at a dose down to a non-effective (or threshold) dose. Based on these data, the non-effective dose

of EMS was calculated to be 1×10^{-3} and that of MMS was 4.2×10^{-3} μl/pupa. Similarly, diethylsulphate (DES) is more mutagenic to the spermatozoa than dimethylsulphate (DMS). Such the tendency is extended to nitrosoguanidines, ENNG and MNNG. These findings suggest that in the silkworm, ethylating agents are generally potent mutagens to the low metabolic spermatozoa as compared with methylating ones. The mechanism responsible for this differential sensitivity of the alkylating agents in the spermatozoa is not clear. To understand the mechanism, we intended to study the mutagenic activity of EMS and MMS in highly metabolic premeiotic oocytes at the mid-stage of pupae.

Freshly prepared EMS and MMS solution (1×10^{-3} to 1×10^{-1} μl/pupa) in 0.85% NaCl were administered by the injection method into the body-cavity of mid-stage wild-type F_1 (*C108*$\times$*Aojuku*) female pupae. Controls were applied with 0.85% NaCl solution alone. After emergence, they were mated with the marker stock homozygous for *pe* and *re* gene loci to detect the recessive visible mutations.

The dose-response kinetics in the premeiotic oocytes with EMS showed a sigmoidal fashion regardless of loci. The frequency of mutations in this germ-cells treated with a nearly killing dose of MMS was just increased above the control level. This situation was almost similar to dialkylsulphates, DMS. The mutagenic activity of these chemicals in oocytes at the late stage of pupae had also been made by the specific-locus method. In the EMS treatment group, a marked increase of the mutational incidence was found in this stage oocytes as compared with the oocytes at the early or mid stage pupa. While a very low frequency of the mutations was detected following the MMS treatment in the oocytes at the mid-stage pupa, the incidence of mutations was slightly increased in the late stage oocytes.

The present data and others clearly indicate that the ethylating agent treatment, which has proven to be highly mutagenic at inducing the specific-locus mutations in matured spermatozoa, was slightly mutagenic in this respect in the oocytes. This situation was effectively pronounced in the treatment with the methylating agents. The differential sensitivity between oocytes and spermatozoa to the alkylating agents may be interpreted being due to a different repair activity for the premutational damage in their repair system. The weak mutagenicity following exposure of the alkylating agents to oocytes may be dependent on the effective repairability for the premutational damage. Especially, the damage with the methylating agents

seems to be more susceptible to the maternal repair system in ooplasma as compared with the ethylating ones. The differential sensitivity of germ-cell types to any form of genetic damage by the kind of alkylating agents would be also likely to be one of the interpretations. Another possibility may be still considered that the methylating compounds were more effectively subjected to inactivation in oocytes (or pupae) before the compound (or its metabolite(s)) reaches the target cells. In any case, further studies are required to check these possibilities to elucidate the differential mutagenic activity between methylating and ethylating agents in a various type of silkworm germ-cells.

VII. RADIATION GENETICS AND CHEMICAL MUTAGENESIS IN MICROORGANISMS AND PLANTS

Analysis of DNA Damage Induced by β-Rays of Tritiated Water

Tsuneo KADA, Yoshito SADAIE and Tadashi INOUE

We previously reported that the efficiency of inactivation of DNA by tritiated water (HTO, upto 100 μCi/m*l*) increases by lowering the HTO concentration and that such an increase in efficiency of inactivation leveled off at concentrations of 0.01–0.001 μCi/m*l* (Sadaie *et al.*, J. Rad. Res. **22**: 387, 1981; Proc. 7th Inter. Congr. Rad. Res., B1–18, 1983). It is probable that the efficiency of damage production in DNA might be reduced at high concentrations of the isotopes due to the "ineffective" recombination of radicals. It is also possible that certain primary potential damages in DNA might be efficiently progressed into fixed damages which would lead to the inactivation of molecules.

In order to obtain more information about the nature of DNA damages involved in the above dose-rate effects of tritium β-irraidation, several types of experiments were carried out.

First, strains carrying DNA-repair deficiencies such as *uvr-19* and/or *rec-43* were constructed and used as recipients of irradiated transforming DNA. UV-irradiation on transforming DNA using recipients carrying a simple repair deficiency (*uvr* or *rec*), moderately reduced the yield of transformants. This was in contrast to the results obtained using wild reci-recipients. The double deficient strain (*uvr rec*) produced markedly reduced transformants. No such difference was found when transforming DNA was irradiated to ^{3}H-β-rays or ^{32}P-β-rays. It is suggested that tritium irradiation caused DNA damage which can not be repaired by cellular repair functions working in the UV-repair.

^{14}C-labelled covalently close circular (ccc) DNA of colicin E_1 was prepared and kept at 0°C in a 100 mM NaCl–10 mM potassium phosphate buffer (pH 7.0) containing 439 μCi/m*l* of HTO (β-irradiation at a dose rate of 128 rad/day). Since ccc DNA changes its sedimentation coefficient by a single nick in one of the DNA strand, we carried out neutral SDG analysis every 2 days on the irradiated samples with or without a following treat-

ment of AP-endonuclease. Results of the above analysis indicated that the dose of β-irradiation producing one AP-endonuclease-sensitive site is 0.24 kR. When similar experiments were carried out with γ-irradiation of ^{137}Cs, it required 0.19 kR to produce one AP-endonuclease-sensitive site. Therefore, RBE for production of apurinic or a pyrimidinic is 0.79 (=0.19/0.24).

One of the marked effects of tritium-irradiation concerns an *in vitro* repair susceptibility. We previously reported that an appropriately diluted crude homogenate of human placenta can repair γ-irradiated transforming DNA of *Bacillus subtilis*. No such repair was observed with ^{3}H-β-rays.

Development of an *In Vivo* Germinal Mutation Detection System in Mice

Hideo TEZUKA, Tadashi INOUE and Tsuneo KADA

We are trying to examine the mutation induction with beta-rays from tritiated water in mice using the Malling's method (Ansari *et al.*, Proc. Natl. Acad. Sci. **77**: 7352–7356, 1980). Alteration of Lactate dehydrogenase-X (LDH-X; LDH-C4) from the original mouse-type to rat-type can be detected by an immunofluorescent antibody technique which enables to examine one mutant sperm among 10^6 sperms on a microscopic slide. There is a discrete species-difference with LDH-X but no strain difference is detected so far.

LDH-X was purified from testes of matured male young rats (2.2 mg of LDH-X from 39 animals) according to the Ansari's methods (Ansari, Biochem. J. **199**: 75–79, 1981). Rabbits were immunized with several injections of rat LDH-X. Rabbit antiserum against rat LDH-X was prepared (titer $\times 128$ to $\times 256$) and absorbed extensively with mouse sperms. The resulting preparation was specific for rat LDH-X judged by the fluorescent antibody technique for artificial mixture of mouse and rat sperms. We are now examining the mutation frequency in mice treated with procarbazine or ethylnitorosourea.

Using this method, 10 mice per experimental groups give reliable data and therefore 50 animals for one experiment may be enough for evaluation of the mutagenicity due to an agent.

Decrease in γ-Ray-Induced Mutations by PLD Repair Inhibition in Chinese Hamster V79 Cells

Akiko YOKOIYAMA and Tsuneo KADA

Since the first demonstration of the repair of potentially lethal damage (PLD) in irradiated mammalian cells in culture (Phillips and Tolmach, 1966), a number of studies have been carried out on factors and conditions which modify repair efficiencies. Recently Nakatsugawa and Sugahara (1980 and 1982) found that purine analogues such as 3′-deoxyadenosine (3′-dA) significantly inhibited the repair of PLD, suggesting promising uses of these chemicals to sensitize tumors in radiation therapy.

We studied the effect of 3′-dA on the frequency of radiation-induced 6-TG resistant mutations. The plateau phase cells of Chinese hamster V79 exposed to γ-rays from a ^{137}Cs source were incubated for 0, 3 or 6 hours in Hanks' BSS with or without 3′-dA, then diluted and inoculated to estimate the PLD repair as well as the induced mutation frequency. The expression time of 5 days was adopted for 1000 rad irradiated cells. The presence of 3′-dA in the course of post-irradiation repair incubation markedly reduced the capacity of repair in irradiated cells. The mutation frequency decreased gradually in the course of PLD repair taking place in the cells in Hanks' BSS. In the presence of 5×10^{-5} M and 1×10^{-4} M of 3′-dA, a marked decrease in the frequency of mutations was found, though there were certain variations of mutation frequencies which could be caused by inactivation of 3′-dA by the cellular deaminase.

Adsorption of Pyrolysate Mutagens by Vegetable Fibres

Tsuneo KADA, Masayuki KATO[1] and Shuhachi KIRIYAMA[2]

It has been recently shown that pyrolysates of amino acids and proteins contain highly mutagenic products (for review, see Sugimura, Cancer **49**: 1970, 1982). We have previoysly shown that a number of vegetables suppressed mutabilities of certain pyrolysate mutagens (for review, see Kada, Proc. 3rd ICEM, 355, 1982). A number of vegetables have also been shown to possess a desmutagenic effect on the mutagenic activities of the crude products obtained from broiled fish (Yoshikawa *et al.*, 3rd ICEM 3P19,

[1] Japan Biological Chemistry Centre, Gifu-ken.
[2] Dept. of Agriculture, Hokkaido University, Sapporo.

1981). Later we found that one of the desmutagenic principles in cabbage was a hemo-protein exhibiting peroxidase activity (Inoue *et al.*, Agric. Biol. Chem. **45**: 345, 1981). From a group of vegetables such as burdock which contain heat-resistant factors we isolated a comples compound having a molecular weight higher than 300,000 (Morita *et al.*, 3rd ICEM, 3P20, 1981). We summerize in this communication our results showing adsorption of pyrolysate mutagens by vegetable fibres.

Fibres of vegetables were prepared according the procedures described by Kiriyama *et al.* (The Jour. of Nutrition **109**: 388, 1979). To each aqueous solution of Trp-P-1, Trp-P-2 or Glu-P-1, vegetable fibres were added and kept at room temperature for several hours. The mutagenic activities before and after the addition of fibres were determined using the Ames' strain of *Salmonella* TA98 with S9 activation. The fibres of following vegetables reduced markedly the mutabilities of the above three pyrolysate mutagens: cabbage, burdock, radish, banboo shoot, Japanese onion, carrot, peaman, spinach and soybean sprout. Results of different studies showed that the mutagens were adsorbed irreversibly by the fibres.

Antimutagens

Tsuneo KADA

In the past ten years, a number of mutagens have been detected as a result of mutagenicity assays using microorganisms. They include not only synthetic chemical compounds but also many natural compounds. On the other hand, little information has been available about antimutagenic factors in the environment. We carried our systematic screening of antimutagens in our environment and found a number of new natural antimutagens. They include certain biometal (cobalt and germanium) compounds, ingredients of certain spices and food such an cinnamon and green tea, crude homogenates of mammalian placental tissues, etc. Chemical characterization was done on some active principles such as protoanemonin of Ranunculus and Anemone (Minakata *et al.*, Mutation Res. **116**: 317, 1983).

We previously showed that cobaltous chloride was effective in reducing mutations of the base-change type. However, our recent studies revealed that this metal compound worked as an efficient antimutagen in certain frame-shift mutagenesis (Mochizuki and Kada, Mutation Res. **95**: 145,

1982).

We collaborated with Dr. T. Ohta *et al.* (Institute of Environmental Toxicology, Tokyo) in studies on cinnamaldehyde, an active principle of cinnamon. Cinnamaldehyde markedly suppressed mutations induced by 4NQO or AF2 in *Escherichia coli* WP2 *uvrA*$^-$ *trpE*$^-$. Mutations induced by MMS or EMS were slightly inhibited. However, cinnamaldehyde was not effective on MNNG-induced mutagenesis of MNNG. Different aspects in the mode of action of cinnamaldehyde suggest that this antimutagen might act by reducing errors of an inducible error-prone DNA repair pathway (Ohta *et al.*, Mutation Res. **107**: 219, 198; *Ibid.* **117**: 135, 1983).

DNA Repair in Competent Cells of *Bacillus subtilis*

Izumi MITA, Yoshito SADAIE and Tsuneo KADA

Two main pathways, excision repair and postreplication repair, are known to operate in DNA repair in bacterial cells in the dark. Usually, these pathways have been studied with rapidly growing vegetative cells. Generally, the former process functions without chromosomal replication, and the latter process requires chromosomal replication. The culture of *Bacillus subtilis* contains, in addition to vegetative cells, competent cells which take up exogenous DNA and appear to be derived from early sporulating cells. Competent cells appear to be metabolically less active and nondividing, and have resting chromosomes. Therefore, DNA damage in competent cells might be repaired by an excision repair process but not by a postreplication repair process. Further, DNA repair in these specific cells might have a unique feature related to cellular differentiation, because competent cells seem to be derived from sporulating cells.

To investigate whether or not excision repair and postreplication repair can function in competent cells of *Bacillus subtilis*, a series of isogenic transformable strains of *Bacillus subtilis* carrying the *uvr-19* or *rec-43* mutation or both were constructed. Both mutations are assumed to block excision repair and postreplication repair respectively. The number of competent cells was scored as that of Trp$^+$ transformants.

The mutations *uvr-19* and *rec-43* made both competent and noncompetent cells highly sensitive to UV irradiation and their effects were additive in a doubly deficient strain. We also examined the effects of *uvr-19* and *rec-43* mutations on the repair of UV-irradiated exogenous transforming

DNA in competent cells. Transforming DNA was UV irradiated and used to transform the competent cells of repair-proficient or deficient strains. The *uvr-19* and *rec-43* mutations reduced the repair capacity of the wild-type strain to a certain extent, whereas competent cells with two mutations lost the capacity to repair transforming DNA to a greater extent. Therefore, UV-induced damage in transforming DNA seemed to be repaired efficiently and independently by processes requiring *uvr-19*$^+$ and *rec-43*$^+$ gene products. The strains used in this study carry a long deletion (about 2×10^7 daltons) *fdpAl*, which blocks the utilization of gluconate or inositol as a carbon source. The transformation from Fdp$^-$ to Fdp$^+$ was very sensitive to UV irradiation because of a large target size. The *uvr-19* mutation did not affect the UV sensitivity of Fdp$^+$ transformation, whereas the *rec-43* mutation did. These results indicate that the *uvr-19*$^+$-dependent repair process could not function on transforming DNA (possibly distinct by having been converted to a single strand and looped out at the site of the *fdpAl* mutation of the host chromosome), whereas the *rec-43*$^+$-dependent repair process could function. (J. Bacteriol. **155**, 933, 1983 and **153**, 813, 1983).

Effects of TPA on the Mutagenicity of Caffeine and Gamma-rays in the Soybean Test System

Taro FUJII, Masumi SHIZAKI, Hirota FUJIKI and Takashi SUGIMURA

12-0-tetradecanoylphorbol-13-acetate (TPA), a well known tumor promoter in mouse skin carcinogenesis, was tested for its effects on mutagenesis, using a soybean test system which was indicated by the appearance of variously colored spots on leaves. The mutagenic activity of TPA was tested at concentrations of between 2 and 20 μg/m*l*. No differences in the average number of spots per leaf were observed. When seeds were treated with 0.01 % caffeine solution, averages of 3.7 mutant spots per leaf were observed. These spots were classified into 3 types, yellow (forward mutation), dark green (backward mutation) and double (somatic crossing over) spots. Treatment with TPA at concentrations of 2 and 5 μg/m*l* reduced the mutagenicity of caffeine, giving averages of 2.5 and 2.2 spots, respectively. When seeds were treated with 0.02 % caffeine solution, the total number of mutant spots increased to about 10 spots per leaf. Treatment with TPA at a concentration of 5 μg/m*l* reduced the total number of spots to 4.4 per leaf. However, no reduction of spots was observed at a concentration of TPA

of 2 μg/m*l*. Treatment with 0.03% caffeine solution, treatment with TPA at a concentration of 20 μg/m*l* significantly decreased the total number of spots from 15.8 to 8.9. However, no decrease in spots was observed when seeds were treated with 0.05% caffeine solution simultaneously with 10 and 20 μg/m*l* of TPA. The remarkable finding was that the concentration of TPA modified the mutation frequency greatly, depending of the concentration of caffeine. Interestingly, TPA did not reduced the mutagenicity of 0.05% caffeine solution.

To examine the TPA action on radiation mutagenesis, combined treatment of TPA and gamma-rays were studied. TPA post-treatment did not affected the gamma-ray mutagenesis (500 R). Spotting frequency of 11.3 and 11.9 per leaf observed in 10 and 20 μg/m*l* TPA post-treatment, respectively, were similar to that obtained in water post-treatment plot. Since some chemicals have a potentiating effect of radiation induced damage when they were present in the material, gamma-ray post-treatment were carried out to seeds pre-soaked in water or TPA. Mutant spots increased considerably with gamma-ray treatments, but there were no differences between radiation treatments singly and in combination with TPA.

Because of the difficulties in the handling of the present material, soybean seeds were treated with solutions of TPA plus caffeine. Reduction of mutant spots was actually observed on treatment with these solution. It is unknown how TPA reduced the mutagenicity of caffeine, TPA suppresses non-repair-associated DNA fragmentation in murine and human cells, and also inhibits the appearance of this DNA fragmentation after X-irradiation. Although it is still unknown whether this suppressive effect of TPA is related to reduction of the mutagenic activity of caffeine, the promoter had no effect on the gamma-ray-induced mutation frequency. Therefore, TPA presumably reduced the concentration of active caffeine molecules inside the embryo cells of the seed. The observation that TPA had no effect at the highest concentration of caffeine used is in accord with such a mechanism of action.

VIII. POPULATION GENETICS (THEORETICAL)

The Neutral Theory as a Basis for Understanding the Mechanism of Evolution and Variation at the Molecular Level

Motoo KIMURA

The neutral theory (or more precisely, the neutral mutation-random drift hypothesis) asserts that the great majority of evolutionary changes at the molecular level are caused by the random fixation of selectively neutral or nearly neutral mutants in the species, rather than by positive Darwinian selection. It also claims that much of the intraspecific genetic variability at the molecular level, such as is demonstrated by protein polymorphisms, is selectively neutral so that polymorphic alleles are maintained in the species by mutational input and random extinction. Thus the neutral theory regards protein and DNA polymorphisms as a transient phase of molecular evolution. The essential aspect of the neutral theory is not that the alleles involved are selectively neutral in the strict sense. Rather, the emphasis is on mutation and random drift as explanatory factors in molecular evolution because the selection intensity involved is exceedingly small. The theory does not deny the occurrence of deleterious mutations. In fact, selective constraint due to such negative selection is a very important part of the neutralist explanation of some important features of molecular evolution. The neutral theory does not deny the possibility that some changes are adaptive. Thus it is by no means antagonistic to the Darwinian theory of evolution by natural selection. However, because of its emphasis on mutation and random drift, and also because of its concern with negative selection rather than positive Darwinian selection, the theory clearly differs in its theoretical framework from the traditional neo-Darwinian or "synthetic" theory of evolution.

The neutral theory is accompanied by a well-developed mathematical theory based on the stochastic theory of population genetics. The latter enables us to treat evolution and variation quantitatively, and therefore to check the theory by observations and experiments. The neutral theory in the above sense does not rule out the possibility that the mutant alleles

involved have different effects on form and function. These mutants can become selected under a special circumstance, even if they are selectively neutral (i.e., selectively equivalent) under the prevailing environmental conditions. This means that "neutral" variations can be the raw material for adaptive evolution. Some important topics relating to the neutral theory are; (i) how it explains the main features of molecular evolution; (ii) what kind of mathematical models are employed to treat the mechanism for the maintenance of protein polymorphisms; (iii) how it differs from rival "selectionist" hypotheses in the face of hard facts; and (iv) why neutral mutations are so prevalent at the molecular level, and how they relate to natural selection at the phenotypic level. For details, see Kimura (1982) In *Molecular evolution, protein polymorphism and the neutral theory* (ed. Kimura, M.), pp. 3–56. Japan Scientific Societies Press, Tokyo and Springer-Verlag, Berlin Heidelberg New York.

Linkage Disequilibrium Due to Random Genetic Drift in Finite Subdivided Populations

Tomoko OHTA

Linkage disequilibrium between two linked loci was studied for a finite population with a subdivided population structure. Wright's island model was used; extinction and replacement of colonies were also incorporated. Two alleles with symmetric mutation rates were assumed at each locus (A_1 and A_2 at the first locus, and B_1 and B_2 at the second locus) and equilibrium properties of linkage disequilibrium coefficients were analyzed. In terms of the analogy with the subdivision of inbreeding coefficient, the variance of linkage disequilibrium is divided into several components: D^2_{IS} (variance of within-colony disequilibrium), D^2_{ST} (variance of correlation of A_1 and B_1 on different gametes from the same colony relative to that of the total population), and D^2_{IT} (total variance of disequilibrium). Alternatively, the total variance can be subdivided into D'^2_{IS} (variance of correlation of A_1 and B_1 on one gamete from a colony relative to that of the average gamete of the population) and D'^2_{ST} (variance of the ordinary disequilibrium of the whole population). When migration is limited, the variance becomes large if the correlation of A_1 and B_1 of one colony is taken relative to that of the whole population (D^2_{ST} and D'^2_{IS}). Also, when the rate of extinction-replacement of colonies is high, the whole-population disequili-

brium coefficient (D'^2_{ST}) can become fairly large. The analyses were extended to the infinite allele model, and it was found that the disequilibrium components may get very large when migration is limited if the correlation of alleles at the two loci within a colony is taken relative to that of the entire population. In other words, with limited migration, random genetic drift of gamete types prevails in each colony. A possible test in which the variance components of disequilibrium are compared is suggested; the test discriminates between epistatic natural selection and limited migration, showing the latter to be the main cause of various observed linkage disequilibria. In particular, observed linkage disequilibria, such as those among markers in the major histocompatibility complex of man and mouse, may well be explained by limited migration, without assuming epistatic natural selection. For details, see Proc. Natl. Acad. Sci. USA **79**, 1940–1944, and Genetics **101**, 139–155.

Further Study on the Genetic Correlation between Members of a Multigene Family

Tomoko OHTA

The extent of genetic similarity (in terms of identity coefficients) was investigated among members of a multigene family that is evolving under mutation, unequal crossing-over and random genetic drift. The method of Kimura and Ohta (1979) was used, but the possibility was incorporated that the length of the shift (in terms of the number of genes) involved in unequal crossing-over can be more than one gene unit. Extensive numerical analyses show that, at equilibrium, the identity coefficients between two gene members are practically independent of their distance apart on the chromosome when the mean length of the shift at unequal crossing over is more than 10% of the total size of the family. In that case, the approximate treatment by Ohta (1980) is shown to be valid, but when the mean length of the shift is less than 10%, the average identity coefficient at equilibrium is underestimated. In order to clarify the effect of natural selection responsible for keeping the family size stable, Monte Carlo simulation studies were performed. The results indicate that the identity coefficients are not greatly influenced by natural selection on gene family size, particularly when the number of genes shifted is small compared to the family size. In addition, with sufficiently strong selection on family size, unequal cross-

overs with a large shift (such as with maximum shift of 90% of the family size) contribute very little to evolution and their net effect is almost indistinguishable from those with a small shift. For details, see Genetics **99**, 555–571.

Allelic and Nonallelic Homology of a Supergene Family

Tomoko OHTA

A model to explain the high degree of polymorphism at the major histocompatibility complex (MHC) is described. The model incorporates domain transfer between the different loci in a supergene family by either gene conversion or double unequal crossing-over. Population genetics theory is used to formulate changes in the probabilities of allelic and nonallelic gene identities and equilibrium values are obtained. The observed degree of allelic and nonallelic homology in the complex can be explained by assuming that a domain is converted at a rate of 10^{-5} to 10^{-6} per generation and reasonable values of other parameters. This rate of domain transfer is compatible with the observed high mutation rate at marker loci in the major histocompatibility complex. For details, see Proc. Natl. Acad. Sci. USA **79**, 3251–3254.

Linkage Disequilibrium, Genetic Distance and Evolutionary Distance under a General Model of Linked Genes or a Part of the Genome

Naoyuki TAKAHATA

A general model of linked genes or a part of a genome is proposed which assumes multiple states at each of linked multiple sites (loci) and which enables us to study various problems in molecular population genetics in a unified way. Based on the diffusion approximation method, several formulas with special reference to linkage disequilibrium and genetic distance are derived for neutral mutations in finite populations. In particular, when the r sites are completely linked and the mutation rate v is independent of site, the mutation scheme at each site following the so-called K allele model, the equilibrium identity probability of the r sites within a population of the effective size N_e is given by

$$\hat{F}_r=\frac{1}{1+Kr\theta}+\frac{r\theta}{(1+Kr\theta)(1+K(r-1)\theta)}+\frac{r(r-1)\theta^2}{(1+Kr\theta)(1+K(r-1)\theta)(1+K(r-2)\theta)}+\cdots+\frac{\prod_{j=0}^{r-1}(r-j)\theta^r}{\prod_{j=0}^{r-1}(1+K(r-j)\theta)} \tag{1}$$

where $\theta=4N_e v/(K-1)$. Note in (1) that the first term on the right-hand side is equivalent to the homozygosity expected on the K allele model. Clearly, $\hat{F}_r\simeq 1/(1+Kr\theta)$ when $\theta\ll 1$. On the other hand, the identity probability between two isolated populations of the divergence time t generations is given by

$$J_r(t)=\frac{1}{K^r}\sum_{p=0}^{r}\left[{}_rC_p\left\{\sum_{q=0}^{p}{}_pC_q(-1)^q K^{p-q}J_{p-q}(0)\right\}e^{-2pKvt/(K-1)}\right] \tag{2}$$

where ${}_rC_p$ is the binomial coefficient. Formula (2) reduces to

$$J_r(t)=\left\{\frac{1}{K}+\left(1-\frac{1}{K}\right)e^{-2Kvt/(K-1)}\right\}^r \tag{3}$$

for $J_p(0)=1$ ($p=1, 2, \cdots, r$) and it also reduces to

$$J_r(t)=J_r(0)e^{-2rvt} \tag{4}$$

for $K=\infty$. Formulas (3) and (4) are closely related to Jukes and Cantor's evolutionary distance and Nei's genetic distance, respectively. It is argued that the present model and formulas are potentially useful to analyze DNA sequence data from the standpoint of population genetics. (Genet. Res. 1983, **39**: 63–77).

Sexual Recombination under the Joint Effects of Mutation, Selection, and Random Sampling Drift

Naoyuki TAKAHATA

The advantage or disadvantage of sexual reproduction or recombination for the accumulation of mutant genes in a panmictic population is studied under the joint effects of mutation, selection, and random sampling drift. Three different methods are used; numerical integration of Kolmogorov backward equations, simulation of stochastic difference equations, and

Monte Carlo experiments. Comparison of the results between sexual and asexual populations shows that the effect of recombination depends on initial linkage disequilibrium, mutation rate v, selection intensity s, and population size N_e. The mode of selection is also an important factor and the large effect of recombination is observed when mutant genes are individually deleterious (advantageous) but collectively favorable (harmful). Under a given model of selection, the large effect of recombination is expected when genetic variation is produced not by mutation but by recombination. Extreme values of $N_e v$ and $N_e s$ make the effect insignificant. The long-term benefit of recombination in a panmictic population is expected only under rather stringent conditions; thus, the present results do not support the classical view that the widespread occurrence of sexuality in the living world may be accounted for recombinational advantage for selection. (Theoretical Population Biology, 1982, **22**: 258–277).

The Disappearance of Duplicate Gene Expression

Naoyuki TAKAHATA

Based on a numerical method for integrating Kolmogorov backward equations and a simulation method for solving stochastic differential equations, I studied the process of fixation of null (non-functional) alleles at duplicate loci. Assuming that normal alleles irreversibly mutate to null alleles, and that the population is finite and panmictic, I obtained the mean and median times until fixation measured in unit of generation, the amount of heterozygosity at specified times and the trajectories of null allele frequencies under various types of selection. It is shown that duplicate genes may rapidly return to the diploid condition when only double homozygotes for null alleles are selected against and linkage is tight. On the other hand, a prolonged retention of duplicate gene expression is expected if the heterozygous effect on fitness is pronounced. We have related the results of these analyses to recent observations on amounts of polymorphism, on loss of duplicate gene expression in tetraploid organisms, and on pseudo genes in mammals. Evolutionary implications of duplicate genes are discussed quantitatively from the viewpoint of population genetics. (In Molecular Evolution, Protein Polymorphism and the Neutral Theory (1982) (ed. M. Kimura), pp. 169–190. Japan Scientific Societies Press, Tokyo/Springer-Verlag, Berlin).

Estimation of Evolutionary Distance under a Mathematical Model of Extranuclear DNA Molecule

Naoyuki TAKAHATA

The evolutionary distance between two related populations is studied based on a mathematical model of transmission genetics of extranuclear genomes. Several formulas are derived assuming neutral mutations, which allow us to estimate the distance in the presence of not only intrapopulational variation but also within-cell variation. Disregard of back and parallel mutations in distant populations underestimates the distance while neglect of intrapopulational and within-cell variations in closely related populations overestimates the distance. The formulas take into account the complete linkage between nucleotide sites in question so that they are potentially useful to analyze data obtained by the use of restriction enzymes. In the light of the present study, we can rigorously examine the situations under which the use of several conventional formulas may cause serious bias in estimating the distance. (RIMS, Kokyuroku **457**, 141–156, 1982).

A Condition for Group Selection to Prevail over Counteracting Individual Selection

Kenichi AOKI

The dynamics of a deleterious gene favored by group selection (a gene for altruism) are studied, based on a one-locus two-allele model similar to the models of Levins (1970), Eshel (1972), and Levin and Kilmer (1974). The genetic system is assumed to be haploid, but the main theoretical result can be generalized to the diploid case. Let N be the effective group size, m the migration rate, s the selection coefficient against the gene, and $\alpha(p)$ the non-extinction probability of a group with fraction p of the gene. Given the distribution of p among the (infinite number of) groups with mean $\bar{p}$ and variance $\sigma_p{}^2$, let b and a be the regression of $\alpha(p)$ on p and the mean of $\alpha(p)$, respectively, with $k \equiv b/a$. Then if ε is the maximum of the positive quantities $1/N$, s, and k, and if we can ignore terms of $0(\varepsilon^2)$ (0 indicates same or smaller order), we have as the condition for the increase of $\bar{p}$,

$$f \equiv \frac{\sigma_p{}^2}{\bar{p}(1-\bar{p})} > \frac{s}{s+k} .$$

If we assume further that $\varepsilon = 1/N$ and that recolonization is of the propagule

pool type (Slatkin, 1977) or $a \approx 1$ so that the effect of recolonization on σ_p^2 can be safely neglected, and if, as numerical results indicate, f takes transient values that are within 10% of the equilibrium value in the absence of individual and group selection, this condition is in the simplest case

$$Nm \lesssim \frac{k}{2s}$$

given $k/s \sim 0(1)$. Thus if, for example, individual and group selection are of the same intensity, then the number of "immigrant genes" per group per generation must be less than 1/2, which indicates a fairly strong isolation. In order to check the validity and the range of applicability of the above formulae, and to observe the behavior of the model where selection is strong, the recurrence equations in the probability distribution of Np were iterated numerically, for group sizes of 10 and 100. Monte Carlo experiments were also conducted as a further check and to observe the effect of finite group number. For details, see *Evolution* **36**, 832–842.

Group Selection for a Polygenic Behavioral Trait: A Differential Proliferation Model

James F. Crow and Kenichi Aoki

Conditions for natural selection to increase a polygenic behavioral trait are derived for a model in which the population is divided into a very large number of partially isolated groups of variable and varying size. Specifically, we consider an altruistic trait that is deleterious to the individual but raises the mean fitness of the group. We assume, for each generation, that all groups have the same proportion of males, k, at the time of migration and that each group contributes M_f females and M_m males to a pool of migrants, from which M_f females and M_m males are randomly parceled out to each group. This assumption ensures that, at equilibrium between random drift and a low level of migration and neglecting the small per locus effect of selection, each group has the same expected value of Wright's fixation index, $F_{ST}=F$. At equilibrium, this is approximately $1/(1+4M_e)$, where $M_e=2kM_f+2(1-k)M_m$. The trait will increase when $(b-c)/c > (1-F)/2F=2M_e$, where b is the expected benefit to the group and c is the expected cost of a unit change in the mean value of the altruistic trait. In particular, the group selection analogue of Hamilton's inequality, $c/b<r$, where r is the coefficient of relationship, is obtained. The effect of isolation

is enhanced if migration is mainly between adjacent groups and if group splitting is along family lines, as data on population structure of primates seem to indicate. For details, see *Proc. Natl. Acad. Sci. USA* **79**, 2628–2631.

Additive Polygenic Formulation of Hamilton's Model of Kin Selection

Kenichi AOKI

Hamilton's condition for the evolution of altruism by kin selection is rederived on an additive polygenic model. The approach uses intraclass correlation. It is then shown that the mean inclusive fitness is non-decreasing. A common ground for the comparison of kin and group selection is provided, permitting a discussion of the differences between and the relative efficacy of kin and group selection. For details, see *Heredity*, **49**, 163–169.

Polygenic Altruism and Extinction Group Selection

Kenichi AOKI

Conditions are derived for altruism to evolve by the differential extinction of groups. The altruism is assumed to be determined by many loosely linked genes, acting additively within and between loci, and an independent environmental component. The results confirm those obtained on a monogenic model by a different method in a previous paper (*Evolution* **36**, 832–842). For details, see *Jpn. J. Genet.* **57**, 297–300.

IX. HUMAN GENETICS AND HUMAN CYTOGENETICS

Hereditary Retinoblastoma: Lack of Maternal Effect

Ei MATSUNAGA

The question of possible maternal effect on variable penetrance and expressivity in hereditary retinoblastoma is of interest for two reasons. First, because a significant proportion of patients with retinoblastoma are diagnosed shortly after birth, tumor formation can be initiated in the intrauterine stage, so that transplacental maternal factors may modify the degree of expressivity. Second, retinoblastoma-like tumors can be produced in rodents and baboons by the injection of human adenovirus 12. Although the transmission pattern of retinoblastoma in certain kindreds showing affected collateral relatives who are connected *via* unaffected persons could be explained by a chromosomal mechanism as well as by successive selection of host resistance genes, the possibility of delayed mutation which assumes a labile premutation cannot be excluded. Such a premutation may involve a vertically transmitting tumor virus that finally becomes integrated into the host genome in the germ cell line. If this is the case, we may expect more instances of transmission through maternal than through paternal lines, at least until the supposed agent is intergrated into the host genome.

Analysis of two-generation data from published pedigrees with familial cases of retinoblastoma showed that, while the proportion of bilateral cases among affected children varied consistently with expressivity of the parents who transmitted the gene, the proportion did not differ with the sex of the parents. These findings support the view that inherited host resistance to the development of hereditary retinoblastoma is determined by modifying genes, and not influenced by transplacental maternal factors, and that the resistance is mainly directed to the induction (promotion) phase. As a by-product of this study, it was found that there was a significant deficit of bilaterally affected mothers relative to bilaterally affected fathers, which could not be accounted for by ascertainment bias or by differential survival or fertility between sexes. It is suggested that female patients who survived bilateral retinoblastoma had much less chance of

marriage as compared to male survivors. Examination of pedigrees with multiple cases of retinoblastoma, transmitted *via* unaffected carriers, revealed no sign of cytoplasmic inheritance associated with a vertically transmitting virus. For details, see Hum. Genet. **62**: 124–128, 1982.

Almost Synchronous Appearance of Bilateral Retinoblastomas

Ei MATSUNAGA

Reexamination was made of previously published data (Matsunaga, E. 1981: Am. J. Med. Genet. **8**: 375–387) concerning distribution of 139 bilateral cases of retinoblastoma by Reese group as ascertained when the diagnosis was first made in either eye. In 124 (89.2%) of the 139, the disease was already bilateral when the diagnosis in one eye was made,although in such cases the tumor in the first eye with the diagnosed disease had usually progressed more than had the tumor in the second eye, resulting in a *negative* correlation between eyes with respect to the Reese group of tumors. However, there was a high *positive* correlation between ages of the patients at diagnosis in the right and left eyes, even after removal of the bias due to the fact that the diagnosis for each eye was never independent. Furthermore, the proportion of simultaneously bilateral cases with tumors of the same group in both eyes (20/139=14.4%) was significantly higher than the expected (6.1%) assuming independent diagnosis for each eye. These two findings seem to contradict the two-mutation model which assumes that in the gene carriers the eyes acquire tumors independently.

One may argue that if tumors in the gene carriers were initiated by a somatic mutation at a very early stage of development, the simultaneous occurrence of bilateral tumors in both eyes would be explained. If this were the case, then assuming equal rate of tumor growth, the age at diagnosis of the patients with tumors of the same Reese group in both eyes should tend to be much lower as compared to the patients with sequentially bilateral cases or with tumors of different Reese group in each eye. Contrary to this expectation, the mean age of the 20 patients at diagnosis (and standard deviation) was 11.7 months (6.4), which was significantly ($P<0.01$) higher than the mean of 9.0 months (8.5) for the remaining 119 patients. This difference is probably due to bias in ascertainment; in the latter group of patients, clinical signs of the eye with the advanced tumor could have been contrasted by the second eye with or without a growing tumor and

so the parents brought their children earlier to a physician than in the case of the former group. For details, see Am. J. Med. Genet. **11**: 485–487, 1982.

Incidence and Prevalence of Genetic Disease (Excluding Chromosomal Aberrations) in Human Populations

Ei MATSUNAGA

Detailed knowledge of the birth frequency or the cumulative incidence over all ages of genetic diseases in human populations is a prerequisite for assessing the magnitude of possible genetic hazards caused by environmental mutagens. However, both theoretical and practical difficulties are involved in precisely measuring the total frequency of these diseases. Two sets of data from large-scale population studies, one from Northern Ireland and the other from British Columbia, are compared with each other and with the results from *ad hoc* surveys for individual monogenic disorders. With due allowance for differences in approach, examination indicates that the data from the large-scale population studies are inadequate. However, it could provide a crude estimate of the total frequency of genetic diseases and a fairly reliable estimate of the individual frequency of certain genetic disorders with early onset that are familiar and readily diagnosed.

In addition to environmental mutagens, there are a number of factors associated with current human activity that may change the incidence of genetic diseases. In order to monitor the human population for environmental mutagens, the change in frequency of sporadic cases of those genetic diseases that arose from fresh mutation and that can be easily detected as early as possible should be followed closely. The mechanism of data collection currently being employed in some countries for childhood cancers, certain congenital malformations, and inborn errors of metabolism could be extended to include the so-called sentinel phenotypes. The rationale and feasibility of using retinoblastoma and Wilms' tumor (nephroblastoma) as examples of such population monitoring are described. For details, see Mutation Res. **99**: 95–128, 1982.

Distribution of Break Points in Human Structural Rearrangements

Yasuo NAKAGOME, Takako MATSUBARA and Hiroko FUJITA*

In 1976, Nakagome and Chiyo (1976) reviewed 117 reported unrelated cases with two-break rearrangements and found that among 220 breakage points, breakages were preferentially located within G-light bands. Similar observations have been made by others (Nielsen & Rasmussen 1976; Yu *et al.* 1978; Evans *et al.* 1978). On the other hand, there have been reports in which the breakages were located at a junction (interface) between adjacent G-dark and G-light bands (Dutrillaux *et al.* 1977; Aurias *et al.* 1978; Brogger 1978).

In the present study, we attempted to resolve the issue on the location of breakages. Three types of structural rearrangements (inverted duplications, isodicentrics and rings) were studied as it was realized recently that they were capable of providing information not obtainable from other types of rearrangements. We found that break points are primarily located within the G-light bands; a small number of breaks are located in G-dark bands. Breakages at the interface were exceedingly rare (For further details: see Nakagome *et al.* Amer. J. Hum. Genet. **35**: 288, 1983).

High Resolution Studies in Patients with Aniridia-Wilms' Tumor Association, Wilms' Tumor, Retinoblastoma and Recklinghausen Syndrome

Yasuo NAKAGOME, Minoru SAKURAI[1], Yasushi ISE[2], Toshio NAKAJO[3], Takako MATSUBARA, Eizo OKAMOTO[4], Shigenori SAWAGUCHI[5], Yoshiaki TSUCHIDA[6], Yutaka NAKAHORI, Ei MATSUNAGA and Sumio SAITO[6]

Since the first cytogenetic report by Francke *et al.*, there have been several confirmatory observations in which an interstitial deletion of the short arm of chromosome 11 was detected in patients with aniridia-Wilms' tumor

* Department of Child Health, Osaka City University, Osaka.
1) Dept. Pediatrics, Mie Univ., School of Med., Tsu.
2) Dept. Pediatrics, National Cancer Center Hosp., Tokyo.
3) Dept. Surgery, National Children's Hospital, Tokyo.
4) Dept. Surgery, Hyogo College of Med., Hyogo.
5) Dept. Surgery, Univ. Tsukuba, Ibaraki.
6) Dept. Pediatric Surgery, Univ. Tokyo, Tokyo.

association. In the present study, high-resolution chromosome analysis was carried out in patients with aniridia-Wilms' tumor association (AWTA) as well as Wilms' tumor, retinoblastoma and Recklinghausen syndrome. As to AWTA, both of two possible hypotheses that the 11p deletion causes transposition of an oncogene next to a promotor in the proximal 11p or the deletion of a specific site within 11p13 band in itself predisposes the tumor, were evaluated based on the distribution of break points. It is also attempted to determine whether every case of AWTA or any of following groups of patients show 11p deletion: cases of Wilms' tumor without aniridia, tumor itself in a case of Wilms' tumor without constitutional 11p deletion and cases of aniridia or hemihypertrophy not associated with Wilms' tumor.

Chromosome studies were carried out by the standard leukocyte-culture as well as the acridine-organge high-resolution banding technique (Nakagome & Matsubara, this report No. 31: 103, 1981; Matsubara & Nakagome, Cytogenet. Cell Gent. **35**: 148, 1983). Small pieces of tumors were cultured for 10 to 14 days. Six cases of aniridia-Wilms' tumor association, 4 cases of Wilms' tumor associated with various congenital abnormalities, 16 cases of Wilms' tumor (in 5 of them cultured tumors were also studied), one case each of aniridia and hemihypertrophy, one case of retinoblastoma (cultured tumor) and 4 cases of Recklinghausen syndrome were analysed. In all cases of AWTA, 11p deletion was detected. Points of breakages were: del(11) (p11. 2p13), del (11) (p11. 2p14.3), del (11) (p1302p p1403), del(11) (p11. 2p13), normal/del (11) (p13 p13) and del (11) (p12 p14.3). In a case of Wilms' tumor with microcephalus and a few minor anomalies, a 46, XX/47, XX, +8 mosaicism was detected. In a case of retinoblastoma (bilat.), blood culture revealed a normal karyotype, however, culture of the tumor revealed a normal/del(13) (q14 q34) mosaicism. All other cases revealed a normal karyotype at 850 to 400 band level.

As to cases of AWTA, proximal break points scattered between p11.2 and p1302 and the distal ones between p13 and p14.3. The finding is inconsistent with the promotor-oncogene hypothesis. The middle part of the p13 band is the common segment of deletion in all 6 cases. Both the proximal 20% and the most distal part of the p13 band was excluded from the common deletion segment. (Blood samples of some cases of Wilms' tumor, with or without aniridia, were kindly made available by Dr. S. Nagahara, Osaka Children's Medical Cneter; by Dr. Y. Takada, Kure National Hospital and Dr. Y. Osawa, Niigata Univ. Further details: Nakagome

et al. submitted).

The Loss of Kinetochores from Chromosomes of Aged Women (A Supplementary Note)

Yasuo NAKAGOME, Tatsuo ABE[1], Shinichi MISAWA[1], Tatsuya TAKESHITA[2] and Kazuso IINUMA[3]

In the Last issue of this report we described that in aged women the frequency of chromosomes that had inactivated kinetochore and lost Cd-band-positive material was significantly increased. Later it was pointed out that our control mostly consisted of infants and children and the difference between the aged and the control group might have been due to developmental effect rather than postpubertal aging.

We added sexually mature women in the control group and tabulated individual control data were prepared. Of 6474 scored chromosomes from aged women, 62 were Cd-negative; this was the case in 12 of 3849 chromosomes from the controls. The difference was highly significant ($p<0.001$). There was no difference within the control group, i.e., between sexually mature women and infants plus children.

We trust that our finding is relevant to the etiology of Down's syndrome and other numerical chromosome abnormalities with either maternal or paternal age effects (for further details: see Nakagome *et al.* Amer. J. Hum. Genet, in press).

A Malformed Girl with Duplication of Major Part of 9q

Yutaka NAKAHORI, Yasuo NAKAGOME and Tsunehiro YOKOCHI

Chromosome abnormalities involving the short arm of a no. 9 chromosome have been repeatedly described. On the other hand, those involving its long arm, especially middle part of it, is very unusual. In the present report, an 8-moth-old girl with almost full 9q trisomy is described.

The patient was born to a 33-year-old mother. A 3-year-old sister was mentally and physically normal. The birth weight was 2,300 g (37 weeks). She was referred to us when she was 8 months of age. She was able to

1) Dept. of Medicine, Kyoto Prefectural Univ., Kyoto.
2) Dept. of Health Sciences, Medical Univ. of Yamanashi, Yamanashi.
3) Dept. of Pediatrics, Ohta Hospital, Tokyo.

control her head but she couldn't sit by herself. Physical examinations revealed growth and metnal retardation, her weight being 5,565 g (−3SD), length 62.5 cm (−3SD) and head circumference 37.5 cm. Other features included white and pale eczematous skin, microdolicocephaly, narrow face, deep orbits, horizontal palpebral fissures, hypotelorism, bilateral inner epicanthal folds, beaked nose, thin upper lip, low set ears, congenital dislocation of the left hip joint, and small hand with relatively long tapering fingers. Both thumbs were held in the flexed position and pressed firmly against the palm. Dermatoglyphic study revealed bilateral simian creases, low axial triradii, and following finger-tip pattern: WL W W W U (r) WLU W WL and L (l).

Chromosome studies were done by the standard leukocyte-culture technique. One of the No. 9 chromosomes was unusually long. Chromosomes were further examined by the C, high-resolution band, and Distamycin-DAPI staining. It was concluded that the abnormal no. 9 chromosome had the duplication of the major part of the long arm of it; dir dup (9) (q12→q32). Both parent had a normal karyotype. (Nakahori et al. in preparation).

X. BEHAVIORAL GENETICS

Selection for the Compound Learning Ability of Mice

Tohru FUJISHIMA

A selection has been made bidirectionally for the discriminated avoidance performance (DAR, %) of mice (60–70 days of age) in the second of two daily training sessions, each comprising fifty learning trials. The fundamental stock was constructed from crossing of four inbred mouse strains (C3H/HeMs, SWM/Ms, C57L/JMs and D103/Ms), each having different characteristics of DAR measured with an automated Y-type maze apparatus (Experimental Animals, **29**(4), 383–390, 1980). The selection has been made on the basis of the within-family level, and the crossing on the circular mating system. Careful attention has been paid to keep the same inbreeding coefficient in both selected lines.

The data for the ninth selective generation were obtained this year. They indicated that the cumulative genetic gain for the average of males and females was 12.9, being statistically significant. As for the component abilities, the difference in avoidance between the DAR High line and the DNA Low line (High line minus Low line) was 23.8, while the difference in discrimination was −3.2 although the difference for the parental population was positive.

These results suggested that there was negative genetic correlation between avoidance and discriminatory abilities, and the selection for hihger DAR resulted in the higher avoidance ability of mice rather than the higher discriminatory ability.

The Behavioral Changes in Mice Continuously Reared under Noise Conditions

Tohru FUJISHIMA

It was found in the previous experiments that continuous rearing under noise conditions caused emotional change as well as decrease of body weight in mice. This year, the experiment was carried out to investigate the effective period of noise to the behavioral change in mice. Mice continuously

reared under non-noise conditions were kept under noise condition from their birth to weaning (21 days old) and then returned to the non-noise condition. Their behavioral performances were compared with those of the 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 results showed that no behavioral change occurred by the exposure during the post natal period. This suggested that the behavioral changes having been observed in the noise treated group so far might have resulted from the effect of noise given to mice before birth.

XI. ECOLOGICAL GENETICS

Variation in Competitive Ability Among Cohabiting Perennial and Annual Types of *Oryza perennis*

Yoshio SANO and Hiroko MORISHIMA

Variation in competitive ability among strains of a common wild rice, *Oryza perennis*, is a complex showing various patterns depending on the competitors. When tested with a perennial or cultivated strain, perennial strains were stronger in competition than annual strains, while annual strains were stronger than perennial strains when tested with an annual strain (Ann. Rep. 31). In the present experiment, two populations in which the plants were differentiated into the perennial and annual types (Ann. Rep. 32) were studied to look into within-populational variation in competitive ability. Competitive ability was evaluated by the difference in plant weight between pure and mixed stands as before.

The Bhubaneswar (India) population was found in a depression, about 30 m in diameter and deeper than 50 cm in the center. The periphery of the depression was covered by an annual type, while the central part was occupied by a perennial type. The perennial and annual types each was represented by a seed bulk from each of 25 individuals. They were grown in pure and mixed stands in the two experimental plots. In one plot, to examine the effect due to water depth, the ground was inclined making difference in water depth ranging from 5 cm to 40 cm. In this plot, the perennial type was a strong competitor against the annual type irrespective of water depth. In the other plot (5 cm deep), to simulate habitat disturbance by men and animals, the plants in the half of the plot were cut at a height of 5 cm and tramped down one month after transplanting, remaining the other half undisturbed. The perennial types showed a marked reduction in plant weight in both pure and mixed stands under disturbed conditions in comparison with the annual type.

The Chiengrai (Thailand) population was found in a strip of roadside depression. It seemed to contain the plants ranging from perennial to annual types growing in clumps. Fourteen progeny lines each representing a single

plant in the original population were grown in pure and mixed stands using annual (W107) and perennial (W120) strains as the associate. Although a range of variation was observed in competitive ability, no significant difference was detected between the perennial and annual types. The same lines were also tested regarding weed tolerance. Comparisons of their performances between weeded and non-weeded conditions showed that perennial strains were generally tolerant to weed.

In general, the perennial and annual types are allopatric. The results obtained from the sympatric populations suggest that the two types are mutually exclusive rather than co-operative, however, to what extent competition between them is important for their differentiation remains unknown.

Variation in Weed Tolerance Among Wild and Cultivated Rice Strains

Hiroko MORISHIMA

A series of experiments to study variation in weed tolerance and the effect of weed-mediated natural selection in rice plants are carried out. In 1981 and 1982, 170 cultivated varieties (*Oryza sativa*) and 37 wild rice strains (*O. perennis*) were grown in a weeded plot (W), a barnyard grass sown plot (E; *Echinochloa crus-galli*), and a sedge sown plot (C; *Cyperus difformis* and other miscellaneous weeds). Weed tolerance of each strain was evaluated by the difference in log-transformed plant weight of rice between weed-free and weed-sown plots (E-W, C-W). Cultivated strains showed a range of variation in weed tolerance, though no significant difference was found between the Indica and Japonica types. In wild strains which show a perennial-annual continuum, the perennial type was generally more tolerant than the annual type. This suggests that the perennial type is adapted to ecologically saturated habitats having strong competitive ability against coexisting species. Sedge tolerance and barnyard grass tolerance were positively correlated ($r=0.52^{**}$ in *O. sativa*, $r=0.57^{**}$, in *O. perennis*). It was found further that weed tolerance was negatively correlated with performance in weed-free plot suggesting a trade-off relationship ($r=-0.37^{**} \sim -0.52^{**}$). These associations might be the products of natural selection, since they were not observed among hybrid progenies which were reared under weed-free condition and tested under the same conditions

mentioned above (Ann. Rep. No. 32).

Neighbor Effects between Two Co-Occurring Rice Species *Oryza sativa* L. and *O. glaberrima* Steud.

Yoshio SANO, Reiko SANO and Hiroko MORISHIMA

Two cultivated rice species, *Oryza sativa* L. and *O. glaberrima* Steud., are reproductively isolated each other but morphologically very similar. In West Africa, these two species are often grown mixed unconsciously in farmers' fields. To study the selective effects of species coexistence in the history of a crop community on the interaction between neighboring plants, two sets of co-occurring *sativa* and *glaberrima* derived from seed samples collected from two fields in Nigeria, one was upland and the other was rainfed lowland, were examined. Neighbor effects between these four strains, two *sativa* and two *glaberrima*, were evaluated by a mixture-diallel (reported in Ann. Rep. No. 30) and a substitutive experiments.

In the substitutive experiment (a replacement series), these four strains were grown under wet and dry conditions in pure stands and in species mixtures with three different proportions keeping plant density constant. Number of seeds produced per plant was recorded. In the mixture of sympatric strains collected from the upland field, *sativa* was a strong competitor under wet condition, but a weak competitor under dry condition against *glaberrima*, suggesting that competitive relationship may change according to water regime. This could be a mechanism to allow coexistence of two species with different adaptability in a farmland with heterogeneous environments prevailing in West Africa. In the mixture of sympatric strains collected from the lowland filed, on the other hand, *sativa* was a strong competitor against *glaberrima* irrespective of water regime. With this combination, however, total yield of mixed plot was higher than that expected from yields of their pure plots. Such cooperative interaction can be explained as the product of natural selection working in the long history of cohabitation. The above results agreed well with those obtained from a mixture-diallel experiment. Further, a trend of frequency dependency that minor component took advantage over major component in reproduction was observed. This can be also a mechanism to help species coexistence. Together with the result from a mixture-diallel experiment, the above result is to be published in J. Appl. Ecology (Vol. 42, in press).

Association of Chromosome and Enzyme Polymorphisms in Natural and Cage Populations of *Drosophila Melanogaster*

Yutaka INOUE and Takao K. WATANABE

The frequencies of a polymorphic inversion, *In(2L)t*, and of *Adh* and *α-Gpdh* alleles were analyzed in three natural populations of *Drosophila melanogaster* from Japan. Significant positive correlations between the frequencies of *In(2L)t* and Adh^S or $\alpha\text{-}Gpdh^F$ were detected due to tight linkage. An analysis of correlation with latitude showed that the negative cline of Adh^S frequency could be explained entirely by its linkage with *In(2L)t*; the frequency of Adh^S on the standard chromosome did not show a latitudinal cline. To the contrary, the cline of $\alpha\text{-}Gpdh^F$ frequency itself was positive, and its linkage with *In(2L)t* makes the positive cline unclear. These results suggest that the two allozymes themselves respond to latitudinal natural selection in different ways. When these populations were transferred to laboratory cages and maintained for a long time, they lost the chromosomal polymorphism but retained stable enzyme polymorphisms, although allele frequencies in the cage were not the same as in nature. The frequencies of *Adh* and *α-Gpdh* alleles were close to those in earlier cage populations of the same geographical origin.

XII. APPLIED GENETICS

Change in Herbicide Tolerance of Barnyard Grass in a Paddy Field

Shin-ya IYAMA

A natural selection experiment of barnyard grass in a rice paddy field has been conducted since 1975 in the experimental plots which consist of the herbicide treatments with (i) propanil and (ii) propanil+MO, and (iii) control without treatment. Plants obtained from each plot in 1981 were grown and harvested individually and then their seeds were used for examination of tolerance to propanil in 1982. Tolerance was measured by the survival frequency of the seedlings treated with a given amount of propanil solution at two weeks old. Average survival frequency of the plants from the treated plots was higher than that of the plants from control, suggesting that the consecutive application of herbicide changed the genetic structure of barnyard grass population regarding the tolerance to propanil (Table 1).

Table 1. Viability of propanil treated seedlings of barnyard grass strains obtained from the paddy field under the application of herbicide since 1975.

Treated with	No. of strains	Viability
Propanil	16	0.40±.04
Propanil+MO	15	0.44±.04*
Control	15	0.31±.04

* Significantly different from control at the 5% level.

Simulation Studies on the Factors Affecting the Pedigree Breeding Method

T. C. YAP* and Shin-ya IYAMA

Computer simulation of the pedigree breeding method was carried out on five different sizes of F_2 populations (5000, 1000, 500, 200 and 100), four

* University of Agriculture, Malaysia.

levels of environmental variance so as to give heritability values in the F_2 as 0.05, 0.2, 0.5 and 0.9 in six dominance effects (complete and partial recessive, no dominance, partial and complete dominance and overdominance) and three recombination values between adjacent loci (1/2, 1/8 and 1/32) in order to study the effects of these factors in relation to genetic gains of the selection. Twenty loci of equal effects were assumed to control the character. The number of plants selected and progenies produced per plant for each generation of selection are given in Table 1.

Table 1. Number of lines and number of plants grown per line at each generation after F_3 in the pedigree selection scheme

	F_3	F_4	F_5	F_6	F_7	F_8	F_9	F_{10}	F_{11}
No. of lines	100 60*	40	20	12	6	5	3	2	1
No. of plants grown/line	10	20	30	50	100	100	100	100	—

F_2 population sizes used: 100, 200, 500, 1,000, 5,000.

* In the case of F_2 population size being 100, 60 F_3 lines were selected, other cases 100 F_3 lines selected.

Table 2. Mean genotypic values after 10 generations of selection for no dominance case with respect to different population sizes and heritability values (Possible maximum value=20 and genotypic value of F_1=10)

Recombination value	h^2	F_2 population size				
		5000	1000	500	200	100
1/2	0.05	16.2	16.0	16.6	15.9	15.7
	0.2	17.6	17.2	17.3	16.9	16.4
	0.5	18.5	17.8	18.0	17.5	17.2
	0.9	19.3	18.8	18.6	18.2	18.0
1/8	0.05	16.4	16.0	16.3	15.8	15.7
	0.2	17.5	16.9	17.0	16.8	16.7
	0.5	18.3	17.9	17.7	17.9	17.3
	0.9	18.7	18.4	18.3	17.8	17.5
1/32	0.05	15.4	15.1	15.0	15.1	15.3
	0.2	16.8	16.1	16.6	15.9	15.5
	0.5	16.9	16.5	16.3	16.2	16.2
	0.9	17.0	17.1	16.8	16.8	16.5

Based on the results of 10 runs, it was found that there was a steady increase of genotypic means in response to selection for complete recessive to partial dominance cases and the genotypes developed were homozygous for all loci. For complete dominance and overdominance cases, the response to selection was erratic and genotypes developed were still heterozygous even after 10 generations of selfing. Results of the response to selection for various degrees of dominance were quite similar and as expected, high heritability values gave relatively high genotypic values after 10 generations of selection or *vice versa* for all the population sizes studied.

With respect to the population size, if the aim is to look for the recombinants which could recover 80% to 85% of the desirable genes from the F_2 generation, perhaps population size of 100 to 200 F_2 plants may be large enough to serve the purpose, judging from that genotypes developed from smaller populations (100 to 200) were not very much inferior to those developed from large population (1000 to 5000) for no dominance case which is of more concern in breeding self-pollinated crop species (Table 2).

Studies on The Breeding Structure of Tree Species in The Tropical Rain Forest. Family Clusters and Intrapopulation Differentiation

Kan-Ichi SAKAI, Toru ENDO, Shin-ya IYAMA, Yasusada MIYAZAKI*, Shigesuke HAYASHI*, Yoshiya SHIMAMOTO*, Lilian U. GADRINAB** and Ulfah JUNIARTI**

Breeding structures of two tropical rain forest tree species, *Altingia excelsa* in Java and *Agathis borneensis* in Kalimantan were investigated. All trees with a diameter at breast height exceeding 6 cm growing in a 50×50 m quadrat set in a forest were recorded for their position. In all 38 among 148 trees recorded were *Altingia excelsa* in Java and 21 among 230 were *Agathis borneensis* in Kalimantan. Twigs with mature leaves were collected from each tree for isozyme study as well as morphological measurements. Peroxidase isozyme pattern of each tree was determined by electrophoretic analysis and "disagreement counts" between trees in the population were calculated based on the zymogram patterns. Assuming that similarity in

* University of Kyushu, University of Kagoshima and University of Hokkaido, respectively.

** BIOTROP, Bogor, Indonesia.

the assortment pattern of the isoperoxidase bands tells genetic relationship between trees, on the one hand, and that inbreeding increases the incidence of smaller values of the disagreement counts, on the other, it has been concluded that inbreeding occurs considerably in *Altingia excelsa* and to some extent in *Agathis borneensis*. Finding that trees showing very low disagreement counts are located close to each other, they were grouped as a family. It was found that different families were quite dissimilar in respect of iso-peroxidase constitution and in several leaf characters as well. The approximate distance between two trees at which they can mate is estimated to be 16.5 meters and the area one family occupies 200 to 250 m^2, assuming that a family cluster can be a single breeding unit in *Altingia excelsa*, within which trees mate at random. Some families were mixedly distributed with each other within the mating distance, but they were found still genetically differentiated from each other. The reason for this reproductive isolation among families might be interpreted to be due to genetic differences between families in flowering time.

In *Agathis borneensis*, there was no indication of family cluster formation. Related trees may have been distributed widely scattering in the forest, and the inbreeding of the species may be due, not to outcrossing between relatives, but to self-fertilization of individual trees.

Effective Population Number of Four Hatchery Populations of Fish

Shin-ya IYAMA and Nobuhiko TANIGUCHI*

Based on the amount of genetic drift, the effective population number of hatchery population of black seabream, *Acanthopagrus schlegeli* was estimated. Four hatchery populations, two of which were F_1 and other two were F_2 progenies were established from the fish captured in the natural waters. From the data of 15 polymorphic isozyme loci in the fish populations, the variance of genetic drift (V_d) was calculated by using the standardized deviation from the original frequency in each locus. Since the original allele frequencies were not equal from locus to locus, each deviation was standardized before calculation: $d_i=(x_i-p_i)/\sqrt{p_i(1-p_i)}$. When the allelic frequency in F_1 population was estimated by N_s samples, the effective number of parents (N_e) was calculated by $N_e=(1-1/2N_s)/2(V_d-1/2N_s)$. For the data obtained from the second generation, N_e was estimated from

* Kochi University.

Table 1. Estimated effective population number (N_e) and range of confidence at 95% probability. n: No. of original parents used in each hatchery

Hatchery	n	V_d	N	Range
Kochi	102	.031881	25.48	15.46–100.85
Okayama	102	.0543956	15.70	5.32– 42.99
Kagawa	71	.047833	27.70	9.42– 72.36
Osaka	44	.052427	24.48	8.47– 61.06

the following relationship: $V_d=1/2N_e+(1-1/2N_e)(1-(1-1/2N_e)^2)$, assuming that the effective number in F_1 and F_2 are equal.

Range of confidence at 95% probability for N was calculated using the confidence intervals of estimated variance V_d,

$$\Sigma d_i^2/\chi^2_{.025}<V_d<\Sigma d_i^2/\chi^2_{.975}\,.$$

assuming that the distribution of d is normal.

The result revealed that the effective population number was smaller than the number of parents used in any of the four populations. This may have resulted from far fewer parents actually contributing to the next generation, or unequal number of male and female parents.

Variation in Nitrogen Fixing Activity among Wild and Cultivated Rice Strains

Kouki OHTA, Yoshio SANO, Taro FUJII and Shin-ya IYAMA

The acetylene reducing activity (ARA) of *Oryza* species including two cultivated species (156 strains) and ten wild species (76 strains) was investigated. A wide range of variation in ARA was observed in wild species and tropical cultivars, especially in the *Sativa* group. The three other species belonging to *Sativa* group, *O. glaberrima*, *O. perennis* and *O. breviligulata* also showed similar variations. As far as the average ARA of each species was concerned, much higher ARA was observed in a cultivated species, *O. sativa* than in its ancestral wild species, *O. perennis*. On the other hand, the species belonging to *Officinalis* and other groups were at the lower level of ARA compared with the species in *Sativa* group so far examined. The difference between the *Sativa* and the other groups was significant statistically. The ARA of four tetraploid species, *O. minuta*, *O. latifolia*, *O.*

eichingeri and *O. ridleyi*, was lower than that of other diploid species. The nitrogen fixing activity in rice species appeared to have no particular relationship with ploidy and origin.

ARA was generally lower in Japanese cultivars than that of wild and tropical strains. Analysis of variance revealed that the variation in the ARA found among the varieties used was statistically significant in each experiment. Correlations among ARA/plant, ARA/g dry root and dry root weight/plant were calculated in the Japanese cultivars. Positive correlation was found either between ARA/plant and root weight or between ARA/plant and ARA/g dry root weight. But no significant correlation was found between ARA/g root weight and root weight. High correlations observed between dry root weight/plant and ARA/plant, and between ARA/plant and ARA/g dry root weight indicated that both the amount of root and the efficiency of root play a role for nitrogen fixing activity. However, insignificant correlation observed between root weight/plant and ARA/g dry root weight indicated that the two characters are independent. This may suggest the possibility of improving nitrogen fixing activity of rice by increasing the amount of root as well as improving the nature of root.

A Sterility Gene between *Oryza sativa* and *O. glaberrima*: Its Association with Photoperiod-Sensitivity

Yoshio SANO

The interspecific F_1 hybrid between the two cultivated rice species, *Oryza sativa* L. and *O. glaberrima* Steud., is male sterile but female fertile. In order to extract and examine elements causing F_1 pollen sterility, a backcross experiment was carried out. The *sativa* (Taichung 65) and *glaberrima* (W025) parents were considered to have S_3^a/S_3^a and S_3/S_3, respectively. The S_3 locus had no deleterious effect on the development of microspore in the homozygote but induced abortion of pollen not carrying it in the heterozygote S_3/S_3^a. There appears to be no adverse effect of S_3 on megaspores or somatic cells, which is different in genic action from those of S_1 and S_2 reported previously. The S_3^a locus was tightly linked with *la* (lazy habit) on the 9th chromosome. In addition, plants homozygous for S_3 proved to be photoperiod sensitive and showed the heading date about 10 days later than those having S_3/S_3^a or $S_3^a/S^a{}_3$ in summer seasons.

As to F_1 sterility barriers in rice, the genes examined so far were of gametophytic type although those of sporophytic type were also possible to be extracted. It is considered that vast numbers of haploid genomes can be screened by intensified gametophytic selection, and adaptive changes could be achieved at relatively little cost. Frequently observed sterility genes of gametophytic type might suggest that gametic selection is favorable for their fixation of genes controlling F_1 hybrid sterility otherwise they are deemed to be quickly extinct (J. Hered. 74, Nov.-Dec., 1983, in press).

Wx Protein in Rice

Yoshio SANO and Akemi MUROFUSHI

The *Wx* locus on the first linkage group in rice controls the production of amylose in the endosperm. Starch produced in the endosperm that carries the *Wx* allele is composed to 15–23% amylose, but homozygous *Wx* endosperm lacks amylose and contains only amylopectin. Reported evidences in maize show that the *Wx* gene product is almost certainly the starch-granule-bound NDP sugar-starch glucosyl transferase. We report here a subunit with 60,000 dalton specific to *Wx* strains in rice. First, seed polypeptides from glutinous (*wx*) and non-glutinous (*Wx*) strains were compared by means of a two-dimensional polyacrylamide gel electrophoresis according to O'Farrell. Comparisons between Taichung 65 and an isogenic line of Taichung 65 carrying *wx* gene revealed that only a difference detected in spot patterns is the presence of a subunit in Taichung 65 (non-glutinous, *Wx*). The molecular weight of the subunit was approximately 60,000. Secondly, SDS extracts of starch granules from dried endosperm contain only three proteins as visualized on polyacrylamide gels. There was a single major protein with 60,000 dalton which is completely missing in 15 *wx* strains examined. More than 100 non-glutinous strains showed the major protein called *Wx* protein in maize. In addition, variation in the quantitative levels of the *Wx* gene product was detected among strains. We intend to ask if regulatory changes of the *Wx* gene is related to differentiation in rice.

Diversity of Rice Cultivars Collected in the Asian Hilly Areas in View of Indica-Japonica Differentiation

Reiko SANO and Hiroko MORISHIMA

Asian rice cultivars tend to differentiate into two varietal groups, the Indica and Japonica types, which can be recongized on the basis of character association pattern. They could be effectively classified into the above two types with few intermediates, using a discriminant function combining four characters, $KClO_3$ resistance, low temperature resistance, apiculus hair length and phenol reaction (Morishima and Oka, 1981). These two varietal groups were found to differ markedly in gene frequencies at six isozyme loci (*Px-2*, *Acp-1*, *Cat-A*, *Pgi-A*, *Pgi-B*, *Est-E*) indicating nonrandom allelic association among loci. In 101 varieties randomly chosen from a large number of primitive cultivars collected in various Asian countries, the varieties classified as the Indica type showed 18 different isozyme genotypes in the combination of the above six loci, while those classified as the Japonica type showed only two genotypes which differed from any of those found in the Indica type. Thus, the Indica and Japonica types defined by character association pattern have specific isozyme genotypes, respectively.

The Himalayan foothill is known as a region in which a large amount of variability in rice is found. One hundred fifty land races collected in Nepal, Assam, Shikkim and Northern Thailand were studied in the hope of looking into the incipient stage leading to the Indica-Japonica differentiation. When examined by the discriminant function mentioned above, they showed a continuous variation between the Indica and Japonica types with many intergrades, though the Indica type dominated in number in the present collection. In total, 35 different isozyme genotypes (in the combination of six loci) were found among them. Nonrandomness of allelic association among isozyme loci as well as specific association between character set and isozyme genotypes clearly observed in the samples from all Asia were not distinct among these hill rices. Further, intra-populational variations were also surveyed in eight populations. Though most of them were highly polymorphic in characters as well as in isozymes, both variations were not associated.

It was concluded that primitive cultivars grown in the hilly areas contain a large amount of genetic variability between and within populations, but

that differentiation into the Indica and Japonica types has not well developed as in the other areas in Asia. In general, the accumulation and maintenance of variations in a crop species would be due to various factors, such as antiquity of domestication, dissemination, hybridization, isolation followed by selection under different climatic and ethnological conditions. Most probably, varietal diversity and whin-populational heterogeneity found in the hilly areas of Asian continent are a function of long-continued primitiveness in agricultural society.

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* Author not on N.I.G. staff.

ABSTRACTS OF DIARY FOR 1982

January	18	278th Meeting of Misima Geneticists' Club
February	18	279th Meeting of Misima Geneticists' Club
March	4	189th Biological Symposium
May	10	190th Biological Symposium
June	7	191st Biological Symposium
	12	192nd Biological Symposium
	15	193rd Biological Symposium
	21	280th Meeting of Misima Geneticists' Club
August	27	194th Biological Symposium
September	20	195th Biological Symposium
	27	196th Biological Symposium
October	25	197th Biological Symposium
November	8	281st Meeting of Misima Geneticists' Club
	29	198th Biological Symposium
December	3	199th Biological Symposium
	13	200th Biological Symposium
	20	282nd Meeting of Misima Geneticists' Club

FOREIGN VISITORS IN 1982

May 1, '80–Apr. 30, '82		ACHERMANN, Josef, Universität Zürich, Switzerland
Feb. 16, '81–Sept. 30, '82		SUN, C. R., Fudan University, China
January	12–18	HOTTA, Y., University of California, U.S.A.
	16–17	OHTSUBO, E., State University of New York, U.S.A.
February	26–Mar. 7	CROW, James F., University of Wisconsin, U.S.A.
March	24–25	RAJAN, S. S., F·A·O Expert, United Nations, Iraq.
	25–26	SZYBALSKI, W., University of Wisconsin, U.S.A.
April	1–3	WERNEK, Mass., University of New York, U.S.A.
	6–Oct. 4	GONI, Beatriz, University of Uruguay, Uruguay.
	12–Oct. 11	YAP, Thoo Chai, University of Agriculture, Malaysia.
	13–16	JEFFERSON, Roland M., U.S. National Arboretum, U.S.A.
May	10	RAETZ, Cristian, University of Wisconsin, U.S.A.
	20–21	GHYSEN, J. M., Université de Liege, Belgium.
	28	HOPPE, Petter C., The Jackson Laboratory, U.S.A.
June	6–7	SUEOKA, N., University of Colorado, U.S.A.
	11–13	WEISBLUM, Bernard, University of Wisconsin, U.S.A.
	11–13	KADO, C. I., University of California, U.S.A.
	14–15	WATSON, John M., Australian National University, Australia.

July	17–20	MULLER, Andreas J., Akademie der Wissenschafter, Germany
August	22–23	EIGEN, Manfréd, Max-Planck-Institut, Germany.
	23	BROCK, Richard D., S.C.I.R.O., Division of Plant Industry, Australia.
September	20–Dec. 20	YASSUDA, Yatiyo Y., University of São Paulo, Brazil.
	20	KING, Robert C., Northwestern University, U.S.A.
October	4–12	BARADJANEGARA, A. A. A., Pusut Penelition Teknik Nuklir, Indonesia.
	26	Naomani, M. K. R., Benchamin, K. V., Venugopala Pillai, S., Chandrashekharaiah., Central Sericultural Research and Training Institute, India.
November	26	KOKKE, Robert, United Nations University, Tokyo.
	29–30	KÖSSEL, Hans, Universität Freiburg, Germany.
December	13–14	OHNO, S., City of Hope Research Institute, U.S.A.

AUTHOR INDEX

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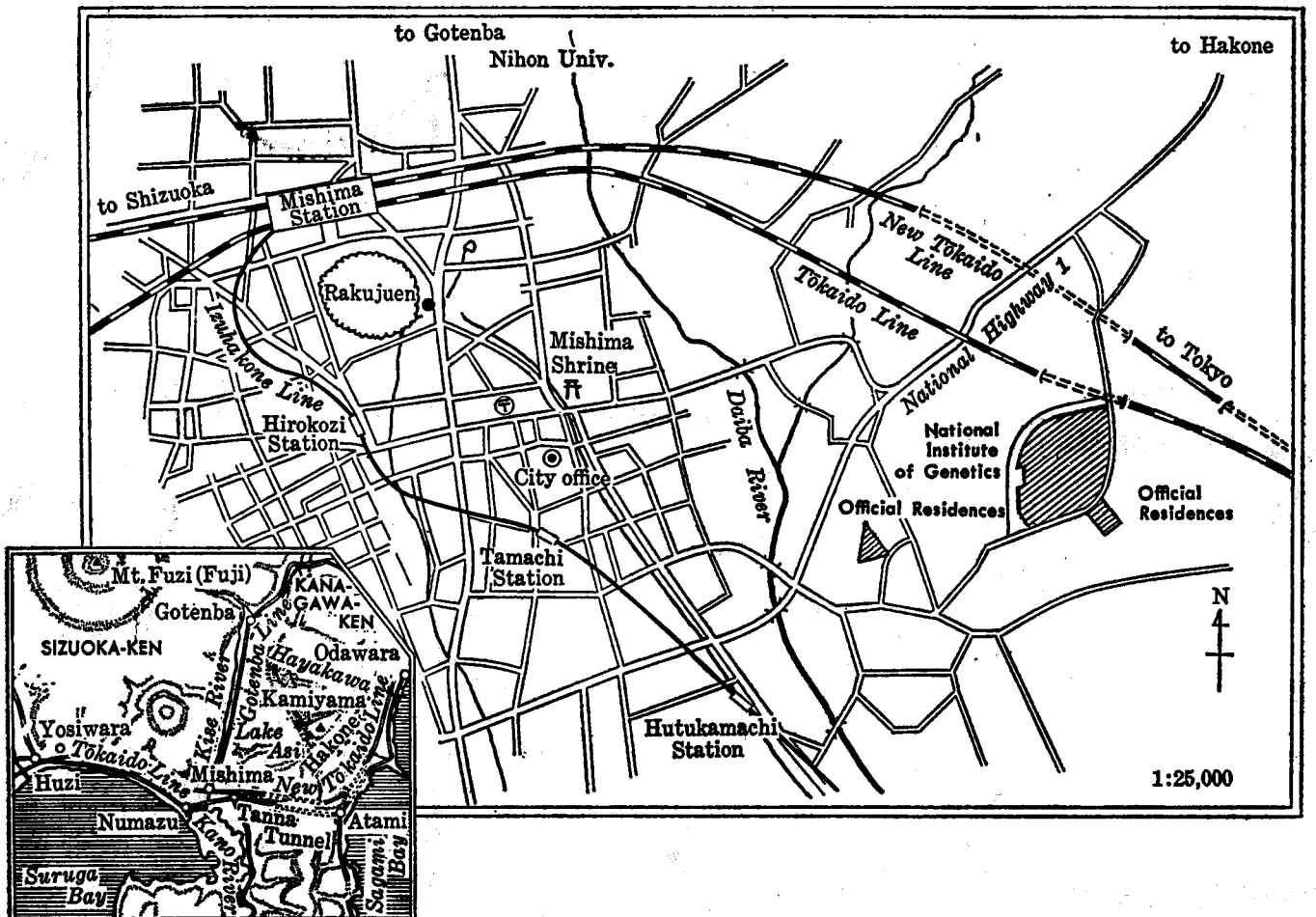
電　話　代表 (0559) (75) 0771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