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Photo by Y. Nakagome

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GENERAL STATEMENT

Science of genetics has made remarkable progress in recent years. Since the foundation in 1949, the Institute has devoted itself to the promotion of fundamental researches on genetics, taking up research subjects of wide range. So far, it has been operated under a policy to keep a balance among each of ten research departments. Such policy permitted us to cope with most research subjects in relevant fields. However, amazingly rapid progress in this science has obliged us to reconsider the policy hitherto adopted. Furthermore, we have been pressed by another unavoidable circumstances. Staff members of the Institute has been reduced in number year by year due to budgetary limitation of the government. Thus, the Institute has confronted serious problems.

In order to find a new turn of the situation we have been discussing for several years about switching over from the present system to other one as research institute for joint use by the universities. There had been several arguments for and against the reorganization. After full discussion, the Future Planning Committee of the Institute concluded at the meeting on June 3rd that adoption of the new system should not be retarded in order to keep up with the rapid progress of the genetic studies. The matter was taken up into deliberation at the annual meeting of the Council held on June 20th. The Council decided that it would be advisable for the Institute to take the new system. Then, having obtained the agreement of all strata of the Institute, I offered our proposal to the Ministry of Education, Science and Culture, and at the same time I requested our Future Planning Committee to embody the plan in detail.

The number of staff members who went abroad decreased to 18 from 32 of the preceding year, however, the visitors to our institute from other countries came up to 62, twice as many as last year. Among them visitors from People's Republic of China were the most in number. Those who have been working as long term visiting researchers under the grant in aid from the Japan Society for the Promotion of Science or with the aid from scientific exchange fund of their own countries are: Dr. Gerard Second from France in Department of Applied Genetics, Dr. C. Pai from Taiwan in Departments of Applied Genetics and Biochemistry, Dr. Josef Acher-

mann from Switzerland and Dr. Nancy L. Wanek from U.S.A. in Department of Biochemistry, Dr. Harold W. Keck and Dr. Hildegard Kraut from West Germany in Department of Microbiology. The Institute is varied in the nationality of the visiting researchers.

Dr. Hiko-Ichi Oka, the head of the Department of Applied Genetics, retired on April 1st by reason of the age limit. He has long been working in this institute as a competent leader of rice research group. I feel regret at his retirement. Dr. Takeo Maruyama, the chief of the first laboratory of the Department of Population Genetics was appointed to the post of the head of the Department of Physiological Genetics which had been left vacant for a year. It is regretful that Dr. Mitsuo Tsujita, honorary member, the former head of the Department of Biochemistry, succumbed to an untimely death on April 7th. May his soul rest in peace!

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OSHIMA, Chozo, Manager,

PROJECTS OF RESEARCH FOR 1980

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 histocompatibility antigens of wild rodents (MORIWAKI)
Cytogenetical study of ants (IMAI)
Cytogenetical studies on *Drosophila* (YAMAMOTO)
Cytogenetical study of fishes (YOSIDA)

Department of Physiological Genetics

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

Department of Biochemical Genetics

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

Department of Applied Genetics

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

Department of Induced Mutation

Molecular mechanisms of spontaneous, chemical- and radiation-induced mutations (KADA, SADAIE and INOUE)
Environmental mutagens and carcinogens (KADA, SADAIE, TUTIKAWA and HARA)
Radiation genetics in mice (TUTIKAWA)
Biochemical factors involved in cellular repair of genetic damage (INOUE and KADA)
Genetics of *Bacillus subtilis* (SADAIE and KADA)

Molecular mechanisms of unicellular differentiation in *Bacillus subtilis* (SADAIE)
Radiation and chemical interaction in the cells (KADA)
Genetic fine structure analysis in maize (AMANO)

Department of Human Genetics

Genetic and epidemiologic studies on certain malformations in human embryos (MATSUNAGA)
Genetic studies on retinoblastoma (MATSUNAGA)
Clinical cytogenetic studies of congenital abnormalities in man (NAKAGOME)
Molecular cytogenetic studies of human chromosomes (NAKAGOME)
Studies on human chromosome variants (NAKAGOME and MATSUNAGA)

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 altruism (AOKI)

Department of Molecular Genetics

Studies on the structure of genome RNA of viruses (MIURA)
Studies on the primary structure of DNA (SOEDA and MIURA)
Studies on structure and function of messenger RNA (MIURA)

Genetical and enzymological studies on *E. coli* polymerase (SUGIURA)
Studies on T_4 RNA ligase (SUGIURA)
Cloning of eukaryotic genes and their structural analysis (SUGIURA and SHINOZAKI)

Genetic Stocks Center

Studies and conservation of germplasm resources in rice and wheat species (FUJII and SANO)
Specificity of mutagen tolerance in higher plants (FUJII)
Exploitation of genetic ability of nitrogen fixation in Graminae (FUJII, SANO, IYAMA and HIROTA)
Studies on genetic differentiation in rice (SANO)
Cytogenetic studies of Norway rats and establishment of the chromosomal mutant stocks (YOSIDA)
Experimental breeding of wild rodents for estabilshment of the new experimental animals (YOSIDA)
Developmental genetic studies on mouse teratomas (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)

RESEARCHES CARRIED OUT IN 1980

I. MOLECULAR GENETICS

Relationship between Structure of the 5′-noncoding Region of Viral mRNAs and Efficiency in the Initiation Step of Protein Synthesis in a Eukaryotic System

Kazuko YAMAGUCHI, Soh HIDAKA and Kin-ichiro MIURA

To determine whether the rate of protein synthesis is controlled by the structure of mRNA near its 5′-terminus, protein synthesizing ability, especially in its initial stage, was compared among RNAs of plant viruses. Those viruses used here contain several definite pieces of single-stranded RNA. Each of these RNAs acts as a messenger. Cucumber mosaic virus (CMV) RNA 5 synthesizes a small amount of a protein, molecular weight 7000, in an *in vitro* protein synthesizing system from wheat germ or reticulocyte. Brome mosaic virus (BMV) RNA 4 synthesizes a large amount of a coat protein under the same conditions. Both RNAs carry the same 5′-cap structure and a short noncoding region (ten nucleotides in CMV RNA 5 and nine in BMV RNA 4) from the 5′-terminus to the initiation codon AUG. A sequence complementary to the 3′-terminal of 18S ribosomal RNA is contained in BMV RNA 4, but not apparent in CMV RNA 5. Formation of the initiation complex for protein synthesis by the 5′-terminal labeled mRNA of cytoplasmic polyhedrosis virus was inhibited by the addition of cold BMV RNA 4, whereas it was only slightly inhibited by cold CMV RNA 5. BMV RNA 4 which has a sequence complementary to rRNA can form the initiation complex more easily than CMV RNA 5. It is concluded that an apparent complementary sequence to the 3′-terminal of 18S rRNA in the 5′ noncoding region of eukaryotic mRNA as well as the 5′-cap structure enhances the rate of initiation complex formation in protein synthesis.

If the 5′-noncoding region is large, tertiary structure of this region seems to arrange mutual relationships among these functional sites. Large hairpin structures can bring the initiation codon near the 5′-cap and/or the

complementary sequence to the 3′-terminal of 18S ribosomal RNA. An example of the possibility is seen in CMV RNA 4 that codes a coat protein and carries 76 bases in the 5′-noncoding region (succeeding report, S. Hidaka and K. Miura). Another example is seen in mRNA of a precursor of corticotropin/β-lipotropin, in which the 5′-noncoding region consists of 128 bases (Eur. J. Biochem. **113**, 531–539 (1981), A. Inoue, M. Nakamura, S. Nakanishi, S. Hidaka, K. Miura and S. Numa).

Terminal Part Structure of Genome RNA of Cucumber Mosaic Virus

Soh HIDAKA and Kin-ichiro MIURA

A genome of cucumber mosaic virus (CMV) is consisted of four pieces (strain O) or five pieces (strain Y) of single-stranded RNA. Molecular weights of these RNA segments are RNA 1: 1.23×10^6, RNA 2: 1.13×10^6, RNA 3: 0.83×10^6, RNA 4: 0.33×10^6 and RNA 5: 0.1×10^6, respectively. Nucleotide sequence near the 5′-terminus and the 3′-terminus of these RNA segments was analyzed. Every RNA segment of CMV carries the same 5′-cap structure $m^7G^{5'}pppG$-.

After the cap part is deleted chemically, the 5′-terminus of RNA is labeled with [^{32}P] phosphate by nucleotide kinase. The RNAs were digested partially by weak alkali and ribonucleases (T_1, U_2). The resulting oligo- and poly-nucleotides were separated by gel electrophoresis and autoradiographed on a X-ray film. By this analysis the sequence of guanine (G), adenine (A) and pyrimidine (Y) was determined. The partial alkaline digest was electrophoresed in pH 3.5 and pH 7 two-dimensionally in a gel, and autographed. By this analysis cytosine (C) and uracil (U) are distinguished. The 3′-terminus of RNA was bound with [^{32}P]pCp by RNA ligase. The 3′-labeled RNA was digested partially by chemical methods and analyzed by gel electrophoresis as carried out in the nucleotide sequencing near the 5′-terminus.

The 5′-terminal nucleotide sequence including the 5′-cap, $m^7G^{5'}pppG$-U-U- is common for all the RNA segments of CMV, but the first initiation codon for protein synthesis, A-U-G, appears at 56th in RNA 1 and 2, at 77th in RNA 4, and at 11th in RNA 5. It is known that RNA 4 carries a coat protein information. The amino acid sequence of the N-terminal of the coat protein was analyzed to determine the location of initiation codon

of coat protein cistron. Since CMV coat protein is acetylated at the N-terminal amino acid as other plant viruses, N-acetylated oligopeptides obtained by pronase digestion were analyzed. The N-terminal amino acid sequence was identified as Ac-Met-(Asx), here Asx means asparagine (Asn) or apartic acid (Asp). In CMV RNA 4, the first initiation codon A-U-G appears at 77th from the 5′-terminus, and the nucleotide sequence following to A-U-G just corresponds to the N-terminal of coat protein: AUG (Met)-GAU (Asp)-AAU (Asn). Therefore, the initiation codon at the 77th nucleotide is confirmed as the true initiation codon for protein synthesis. Upstream (5′-side) of the initiation codon contains nucleotide sequences complementary to the 3′-terminal part of 18S rRNA in CMV RNAs 1, 2 and 4, but not in RNA 5. The 5′-noncoding region of RNA 1, 2 and 4 is the almost same between O strain and Y strain of CMV, though there are several base changes.

The 3′-terminal nucleotide sequence was very similar among the RNA segments of CMV, 10 nucleotides sequence from the 3′-terminus is perfectly common for RNAs 1 to 4. It would mean that the structure near the 3′-terminus functions for template recognition of the RNA replicase.

The N-terminal analysis of protein was coworked with the late Prof. Kozo Narita and Dr. Susumu Tsunazawa of Osaka University. CMV was prepared by Drs. Susumu Kubo and Yohichi Takanami of Central Research Institute of the Japan Tobacco and Salt Public Corporation.

Sequence of a Putative Promoter Region for the rRNA Genes of Tobacco Chloroplast DNA

Naoki TOHDOH, Kazuo SHINOZAKI and Masahiro SUGIURA

The nucleotide sequence of the segment of tobacco chloroplast DNA adjacent to and including the start of the 16S rRNA gene has been determined (766 base-pairs). The region just preceding this gene was found to contain a gene (see Fig. 1, pp 15) and promoter-type sequences similar to those which occur in *E. coli* were found before this tRNA gene. *E. coli* RNA polymerase can recognize these sequences and *in vitro* co-transcribes the tRNA and rRNA genes.

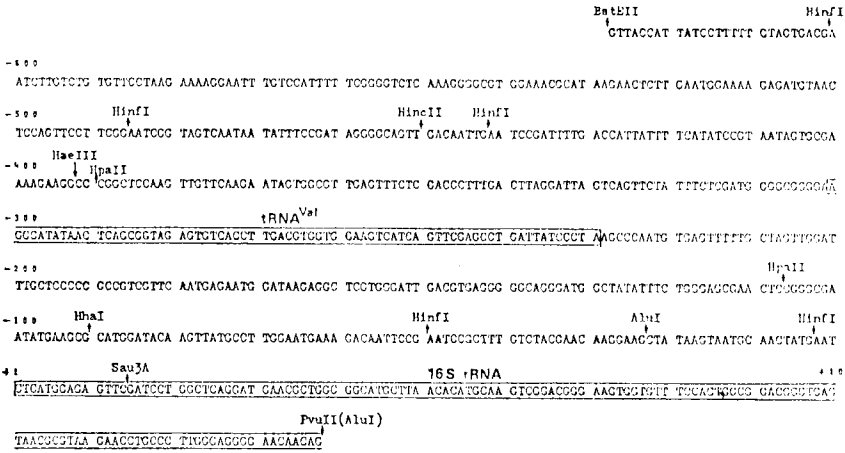


Fig. 1. Nucleotide sequence in the region at the start of tobacco chloroplast 16S rRNA gene.

The Complete Nucleotide Sequence of a 16S Ribosomal RNA Gene from Tobacco Chloroplast

Naoki TOHDOH and MASAHIRO SUGIURA

The complete nucleotide sequence of a 16S ribosomal RNA gene from tobacco chloroplast has been determined (Fig. 1, p. 16). The coding region of the 16S rRNA gene is 1485 base-pairs long. Its nucleotide sequence has 96% homology with that of maize chloroplast 16S rRNA gene and 74% homology with that of *E. coli* one. The 3′ terminal region of this gene contains the sequence ACCTCC which is complementary to sequences found at the 5′ termini of prokaryotic mRNAs. The large stem structure can be constructed from the sequences surrounding the 5′ and 3′ ends of the 16S gene.

The Nucleotide Sequence of 4.5S Ribosomal RNA from Tobacco Chloroplasts

Fumio TAKAIWA and Masahiro SUGIURA

The 4.5S RNA has recently been found in the large subunit of the chloroplast ribosomes from a number of higher plants. In tobacco chloroplasts, the 4.5S RNA is present in approximately equimolar amount as

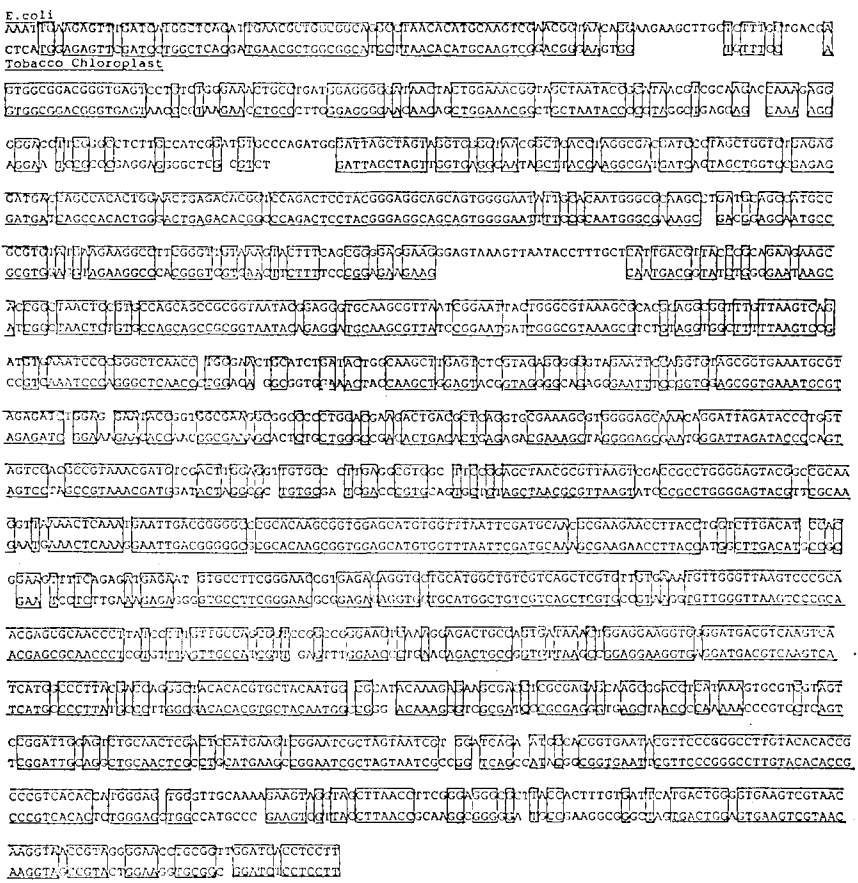


Fig. 1. Nucleotide sequence of tobacco chloroplast 16S rRNA gene. For comparison, sequence of *E. coli* 16S rDNA (upper row) is presented and homologous regions are boxed.

the chloroplast 5S RNA and is coded for by the chloroplast DNA between the 23S and 5S rRNA genes. The nucleotide sequence of tobacco chloroplast 4.5S ribosomal RNA has been determined. The 4.5S RNA is 103 nucleotides long and its 5′-terminus is not phosphorylated. A possible secondary structure for the 4.5S RNA can be constructed as shown in Fig. 1. (p. 17). This work was published in the Nucleic Acids Research **8**, 4125–4129 (1980).

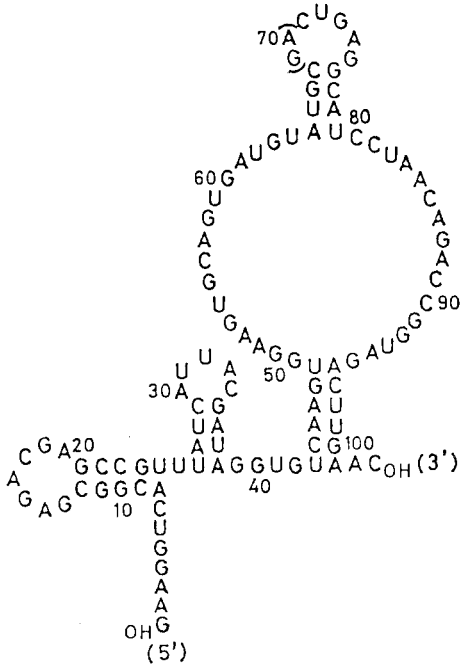


Fig. 1. A possible secondary structure of tobacco chloroplast 4.5S RNA.

The Nucleotide Sequences of two tRNA^{Asn} Genes from Tobacco Chloroplasts

Akira KATO, Hiroaki SHIMADA and Masahiro SUGIURA

Recombinant plasmids which contain EcoRI fragments of tobacco chloroplast DNA carrying tRNA genes were constructed. Plasmids pTC211 and pTC293 contain the base sequences for tRNA^{Asn} in their 1.4 and 1.1 Md EcoRI fragments, respectively. These two tRNA sequences are identical. Fig. 1 (p. 18) shows the cloverleaf structure that can be deduced for the tRNA^{Asn} based on the DNA sequence. Each tRNA^{Asn} gene is located at about 0.9 kb apart from the distal end of each 5S rRNA gene and is coded for by the DNA strand opposite from that of the rRNA genes.

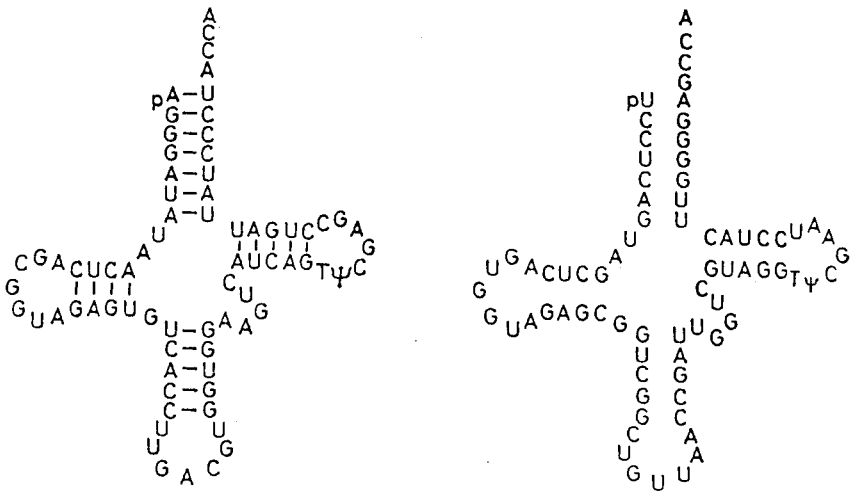


Fig. 1. Tobacco chloroplast $tRNA^{Asn}$ (right) and $tRNA^{Val}$ (left).

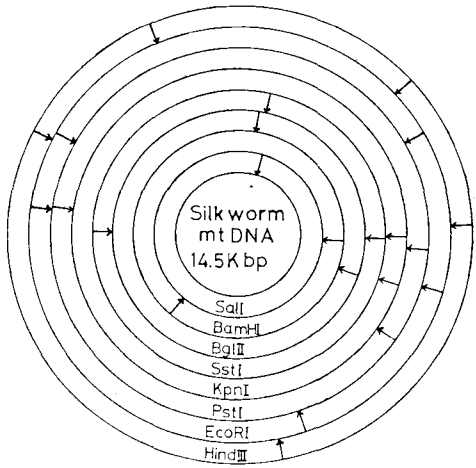
Purification of the T4 DNA Ligase by Blue Sepharose Chromatography

Masahiro SUGIURA

T4 DNA ligase catalyzes the formation of phosphodiester bonds between adjacent 5′-phosphoryl and 3′-hydroxylends in nicked duplex DNA. In addition, it catalyzes the joining of duplex DNA molecules at completely base-paired ends. These activities of T4 DNA ligase have been used to synthesize DNA with defined sequences and to construct recombinant DNA molecules *in vitro*.

As T4 DNA ligase was found to bind to Blue Sepharose CL-6B, Blue Sepharose CL-6B chromatography was introduced at the last step of purification procedure. The purified T4 DNA ligase preparation gave one band on SDS-polyacrylamide gel electrophoresis and the molecular weight was estimated to be 60,000. Thus Blue Sepharose chromatography removed at least nuclease(s) active on $(dT)_{10}$, which could not completely be removed by DEAE- and P-cellulose chromatography and gel filtration. This work was carried out with assistance of Mrs. Mie Kusuda and was published in the Analytical Biochemistry **108**, 227–229 (1980).

The physical map of silkworm mt DNA.



Cloning of Mitochondrial DNA from Silkworm Eggs

Jun KUSUDA and Kimiji ONIMARU

Several hundred of silkworm mutants were isolated and these relative genetic loci were determined on chromosomes as a linkage maps. In contrast to extensive analysis of chromosomes of silkworm, genetic and chemical characters of the extrachromosomes is little known. Silkworm mitochondria show heterologous forms in the midgut or Malpighian tissues. Elucidation of the gene organization of mitochondria has been difficult because of its incapability of mating. Recent advance of gene cloning and nucleotide sequencing techniques allow us to analyse the fine structure of this organella DNA and estimate the genes coded on it. We have started to study the structure of silkworm mitochondrial DNA by the application of these techniques. Mitochondrial DNA isolated from silkworm (strain P-22) eggs were cleaved by Pst I into three fragments of 7.1 kiro base pairs (kbp), 6.2 kbp and 1.2 kbp long. Genome size of this DNA, estimated from the summation of the size of Pst I fragments, was 14.5 kbp. Three fragments were cloned into *E. coli* plasmid pBR322 in order to obtain large quantities of DNA homologous to mitochondrial DNA for further analysis.

Pst I fragments of the mitochondrial DNA were joined to pBR322 cleaved with Pst I using T_4 ligase and transformed *E. coli* HB101. Transformants containing recombinant plasmids were selected by the colony hybridization using ^{32}P-labeled mitochondrial DNA from the Tetracycline resistant and Ampicirine sensitive colonies. Seven colonies hybridized to ^{32}P labeled probe. Gel analysis of Pst I digests of recombinant plasmids revealed that the three types of clones were obtained. pBmt24-2, pBmt13 and pBmt30 contain the Pst I fragments A, B and C, respectively. pBmt24-1 contains both Pst I-B and Pst I-C fragments. pBmt24-1 and pBmt24-2 DNA were digested with Sal I, Bam HI, Bgl II, Sst I, Pst I, Kpn I, EcoRI and Hind III. The location of cleavage sites were deduced from the electrophoretic patterns of double and triple digests with these enzymes. A physical map of whole mitochondrial DNA was constructed by the combination of pBmt24-1 and pBmt24-2 maps. As illustrated, mitochondrial DNA from silkworm eggs contains 23 cleavage sites of 8 restriction enzymes tested. Sixteen of them were located on the region of 7 kbp long corresponding to half genome. Since 6 among 8 enzymes used are specific for hexanucleotides including 4 GC base pairs, we assumed that the nucleotide sequence of this region was rich in GC base pairs and the replication origin could be located in the another half region containing few restriction sites, because this region is assumed to be rich in AT base pairs.

II. MICROBIAL GENETICS

On the Biological Role of Penicillin-binding Proteins 4 and 5

Miguel Angel de PEDRO[1], Uli SCHWARZ,[1] Yukinobu NISHIMURA, and Yukinori HIROTA

Recent studies *in vivo* of murein biosynthesis in *E. coli* show that newly synthesized murein differs in its chemical composition from preexisting murein. Murein newly inserted into growing cells is less cross-linked than the preexisting murein and it carries pentapeptide subunits. The pentapeptide subunits act as donors in a secondary maturation process which involves further transpeptidation by which the final stage of cross-linkage in murein is achieved. This reaction seems to involve penicillin-binding protein 4. In mutants with a defective penicillin-binding protein 4, the secondary transpeptidation is abolished and the murein, in this case, is not processed further and remains poorly cross-linked. This indicates that penicillin-binding protein 4, which *in vitro* has carboxypeptidase IB and DD-endopeptidase activity, may act as a transpeptidase in the intact cell.

First hints on the possible role of penicillin-binding proteins 5 and/or 6, which show carboxypeptidase IA activity *in vitro* come from an accumulation of pentapeptide side chains in intact sacculi. These pentapeptide subunits were identified by their strong and stable interaction with vancomycin. Exponentially growing cultures of appropriate mutants were either pulse-(5 min) or uniformly labelled with tritiated diaminopimelic acid and the amount of pentapeptide-containing subunits and the extent of cross-linkage in the murein were determined.

As listed in Table 1, mutants defective in penicillin-binding protein 5 show an increase of pentapeptide subunits, both after pulse- and uniform-labelling of the murein. All mutants tested with defective carboxypeptidase IA show a dramatic accumulation of pentapeptide in the murein. Thus, penicillin-binding protein 5 seems to function as a carboxypeptidase not only *in vitro* but also *in vivo*. It converts pentapeptide subunits, the activated substrate for transpeptidation, into tetrapeptides.

The double mutants with defects in penicillin-binding proteins 4 and 5

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Table 1. Cross-linkage and accumulation of pentapeptide subunits in the murein of wild-type and mutant strains of *E. coli*

Strain	Cross-linkage[a]		Pentapeptide[b]	
	Pulse-labelled 5 min	Uniformly labelled	Pulse-labelled 5 min	Uniformly labelled
W-7 (Wild type)	0.22±0.012	0.28±0.013	3 ±0.45	0.3±0.18
5509 (Wild type)	0.20±0.01	0.27±0.011	2.6±0.6	0.4±0.15
DL64 (*dacB*) 4	0.20±0.009	0.21±0.011	10 ±1.4	0.9±0.1
1191 (*dacA*) 5	0.22±0.014	0.29±0.003	29 ±6.0	5.6±1.49
5683 (*ponA*, *dacA*, *dacB*)[c]	0.27±0.002	0.29±0.006	42 ±0.88	15 ±0.96
5684 (*dacA*, *dacB*)	0.26±0.01	0.27±0.009	36 ±1.05	15 ±0.4

[a] Cross-linkage is expressed as molar fraction of cross-linked dimers in total murein.
[b] Expressed as percentage of total monomeric lactyl-peptides.
[c] Leaky for carboxypeptidase 1b activity; no penicillin-binding protein 4 detectable.

as well (strain JE5683 and JE5684) have a highly cross-linked murein both after pulse- and uniform-labelling of the murein. This is unexpected because a defect in penicillin-binding protein 4 alone results in decreased cross-linkage (Table 1); maturation of murein *via* transpeptidation is abolished. However, the enormous accumulation of pentapeptide subunits in the mutants may favour an abnormal formation of peptide cross-links in the initial step of murein insertion which under normal conditions yields poorly cross-linked murein. (For details, see FEMS Microbiology Letters **9**, 219–221 1980).

On the Process of Cellular Division in *Escherichia coli*: Isolation and Characterization of Penicillin-binding Proteins 1a, 1b, and 3

Toshihide TAMURA, Hideho SUZUKI, Yukinobu NISHIMURA, Junzo MIZOGUCHI, and Yukinori HIROTA

Multiple mutants of *Escherichia coli* defective in penicillin-binding proteins (PBPs) were constructed, and into these strains ColEl plasmids carrying the genes for PBP-1a, -1b, or -3 were introduced. From these plasmid-carrying strains, PBP-1a and -1b were purified by ampicillin-Sepharose affinity chromatography and PBP-3 by cephalexin-Sepharose affinity chromatography. Improved purification was achieved by differential elution

with NH_2OH. Purified PBP-1b synthesized murein when added to the membrane fraction of a PBP-1b-defective mutant, which by itself failed to support murein synthesis *in vitro*. The PBP-1b preparation was able to synthesize murein from the lipid intermediate extracted with chloroform/methanol but was unable to utilize UDP-linked precursors for murein synthesis. Murein synthesis was inhibited by vancomysin, ristocetin, moenomycin, and endruacidin, but not by β-lactam antibiotics. The synthesized murein was shown to contain crosslinked muropeptides. Their crosslinking was abolished by action of β-lactam antibiotics. The PBP-1a and -3 preparations showed substantially no activity for murein synthesis in the same reaction system. None of the three PBPs showed D-alanine carboxypeptidase activity with UDP-N-acetylmuramyl-pentapeptide as substrate or endopeptidase activity with bis(disaccharide-peptide) as substrate. (For detail, see Proc. Natl. Acad. Sci. USA 77, 4499–4503, 1980)

A Mutant of *Escherichia coli* defective in Penicillin-binding Protein 5 and Lacking D-alanine Carboxypeptidase IA

Yukinobu NISHIMURA, Hideho SUZUKI, Yukinori HIROTA, and James T. PARK[1)]

A mutant of *Escherichia coli* defective in penicillin-binding protein 5 activity was isolated. The mutation (*pfv*) was shown to be located at 14.0 min on the *E. coli* chromosome map. Loss of penicillin-binding protein 5 in the *pfv* mutant was associated with the loss of D-alanine carboxypeptidase IA activity and increased sensitivity to β-lactam antibiotics. We conclude that penicillin-binding protein 5 catalyzes the major D-alanine carboxypeptidase IA activity and that the enzyme activity, *in vivo*, protects *E. coli* cells from killing by low inhibitory concentrations of β-lactam antibiotics. (For detail, see Journal of Bacteriology, July 1980, 531–534)

Synthetic ColEl Plasmids Carrying Genes for Penicillin-Binding Proteins in *Escherichia coli*

Yutaka TAKEDA, Akiko NISHIMURA, Yukinobu NISHIMURA, Masao YAMADA, Seiichi YASUDA, Hideho SUZUKI and Yukinori HIROTA

Clarke and Carbon's collection of 2,000 *Escherichia coli* strains which

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harbor ColEl plasmids carrying small random segments of the *E. coli* chromosome was screened for the correction of mutational defects in penicillin-binding proteins (PBPs): *ponA* (PBP-1a), *ponB* (PBP-1b), *dacB* (PBP-4) and *pfv* (PBP-5). We found the plasmids carrying the chromosomal segments containing *pon*A$^+$-*aro*B$^+$ (pLC29-47), *pon*B$^+$-*ton*A$^+$ (pLC4-43, pLC-4 44 and pLC19-19) and *arg*G$^+$-*dac*B$^+$ (pLC10-46 and pLC18-38). Characters of these plasmids were analyzed. Two other plasmids (pLC26-6 and pLC4-14) previously found to correct an *fts*I mutation (Nishimura *et al.*, 1977) were also investigated further. Restriction maps of chromosomal DNAs carried by pLC29-47, pLC4-44, pLC19-19, pLC18-38, pLC26-6 and pLC4-14 were constructed. The regions of *pon*B-*ton*A on pLC4-44 and pLC19-19, and of *leu*A-*fts*I-*mur*E & F on pLC26-6 were located on the restriction maps. Although both pLC26-6 and pLC4-14 corrected a thermosensitive mutation, *fts*I, which causes a defect in cell division due to abnormal PBP-3, only pLC26-6 led an *fts*I mutant to restore production of PBP-3, while pLC4-14 did not. Restriction and hetero-duplex analyses of pLC26-6 and pLC4-14 have shown the absence of nucleotide sequence homology between them. The plasmids, pLC29-47 carrying *pon*A$^+$ and pLC4-43, pLC4-44 and pLC19-19 carrying *pon*B$^+$ led the host cell to overproduce the respective PBP. (For details, see Plasmid, 1981)

Cloning of *pon* B and *fts* I Gene of *Escherichia coli* and Purification of Penicillin-Binding Proteins 1B and 3

Hildegard KRAUT, Wolfgang KECK and Yukinori HIROTA

In the inner membrane of *Escherichia coli* at least 7 penicillin-binding proteins were detected, to which penicillin binds covalently (Spratt, B.G. 1975, Proc. Natl. Acad. Sci. **72**: 2999–3003). Since β-lactam antibiotics are known to inhibit cell division and cell elongation, the role of these penicillin-binding proteins with respect to the murein metabolism has been investigated. In the meantime, with the exception of penicillin-binding protein 6, mutants for all these PBPs had been isolated (Suzuki *et al.* 1978, Proc. Natl. Acad. Sci. **75**: 664–668, Spratt, B.G. 1977, J. Bacteriol. **131**: 293–305, Matsuhashi *et al.* 1978, Proc. Natl. Acad. Sci. **75**: 2631–2635). By studying these mutants it was suggested that PBP-1B is directly involved in the final polymerization steps in murein biosynthesis (Suzuki *et al.* 1977, Ann. Rep. Natl. Inst. Genet. **28**: 23–24). An enriched fraction of PBP-1B synthesized

crosslinked murein from lipid linked precursors (Tamura *et al.* 1980, Proc. Natl. Acad. Sci. **77**: 4499–4503).

The activity of PBP-3 is essential for cell division, which can be demonstrated by the follwing evidence. Specific β-lactam antibiotics, like benzylpenicillin or cephalexin which cause filament formation, bind at the effective concentration preferentially to PBP-3 in the inner membrane (Spratt, B.G. 1977, Eur. J. Biochem. **72**: 341–352). Mutants with a genetic defect in PBP-3 possess a thermolabile PBP-3 and a temperature sensitive defect in septum formation in parallel. Our main effort focused on these two PBPs because of their essential functions in cell elongation and cell division.

Clarke and Carbon's synthetic Col El plasmid collection was screened for the correction of mutational defects in penicillin-binding proteins and the restriction maps of the plasmids were established (Takeda *et al.* 1981, Plasmid, in press). *Escherichia coli* strains carrying pLC 19-19 (pon B) or pLC 26-6 (*fts* I) show about 10-fold overproduction of PBP-1B or -3 respectively (Tamura *et al.* 1980, Proc. Natl. Acad. Sci. **77**: 4499–4503).

Starting from these plasmids we cloned the two structural genes *pon* B and *fts* I independently into a plasmid with temperature dependent copy number. This high copy number plasmid pSY 343 is derived from the miniplasmid pBEU 17 which was constructed from a runaway replication mutant pKN 402 of the antibiotic resistance factor R1 (Uhlin *et al.* 1979, Gene **6**: 91–106). Seiichi Yasuda exchanged the ampicillin resistance in pBEU 17 with kanamycin resistance to get the plasmid pSY 343. This plasmid replicates at temperatures above 35°C without copy number control during 2–3 hours. This gives a 500–1000 fold increase in gene dosage of plasmid-coded genes compared to an average chromosomal gene. During gene amplification, cell growth and protein synthesis continue at the normal rate and lead to an overproduction of plasmid gene products.

The *fts* I gene was cloned from pLC 26-6 into the high copy number plasmid pSY 343 by using the *Bam* HI and *Eco* RI site (Fig. 1). A mutant strain (JE7627) with defective PBP-1B and -4 was transformed with this new plasmid pWK 7. For overproduction of PBP-3 the cells were grown in the presence of kanamycin at 30°C, shifted to 37°C in the early logarithmic phase and cultivated for 4 more hours. During this time the cells reach 150-fold overproduction of PBP-3 compared to wild type cells, all of which is inserted into the inner membrane.

The cloning of *pon* B gene was a little more complicated, because there

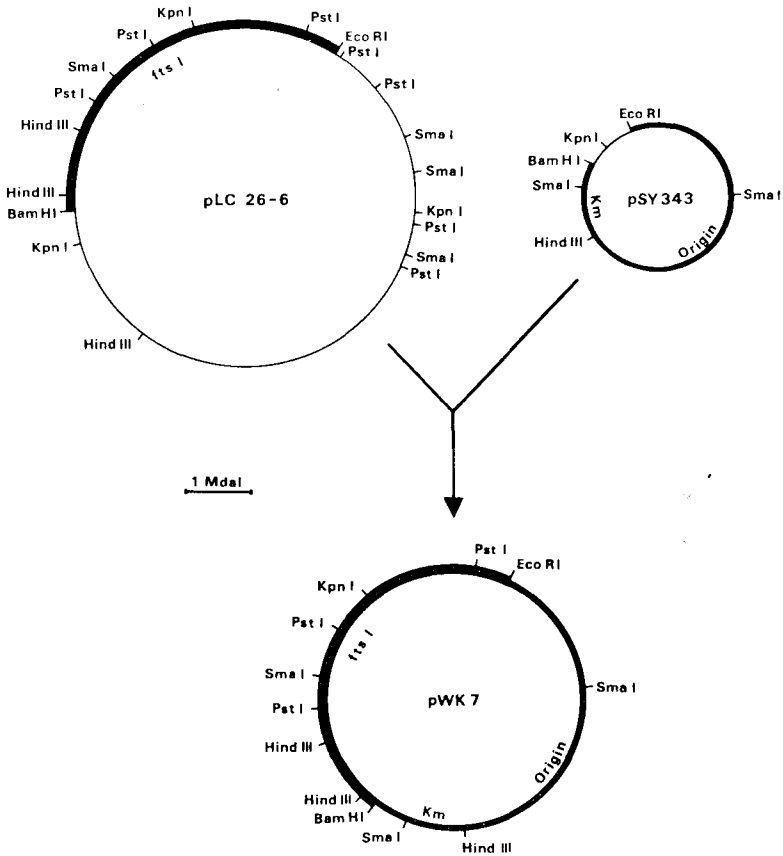

Fig. 1. Cloning of *fts* I gene into the high copy number plasmid pSY 343.

are no suitable restriction sites in the plasmid pLC 19-19, which could be used for direct cloning into the high copy number plasmid pSY 343. We first used the *Pst* I sites to clone the *pon* B gene from pLC 19-19 into the *Pst* I site of the vector pBR 322 (Fig. 2). Plasmids with both possible orientations of the integrated fragment could be isolated (pHK 1811, pHK 1824). Both types of plasmids lead to an unexpected high overproduction of PBP-1B, about 120-fold compared to the wild level. For the vector pBR 322 only 30 copies per cell are normally described. Recently it was found that the deletion of a given non-essential region from Col El-like

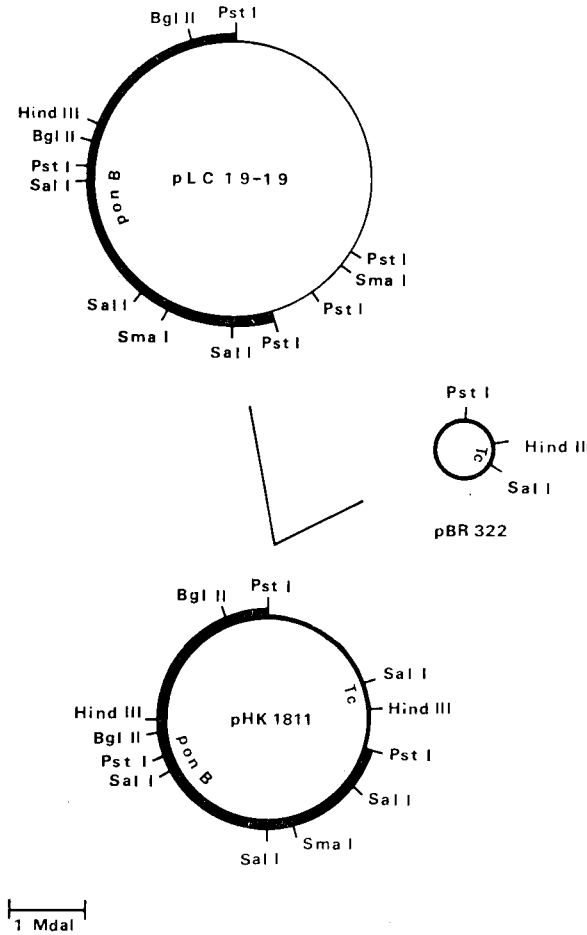

Fig. 2. Cloning of *pon* B gene into the plasmid pBR 322.

plasmids results in an increased copy number. Moreover, other Col El sequences, which might be lost in the plasmids pHK 1881 and pHK 1824, are involved in determining plasmid copy number (Twigg, A. J. and D. Sherratt, 1980, Nature **283**: 216–218). For further cloning of *pon* B gene from pHK 1811 into the high copy number plasmid pSY 343 the *Hin*d III sites were used (Fig. 3). The plasmid pHK 231, which contains the complete DNA of pHK 1811, pBR 322 and pSY 343, was obtained. A multiple

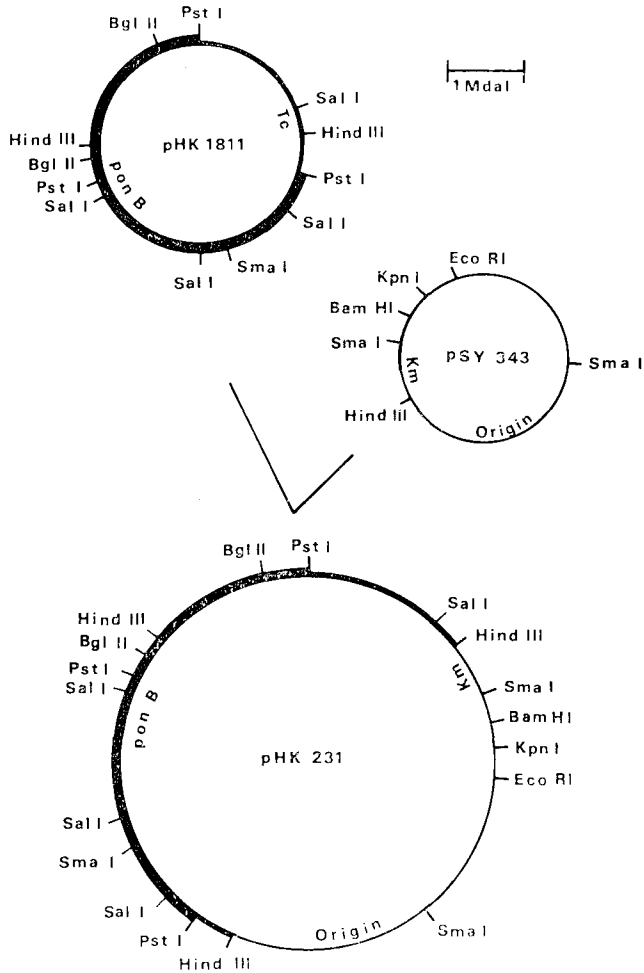

Fig. 3. Recloning of *pon* B gene into the high copy number plasmid pSY 343.

mutant strain with temperature sensitive PBP-1A, which was also defective for PBP-1B and -4, was transformed with this new plasmid pHK 231. Cells are grown as already described in the case of PBP-3. In these cells PBP-1B was overproduced 150-fold or more, compared to wild type level. All this protein was membrane bound.

By cloning and amplification of these two structural genes and the overproduction of the gene products we are able to isolate both penicillin-binding proteins and study them on the protein level, and we can isolate the DNA-fragments, that code for the structural genes of these two proteins and study them on the DNA level. For the purification of PBP-1B and -3 we used overproducing cells with defects in other penicillin-binding proteins. After opening the cells the PBPs were extracted from the membrane fraction and the crude extract was applied to covalent-affinity-chromatography. For the isolation of PBP-1B ampicillin was coupled to activated CH-Sepharose 4B (Pharmacia) and for PBP-3 cephalexin was used. After loading the columns with crude extracts the columns were washed extensively with buffer containing 2M NaCl. The covalently bound penicillin-binding proteins are then eluted with buffer containing 1M hydroxylamine. Both proteins could be obtained by this single step purification in pure form. Starting with 25 g wet weight of cells we could obtain, in a typical purification procedure, about 5–10 mg of pure PBP-1B and about 2 mg of pure PBP-3.

With the purified proteins we are now able to study the structural properties, the binding-sites and hydrophobic sequences of these two proteins. We will also continue to unravel their enzymatic activities, their binding- and complex-stability with different β-lactam antibiotics. All this information will provide insight into the very complex process of cell growth and cell division in *Escherichia coli*.

Isolation of an *E. coli* DNA Topoisomerase I Mutant

Rolf STERNGLANZ,[1] Stephen DINARDO,[1] James C. WANG,[2]
Yukinobu NISHIMURA and Yukinori HIROTA

A collection of temperature-sensitive *E. coli* mutants has been screened in order to find a DNA topoisomerase I (ω) mutant. Two such mutants have been found, both with a significant reduction in ω activity. One mutant has about 10% residual topoisomerase activity and the other has 1–5% the activity of wild-type. The remaining activity is resistant to oxolinic acid and therefore is presumably due to ω. The temperature sensitivity of the mutants can be removed by P1 transduction or conjugation,

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and the resultant strains still lack ω activity. Therefore, either ω is not essential for *E. coli* or the residual activity present in the mutants is sufficient for viability. The mutation causing the lack of ω activity maps near *trp* at about 27 min on the *E. coli* genetic map. (For details, see Mechanistic Studies of DNA Replication and Genetic Recombination, 1980)

Mutations in the Gene Coding for *Escherichia coli* DNA Topoisomerase I affect Transcription and Transposition

Rolf STERNGLANZ,[1] Stephen DINARDO,[1] Karen A. VOELKEL,[1]
Yukinobu NISHIMURA, Yukinori HIROTA, Kathleen BETHLERER,[2]
Louis ZUMSTEIN,[2] and James C. WANG[2]

Mutations in *top*, the structural gene for *Escherichia coli* DNA topoisomerase I, have been identified and mapped at 28 min on the chromosome, near *cysB*. Strains carrying deletions of the *top* gene are viable. The *top* mutations, however, do exert pleiotropic effects on transcription and transposition. Mutants lacking DNA topoisomerase I have a more rapid rate of induction and a higher level of catabolite-sensitive enzymes including tryptophanase and β-galactosidase. This general activation of transcription by *top* mutations can be attributed to an increase in the negative superhelicity of the DNA *in vivo* when the topoisomerase activity is abolished. The frequency of transposition of Tn5, a transposon carrying kanamycin resistance, is decreased by a factor of 40 or more in *top* mutants. A direct or indirect role of the topoisomerase in transposition is discussed. The transposition frequency of Tn3, however, is not dependent on *top*. Based on the studies of the *E. coli top* mutants, it appears that the *supX* gene, which was originally studied in *Salmonella typhimurium* [Dubnau, E. & Margolin, P. (1972) Mol. Gen. Genet. **117**, 91–112] is likely to be the structural gene for DNA topoisomerase I. (For detail, see Proc. Natl. Acad. Sci. USA **78**, 2747–2751, 1981)

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Characterization of the *dnaA* Gene Carried by Lambda Transducing Phage

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Haruo OZEKI, and Hideo YAMAGISHI

Specialized transducing phages *λdnaA* were obtained by inducing lysogens in which *λtna* was integrated at the *tnaA* region of the *Escherichia coli* chromosome; the *tnaA* region is located in the vicinity of the *dnaA* gene. The *dnaA*$^-$ deletion derivatives of *λdnaA* were isolated from the lysate of *λdnaA* grown on bacteria carrying a transposon Tn3.

The structures of various transducing phages thus obtained were determined by heteroduplex DNA mapping. From these results, the transducing fragment of 13.8-kb-long was divided into nine domains. Upon infection of UV-irradiated cells with the phage, production of polypeptides of 49 kD and 42 kD was specifically associated with infections by the *dnaA* and *recF* transducing phages. Polypeptides of 49 kD and 42 kD appeared to be coded for by *dnaA* and *recF* genes, respectively. The *dnaA* gene was assigned to the region of 2.8-kb-long which extends by 2.4 kb in the counterclockwise direction on the *E. coli* genetic map and 0.4 kb in the opposite direction, as measured from the nearest *Hin*dIII site close to the *tnaA* gene. The *recF* gene was also discovered to lie very close to *dnaA* in the order of *tnaA-dnaA-recF*.

Merogenotes heterozygous for the *dnaA* gene were constructed by introducing F′100-12 carrying *λdnaA* into the recipients with different mutations at or near *dnaA*. For combinations, F′(*λdnaA*$^+$)/*dnaA*46 and F′(*λdna*$^+$)/*dna*-83, *dnaA*$^+$ was trans-dominant, whereas the *dnaA*$^+$ was recessive for F′(*λdnaA*$^+$)/*dna*-5. For F′(*λdnaA*$^+$)/*dna*-167, the result of the transdominance test was affected by the growth media employed; *dnaA*$^+$ was dominant on a *λ*-broth plate, and *dna*-167 was dominant on an M9-minimal plate. Thus, transdominance of *dnaA*$^+$ in heterozygotes is affected by difference in mutations and growth media. (For details, see Mol. Gen. Genet. **180**, 235–247, 1980)

Globomycin Sensitivity of *Escherichia coli* and *Salmonella typhimurium*: Effects of Mutations Affecting Structures of Murein Lipoprotein

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The sensitivity of strains of *Escherichia coli* and *Salmonella typhimurium* to globomycin is increased in mutants defective in the lipopolysaccharide structure. *E. coli* mutants altered in the structures or biosynthesis of murein lipoprotein are more resistant to globomycin than the parental strains. (For details, see Journal of Bacteriology, Jan. 1981, 657–660)

Cloning and Nucleotide Sequence of Asparagine Synthetase Gene of *Escherichia coli*

Masataka NAKAMURA, Masao YAMADA, Yukinori HIROTA, Kazunori SUGIMOTO, Atsuhiro OKA and Mituru TAKANAMI

We have subcloned the *asnA* gene of *E. coli* K-12, a gene coding for asparagine synthetase, from a previously cloned 6 magadalton segment of *E. coli* chromosome containing the DNA replication origin, *ori*, and *asnA* (Yasuda, S. and Y. Hirota. 1977 Proc. Natl. Acad. Sci. USA **74**: 5458). The complete nucleotide sequence of the *asnA* gene was determined: the region of the structural gene extends 990 base-pairs which codes for a polypeptide of 330 amino acid residues with a molecular weight of 36,688 daltons. The nucleotide sequences of the promoter and the ribosome-binding site of the gene are also assigned. (For details, see Nucl. Acid Res. 1981)

Conjugative Transfer of pBR322 with a Chromosomal DNA of *Escherichia coli* by Hfr

Masao YAMADA and Yukinori HIROTA

It is well known that the non-autotransmissible plasmid, ColEl, can be transferred efficiently in the presence of F plasmid, while the common

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cloning vectors derived from ColEl-like plasmid, such as pBR322, pBR313 and pMB9, could be transferred barely in the presence of F, since they lack the region corresponding to *mob* (mobility) gene of ColEl (Clarke, L. and J. Carbon, 1976, Cell **9**: 91, Young, I. G. and M. I. Poulis, 1978, Gene **4**: 175). However, we found that such cloning vectors could be transferred from Hfr to recipient cells by a bacterial mating if they had a chromosomal DNA of *E. coli*. When the chromosomal DNA in the plasmid corresponded to the region transferred early by the Hfr, the frequency of the transfer was significantly high. The transfer depended on the *recA* function of the donor and recipient cells.

We speculate the transfer mechanism mediated by Hfr as follows. All the transfer machinary is provided by the Hfr. Hybrid plasmid may associate with the specific region of the chromosome with the homology between them. When the chromosome was transferred by Hfr, the associated plasmid simultaneously transferred to recipient cells. Any non-autotransmissible plasmids may be transferred to recipient cells by mating, as long as they have the homologous sequence with the DNA to be transferred by another plasmid or Hfr.

This method will be a powerful tool for determination of chromosomal site of the unidentified fragment cloned on plasmids, since we can test whether or not the plasmid in question is transferred by a brief mating with a particular Hfr. Many Hfr strains with different transfer origin and direction are available for the mating. This method will be also useful to enrichment of desired plasmids. Passage of cloned plasmids from appropriate Hfr strain to any recipient, enriches the plasmids with downstream region of transfer origin of the Hfr.

Isogenic and Allogenic Suppression of *fts* Mutation of *Escherichia coli*

Masao YAMADA and Yukinori HIROTA

In order to elucidate the molecular mechanism of cell division, we are studying on the temperature-sensitive (ts) mutation of *E. coli* defective in cell division and the hybrid plasmids suppressing the ts mutation.

Nishimura *et al.* (1977, Plasmid **1**: 67) found 5, 4 and 2 plasmids correcting *ftsB*, *E* and *I* mutaions, respectively, among the gene bank of *E. coli* prepared by Clarke and Carbon (1976, Cell **9**: 91). We analyzed the plasmid

Table 1. pLC plasmids that suppressed ts phenotype of *fts* mutation.

fts mutant	pLC plasmid	isogene	allogene	other gene carried by the plasmid	chromosomal location
MFT 84 (*ftsB*$^-$)	pLC 3-46	*ftsB*		(*nrdB*) *glpT*	48 min
	pLC 19-24	*ftsB*		*nrdAB glpT*	
	pLC 42-17	*ftsB*		(*nrdB*) *glpT*	
	pLC 1-41		*sufB*		53
	pLC 6-16		*sufB*		
MFT 1181 (*ftsE*$^-$)	pLC 31-16	*ftsE*			74–79
	pLC 31-32	*ftsE*			
	pLC 19-48		*sufE*	*aceE*	3
JE 10730 (*ftsI*$^-$)	pLC 26- 6	*ftsI*		*leuA murEF*	2
	pLC 4-14		*sufI*		64

DNAs with restriction enzymes, constructed their restriction map and subcloned appropriate fragment of the plasmids. By these experiments, we found that the plasmids correcting *fts* mutation could be classified into 2 groups. One had the gene itself that was defective in particular *fts* mutant (isogenic suppression). The other had an entirely different segment from the isogene with respect to the restriction map and the homology revealed by hybridization analysis. It was confirmed that the segment was derived from chromosome of *E. coli* by Southern blotting technique. The chromosomal location site of the segment was determined. The summary of the results is shown in Table 1.

We named this phenomenon as *allogenic suppression* and the gene responsible for the suppression as *allogene*. It is probable that the allogene could suppress ts mutation because of the elevated gene dosage of the cloned material or that it was the normally silent gene which turned on as a consequence of being cloned.

Electron Micrographic Observation of *E. coli* Flagellar Precursors

Yoshibumi KOMEDA

Escherichia coli mutants with defects in 29 flagellar genes identified so far were examined by electron microscopy for possession of incomplete flagellar structures in membrane-associated fractions.

In vivo gene fusion technique has revealed the transcriptional interaction of these flagellar genes in *E. coli* K-12 (Komeda, US-Japan Cooperative Microbiology Congress, 1979). The flagellar genes were divided into six groups for their transcriptional control. The *hag* gene is expressed after the expression of other flagellar genes. The mutants with defects in the *hag* genes have the most complex structures with hook-basal body structures. Accordingly, it was plausible to expect that the early genes in the transcription sequence were responsible for the simpler structure of a flagellum; the late genes were responsible for more complex structure. Identification of the precursor structure of each mutant will elucidate the role of transcriptional control for flagellar assembly. Therefore, precursor structures of each mutant were examined on the basis of their possible role as assembly intermediates of a flagellum. Suzuki *et al.* (1978) succeeded to identify these precursor structures of flagella by the aid of electron microscope in *S. typhimurium*. So far, the electron microscopy has given us the most reliable data for analysis of flagellar precursors. We applied this method to identify precursor structures of *E. coli* flagellar mutants.

Hook-basal body structures were detected in *flaD*, *flaS*, *flaT*, *flbC*, and *hag* mutants. The *flaE* mutant had a polyhook-basal body structure. An intact basal body appeared in *flaK* mutants. Putative precursors of the basal body were detected in mutants with defects in *flaM*, *flaU*, *flaV*, and *flaY*. No structures homologous to the flagellar basal body or its parts were detected in mutants with defects in *flaA*, *flaB*, *flaC*, *flaG*, *flaH*, *flaI*, *flaL*, *flaN*, *flaO*, *flaP*, *flaQ*, *flaR*, *flaW*, *flaX*, *flbB*, and *flbD*. One *flaZ* mutant had an incomplete flagellar basal body structure and another formed no significant structure, suggesting that *flaZ* is responsible for both basal body assembly and the transcription of the *hag* gene.

The result was compared with that of *fla-lac* study (last issue of this report). Except the *flaU* mutants, it was noted that defects of the genes in the late group (*hag*, *flaS*, *flaT*, *flbC*) had the most complex precursor: hook-basal body structure. I believe the scheme (last issue of this report) reflects at least some aspects of the interaction of flagellar gene products for their synthesis and assembly.

Characterization of Ribosomal RNA Genes from Blue-Green Algae

Noboru TOMIOKA and Masahiro SUGIURA

Blue-green algae are micro-organisms with chlorophyll-*a* as a photosynthetic pigment. Many species possess the ability of N_2-fixation, though they evolve O_2 by their photosynthesis. About two thousand species are widely distributed over the world and the algae have a long history during the biological evolution on the earth. An endosymbiotic theory explains that chloroplasts were derived from an ancestral photosynthetic prokaryotes related to the blue-green algae. In spite of their interesting characters, the present knowledge of genetics in the organisms is limited. Recent progress in molecular genetics, however, enable us to analyze fine gene structures. Thus, we began to study rRNA genes of the organisms using gene cloning and DNA sequencing techniques. First, we isolated total DNAs from *Anacystis nidulans*, *Anabaena variabilis* and *A. cylindrica*, then purified by CsCl equilibrium density centrifugation. Purified DNAs are digested with several restriction enzymes and fractionated by 0.7% agarose gel electrophoresis. DNA fragments containing rRNA genes were detected by Southern hybridization method. (5′-^{32}P) rRNA was used as a probe. The results showed that 5.5 and 4.2 Md Pst I fragments of *Anacystis nidulans*, 2.6 and 2.4 Md EcoRI fragments of *Anabaena variabilis* and 1.9 and 1.7 Md EcoRI fragments of *A. cylindrica* contained rRNA gene(s). For further analysis of the DNA fragments, it is necessary to clone these genes.

III. BIOCHEMICAL GENETICS AND IMMUNOGENETICS

Effect of Major Histocompatibility Gene Complex (MHC) on the Susceptibility to Urethane Induced Lung Tumors in Mice

Kazuo MORIWAKI and Nobumoto MIYASHITA

In addition to a pulmonary tumor resistance (ptr) gene probably located on chromosome 11, MHC seems to function in the processes of tumorigenesis. This study aims to reveal the effect of MHC on urethane-induced lung tumor incidence by using A H-2 congenic strains. In susceptible A/J mouse, average number of lung tumor foci is 30.6. If H-2^a complex of A/J strain is replaced by H-2^b, H-2^f and H-2^s, those number reduced to about 15 (Table 1). These results clearly show the profound regulatory function of MHC for the lung tumor development by urethane in mouse strains with susceptible ptr gene. Suppressive effect of wild-derived chromosomes is also investigated in F_1 hybrids between ICR mouse and MOL. TEN inbred strain derived from Japanese wild mouse.

Table 1. Effect of H-2 complex on the lung tumor incidence induced by urethane

Strains	H-2 haplotypes	Number of pulmonary tumor foci per mouse	
		Female	Male
A	a	26.7±4.6(12)	34.5±6.1(12)
A. SW	s	9.8±3.4(17)	13.6±5.3(12)
A. BY	b	14.9±5.3(19)	13.0±5.2(25)
A. CA	f	17.5±4.5(14)	15.3±6.5(14)
B10. A	a	1.9±1.5(8)	1.7±1.5(7)
B10	b	1.0±1.0(12)	0.5±0.9(10)
B10. TEN1	w^{mol-1}	0.9±1.2(7)	1.1±0.9(9)
B10. TEN2	w^{mol-2}	0.1±0.2(8)	0 (7)

High Frequency of Intra-H-2 Recombination in B10.A/B10.MOL-SGR Heterozygote

Toshihiko SHIROISHI, Tomoko SAGAI and Kazuo MORIWAKI

Intra-H-2 recombinant has been one of the most powerful tools for the current genetic analyses of the H-2 complex and traits it controls.

We have produced several B10.H-2 congenic strains which carry the H-2 complex derived from Japanese wild mouse. Although these strains are characteristic and useful materials themselves, further precise studies require intra-H-2 recombinants in which one end of H-2 complex is derived from well known H-2 haplotype of inbred strains and the other end from those of wild mouse. For this reason, we attempted to produce the intra-H-2 recombinants, using B10.A and B10.MOL-H-2 congenic strains.

First, the males of three B10.MOL-H-2 congenic strains, B10.MOL-TEN1, B10.MOL-SGR and B10.MOL-YNG2 were crossed with B10.A females. Then, females of the F_1 hydrids were backcrossed to the respective parental B10.MOL-H-2 congenic mice. The offsprings obtained in backcross were tested for H-2 genotype by hemagglutination. H-2 antisera used in H-2 typing were D-23 (anti-H-2K.23) and D-4 (anti-H-2D.4), which direct private antigens encoded in H-2K and H-2D end of B10.A strain. Since each B10.MOL-H-2 strain does not have both of H-2K.23 and H-2D.4 antigens, backcross offsprings carrying either one of them are recombinants.

We have screened 722 mice and already found 13 recombinants. Recombination frequency in three combinations of H-2 heterozygotes are different remarkably each other (Table 1).

Table 1. Frequency of intra-H-2 recombination

H-2 heterozygous parents	No. of mice screened	No. of mice recombinants	Recombination frequency (%)
B10. 1/B10. MOL-TEN1	209	1	0.48
B10. A/B10. MOL-SGR	396	12	3.0
B10. A/B10. MOL-YNG2	117	0	0.0

Among them, B10.A/B10.MOL-SGR heterozygote shows extraordinary high recombination frequency. On the other hand, the other two combinations show ordinary value.

Table 2. H-2 genotype of new intra-H-2 recombinants

H-2 heterozygous parents	Genotype H-2K		H-2D	No. of recombinants
B10. A/B10. MOL-TEN1	w*	—	d	1
B10. A/B10. MOL-SGR	w*	—	d	7
B10. A/B10. MOL-SGR	k	—	w*	5

w*: H-2 genotype derived from Japanese wild mouse.

The H-2 genotypes of new recombinants obtained in the present experiment are shown in Table 2.

In B10.A/B10.MOL-SGR heterozygote, the reciprocal coss-over types, K^w-D^d and K^k-D^w occurred with approximately equal frequency.

Klein (1975) reported that overall frequency of intra-H-2 recombination is 0.41 percent based on 12,629 mice. Accordingly, the value of recombination frequency observed in B10.A/B10.MOL-SGR heterozygote is almost one order higher than that in previous reports. Since all mice used in the present experiment were B10.H-2 congenic strains, the difference of recombination frequency observed here can be attributed exactly to the H-2 region on 17th chromosome.

Allozyme Studies on the Japanese Wild Populations of Medaka, *Oryzias latipes*

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

Allozymic variation at 21 loci in Japanese wild populations of the freshwater fish, *Oryzias latipes*, collected at 47 localities, was studied. By means of unique alleles at the *Adh*, *Pgm*, *Sdh*, and *Sod* loci, the Japanese wild populations could be divided into two major groups, the 'Northern Population' (northern coast of the Sea of Japan) and the 'Southern Population' (the rest part of Japan). No clinal distribution was observed at these loci, but the boundary of two regions was very distinct. The Southern Population was further divided into five subpopulations (the Eastern Subpopulation, the Inland Sea Subpopulation, the San-in Subpopulation, the Kyushu Subpopulation, and the Ryukyu Subpopulation) by means of the unique alleles at *Acp*, *Amy*, *Got*, *Mod*, and *Pgd* and the electrophoretic pattern of muscle lactate dehydrogenase. In this case also, the boundary was clear.

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The heterozygosity value of the Northern Population was very small (H=0.034). On the other hand, the Southern Population was variable as a whole, though the values were different among subpopulations (0.046–0.095). The genetic identity values between the Northern Population and each subpopulation in the Southern Population were 0.71–0.75. These values between four subpopulations in the Southern population were 0.87–0.93.

These results show that Japanese wild populations of medaka are remarkably differentiated regionally. As little clinal distribution is observed, it is supposed that the differentiation is primarily due to geographical isolation. Based on the results, the origin of the orange-red variety of madeka is thought to be the Eastern Subpopulation.

Conventional and Developmental Null Forms Coded by the *Acp*-1 Locus in Rice

Toru ENDO

A new type of null form isozymes was discovered in rice and was termed "developmental null form," though several other kinds of null forms, either associated with active forms or not have been previously known. Each of the active alleles at the *Acp*-1 locus produces a different set of three major and three minor isozyme bands of acid phosphatase in the leaf and some other organs (Endo, Biochem. Genet. **19**: 373, 1981). Two null strains, 1707 (Indica type: spontaneous origin) and N8-409 (Japonica type: induced origin), did not show any band coded by the *Acp*-1 locus in old mature leaves. However, in young leaves of the former strain three appreciably intense but blurred bands were present near the position of the major band A specified by the active allele of Acp^{-4}. The intensity of the three bands, however, gradually decreased during the maturation of the leaves.

This difference between the strains described above may be explained as follows: in N8-409 a termination mutant site was formed at the proximal region in the base sequence of the structural gene locus. On the other hand, in 1707 the mutant site was formed at the distal region in the base sequence of the locus, and so an incomplete polypeptide subunit containing only a part of the active site for acid phosphatase was produced. This incomplete phosphatase might have formed dimers via incomplete modification and expressed atypical bands in young leaves. However, this in-

complete enzyme molecules could not have lasted until the leaf maturation, removing the bands from old leaves. The phosphatase might not have formed in N8-409 since the termination occurred before the synthesis. Under this assumption, the alleles for null forms of N8-409 and 1707 were designated as the conventional null form (Acp^{null}) and the developmental null form ($Acp^{dev\text{-}null}$), respectively.

An Extraction System for Leaf Zymographic Analysis in Woody Plants

Toru ENDO

There are many reports on gel electrophoresis for plant isozyme analysis describing buffer systems for the gel and electrode as well as a number of reacting systems for the band development of each enzyme species. Extracting systems for enzymatic ingredients from plant materials, however, have not yet been developed sufficiently to cover a range at different materials. In general, distilled water or simple buffer solutions around neutral in acidity have been successfully used as extractants for enzymes from annual crop plants.

On the other hand, water and the common buffers were not efficient as extractants for several woody plants such as *Camellia*, *Prunus* and *Shorea*. Therefore, I have examined about one hundred extraction systems for leaf of *Shorea*. At present, the extractant called EE 7.5, a modified Berthou's buffer for coffee leaves (Communication at 9th ASIC Colloquium, London 1980), appears to be most useful. Its composition was 10% Triton X-100, 0.5 M sodium chloride, 0.5 M Tris, 0.2 M ascorbic acid, 10 mM EDTA and 1 mM potassium ferricyanide adjusted at pH 7.5 with glacial acetic acid of 1.6% at final concentration. Leaf cuts of 0.1 g were mixed with 0.5 ml of the EE 7.5, 0.1 g quartz sand and 0.05 g polyvinylpolypyrrolidone (Polyclar AT), and were crushed thoroughly in a mortar. The resultant exudate was either diretly adsorbed with filter paper strips or adsorbed after centrifugation with hematocrit tubes. In some cases, Tergitol NP-40 may be substituted for Triton X-100, though the zymographic patterns were slightly different in *Shorea*.

Modification Gene for Peroxidase Isozymes Coded by *Px*-1 Locus in Rice

Chiang PAI and Toru ENDO

There are several reports showing the appearance of only the S band in F_1 hybrids when two allelic isozyme bands, F (faster moving to the anode) and S (slower moving), are present in different homozygous strains: that is, the case of alkaline phosphatase and aminopeptidase in fowl. This phenomenon was found to be due to the presence of modifier enzymes such as neuraminidase, by which the F band is changed to a S band, and the gene specifying the modifier enzyme is assumed to be dominant in inheritance (Law, Science **156**: 1106, 1976).

Another such case was detected in the *Px*-1 locus specifying peroxidase in rice. This was that the bandmorphs under the presence of a modifier enzyme were unique in the heterozygotes at the *Px*-1 locus, of which isozymes were of dimeric form. Four alleles, Px^{2A}, Px^{4A}, Px^{0C} and Px^{null}, have been detected so far at this locus. Under the dominant modifier gene, *Mo-Px*, these alleles produce 2A, 4A and 0C bands, as well as null form, respectively. While under the recessive modifier gene, *mo-Px*, the null allele does not code for any band and the rest produce 2mA, 4mA and 0mC, the migration rates of which are down about 4–5 mm from each of the respective normal bands.

In the cross between 2mA, and 2A strains, the F_1 showed only 2A and the segregation of 3(2A): 1(2mA) was seen in the F_2 generation. From the cross between 2mA and null form strains, the F_1 showed only 2A, and the segregation ratio in the F_2 was 9(2A): 3(2mA): 4(null). This segregation ratio agrees with that expected when recessive epistasis is present. In the cross between 2mA and 4A, the F_1 produced 2A-3A-4A bandmorph, the same as that from the Px^{2A}/Px^{4A} heterozygote. This ratio is due to the recombination of codominant and conventional heterozygotes, and may be a new type of Mendelian segregation regarding the interaction of two genes of different loci.

IV. DEVELOPMENTAL GENETICS AND SOMATIC CELL GENETICS

The Growth and Differentiation of Germ Cells of *Drosophila melanogaster* in Culture

Yukiaki KURODA

Analysis of the process of germ cell maturation in *Drosophila* is fundamentally essential to understand the nature of the maternal effect in the cytoplasm for some sex-linked recessive lethal genes and to analyze the localized specificity in the egg cytoplasm in embryogenesis. In the present study, the testes and ovaries obtained from pupae were cultured *in vitro* and the process of germ cell maturation was examined.

Testes and ovaries were obtained from 48-hr pupae which were grown under sterile conditions. The testes had attached to the vas deferens derived from the genital discs and were in the spiral shape. When these testes isolated were cultured at 28°C in T-5 flasks in medium K-17 supplemented with 0.1 μg/ml fetuin and 15% fetal calf serum, they grew and increased in length and thickness.

Spermatogonia in the anterior part of testes differentiated to form the cyst of spermatocytes. A single cyst of the primary spermatocytes was isolated from testes by using the fine needles and cultured by the same procedure. The first meiotic division was observed to occur synchronously in all spermatocytes in the cultured cyst. The cysts of spermatids in culture elongated extensively from the initial round shape to a rod-like shape. Each process of spermiogenesis which involves an extensive elongation of sperm bundles was clearly observed under a phase contrast inverted microscope.

On the contrary, in ovaries obtained from 48-hr pupae no noticeable growth and differentiation were observed in the inside germ cells under the same culture conditions, Muscle cells and fibroblastic cells migrated from the peritoneal sheath of testes and ovaries, and grew rapidly around the original explants. Some mutant strains relevant to the germ cell maturation are used to analyze the gene actions in the development of germ cells.

Effect of Lectins on the Cartilage Nodule Formation of Embryonic Quail Limb Bud Cells in Culture

Yukiaki KURODA and Etsuya MATSUTANI

In the process of limb morphogenesis of avian embryos, the condensation of cells occurs in the central region of limb buds, and then cells in high cell density region differentiate into cartilage cells. This suggests that the cell condensation may be prerequisite to cell differentiation in the process of limb morphogenesis. In our previous works, we have analyzed the early step in cartilage differentiation, and found the role of cell aggregation in this step. In the present work, we examined the effect of several lectins, which had been known to promote cell aggregation in various systems, on the cartilage nodule formation of mesenchyme cells from limb buds.

Mesenchyme cells were obtained from hind limb buds of quail embryos (*Coturnix coturnix japonica*) at stages 20–21. An inoculum of 5×10^4 cells in 10 μl of medium 199 with 15% serum was placed on a plastic perti dish. After allowing 2 hr for cell attachment, the dish was flooded with 2 ml of culture medium. Lectins used were ConA, succinyl ConA (s-ConA), DBA and WGA. Cells were fixed on the fourth day and stained with 0.05% toluidine blue for 4–5 hours. The cartilage nodules were stained purplish-red with toluidine blue, then the number of cartilage nodules stained was scored to estimate the degree of cartilage differentiation.

First, the treatment period of cells with lectins was determined using s-ConA. It was found that the 24-hr treatment after the initial 24 hours of incubation was most effective in promoting the cartilage differentiation of mesenchyme cells.

Next, four lectins were tested at various concentrations (5, 10, 20, 50 and 100 μg/ml) for their effect on cartilage nodule formation. The results showed that ConA and DBA had almost no effect at low concentrations, while s-ConA and WGA increased the number of cartilage nodules at middle range of concentrations. However, in the case of WGA, the arrangement of cells in cartilage nodules was abnormal. More detailed effects of lectins were examined with s-ConA.

The effect of s-ConA on the aggregation of mesenchyme cells was examined by measuring the diameters of cell aggregates formed in gyratory shaking cultures. The results indicated that s-ConA promoted cell aggregation of mesenchyme cells. More detailed examination on the time-lapse

changes in the number of cartilage nodules indicated that the lectins had an activity to reduce the time required for the appearance of cartilage nodules. This supports the idea that the lectin may promote cell condensation during the early step in the cartilage differentiation.

The effect of the lectin on cell proliferation was also examined. Cells were cultured in 50 μg/ml of s-ConA medium for 24 hours at different times after inoculation. The results showed that the treatment at an initial 24 hours caused a decrease in the number of cells. The treatment at a middle 24–48 hours and late 48–72 hours period had no effect on cell proliferation. As a result, it is suggested that lectins may have an activity to enhance the cell adhesion of mesenchyme cells, then their tight contacts may be established in cell aggregates, and resulted in the increase of the number of cartilage nodules.

Mutagenic Activity of Trp-P-2 and Glu-P-1 on Embryonic Human Diploid Cells in Gulture

Yukiaki KURODA

Trp-P-2 and Glu-P-1 are pyrolysis products of trypotphan and glutamic acid. Both have strong flame-shift mutagenic activities and weak base-change mutagenic activities in the *Salmonella* system. In the present experiments, the effects of Trp-P-2 and Glu-P-1 on survival and mutation induction in embryonic human diploid cells were examined.

Cells were treated with Trp-P-2 at various concentrations for 1, 2, 4, 8, 16 and 24 hours and the colony-forming activities of cells after 14 days in normal medium were determined. Surviving fractions of cells decreased depending to concentrations of Trp-P-2. The cytotoxic effect of Trp-P-2 giving the same product of concentrations and exposure times was stronger in cells treated with higher concentrations for shorter exposure times than in cells treated with lower concentrations for longer exposure times, as shown previously in the effect of Trp-P-1. Since Trp-P-2 is relatively stable for incubation at 37°C, this dose-rate effect suggests the presence of repair activity of cells from sublethal damages produced by chemicals.

Cells were treated with Trp-P-2 at various concentrations for 4 hours and cultured in normal medium for the mutation expression time of 7 days. The cells were replated and selected with 30 μg/ml of 8-azaguanine (8AG). After cultivation for 14 days, the frequency of 8AG resistant mutations

was calculated. In cells treated with Trp-P-2 at concentrations of 0.1 and 0.3 μg/ml, no 8AG-resistant mutations were induced. At cencentrations of 1.0 and 3.0 μg/ml, the induced mutation frequencies of 8AG-resistant cells were 2.8 and 1.4 per 10^5 survivors, respectively.

Glu-P-1 had a weak cytotoxic effect on cultured human diploid cells. Cells treated with Glu-P-1 at a concentration of 100 μg/ml for 4 hours, still gave the surviving fraction of cells of 0.92. When cells were treated with Glu-P-1 at various concentrations ranging from 0.3 to 30 μg/ml for 4 hours, the induced mutation frequencies of 8AG-resistant cells were 0.9 to 2.7 per 10^5 survivors. These results suggest that both Trp-P-2 and Glu-P-1 may require the metabolic activation by microsomal enzymes for revealing their active mutagenicity.

Metabolic Activation of Tryptophan Pyrolysis Products by S-9 Mix in Induction of Mutations in Cultured Chinese Hamster Cells

Yukiaki KURODA and Masumi ASAKURA

For quantitative studies on dose-response relationship of chemical mutagens, cultured Chinese hamster cells have some advantages: 1) They maintain normal diploid or near diploid karyotype for relatively long period. 2) They show the high growth activity and colony-forming activity. In the present experiment, the effect of S-9 Mix on the frequency of mutations induced by Trp-P-1 and Trp-P-2 was examined in Chinese hamster V79 cells.

The concentrations of Trp-P-1 and Trp-P-2 lowering the colony-forming activity of cells to 50% of control cultures were 2.6 and 12.3 μg/ml, respectively. When the rat liver microsomal fraction and coenzymes (S-9 Mix) were added to medium, no noticeable enhancement of cytotoxic effects of Trp-P-1 and Trp-P-2 was detected.

Cells were treated with Trp-P-1 and Trp-P-2 for 4 hours and the frequency of 8-azaguanine (8AG) resistant mutations induced by both chemicals was examined. At a concentration of 0.02 μg/ml, both chemicals induced mutations slightly, but at higher concentrations than 0.06 μg/ml no dose-dependent increase in induced mutation frequency was observed. The addition of S-9 Mix to medium enhanced the frequency of mutants induced by Trp-P-1 three times that of cultures without S-9 Mix.

When ouabain (OU) resistance was used as the genetic marker for detecting mutations induced by Trp-P-1 and Trp-P-2, the frequency of induced mutations was approximately one tenth the frequency of 8AG resistant mutations induced by these two chemicals at the same concentrations. No marked dose-response was observed between concentrations of Trp-P-1 and Trp-P-2 and the frequency of OU resistant mutations. When S-9 Mix was added to medium in which cells were treated with chemicals, the frequency of OU resistant mutations increased approximately five times that in cultures without S-9 Mix. These results indicated that mutagenic activity of Trp-P-1 and Trp-P-2 was enhanced by metabolic activation with S-9 Mix.

Developmental Effects of Radiation on Embryos of the Silkworm (*Bombyx Mori* L.)

Akio MURAKAMI

As a laboratory insect, the domestic silkworm posseses various attractive features for biological studies of embryos because of their structural simplicity, independence, and minor sensitivity to the influence of environment. In addition, it can be easily collected a large number of synchronized cell populations during the early embryonic development stage providing a useful *in situ* system for the quantitative analysis of the cellular phenomena in the insect. These silkworm advantages which make it possible to analyze the change in stage-radiosensitivity, either embryonic mortality or mutagenicity, within the nuclear division cycle that occurs during the early embryonic development, as well as the change in age-radiosensitivity throughout the whole course of embryogenesis and/or morphogenesis take place from simple to complex form. In the silkworm egg, the serosa membrane of some mutant stocks in the all embryonic development is colorless and transparent as well as that of wild-type stocks in the early embryonic development permitting effective exposure to the UV light. This makes possible to compare the biological effects of UV light and ionizing radiation on embryonic nuclei and other cellular constituents. Moreover, comparison of the radiosensitivity among different X-ray-sensitive strains is possible and thus may shed light on mechanisms for the changes in radiosensitivity within the time course of cleavage division cycles.

In this paper, I shall attempt to assemble and present the available in-

formation in the silkworm obtained by myself and by others with regard to the relationship between radiosensitivity and nuclear division cycles, the nuclear-cytoplasmic interaction, and the relationship between radiosensitivity and embryonic morphogenesis *et cetera.* I shall further refer to the aspects of radiation effects on determination and differentiation in the embryonic process of silkworms, if possible. (Abstarcts of a paper presented at the Kyoto University Radiation Biology Center Workshop "Radiation Effects on Development and Differentiation". August 18–20, 1980: Misaki Marine Biological Station, Miura-shi)

Roles of the Head-activation Potential and Head-inhibition Potential Gradients in Hydra Pattern Formation

Tsutomu SUGIYAMA, Josef ACHERMANN and Jun TAKANO*

Hydra has a strong regenerative capacity. After amputation of the head and foot, the end of the body column which originally had the head always regenerates a new head, while the other end which originally had the foot always regenerates a new foot. It has been generally thought that this rigid polarity of regeneration is governed by a "polarity gradient" which exists along the hydra body axis (Child, 1941).

Wolpert *et al.* (1974) proposed that the polarity gradient in hydra consists of at least 2 factors. One is the potential to activate the formation of a head (head-activation potential) and the other is the potential to inhibit head formation (head-inhibition potential). Both potentials are high in the head region and become gradually lower toward the foot. In normal hydra tissue, the activation potential is not expressed due to the counteracting power of the inhibition potential. After amputation of the head, however, the head-inhibition potential falls rapidly at the cut surface due to its instability (diffusibility), allowing the head-activation potential to express itself to regenerate a new head. This model can successfully explain the results of various types of regeneration and grafting experiments carried out with wild type hydra strains. A similar model was also proposed by Gierer and Meinhardt (1974) on a more theoretical basis. They expressed the interaction of the head-activation and head-inhibition potentials in mathematical terms, and showed by computer simulation how the gradients of the two potentials are produced and maintained in normal, budding

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and regenerating hydra.

In an attempt to test these models experimentally, we have devised a procedure to semiquantitatively compare the relative strengths of the head-activation and head-inhibition potentials of different hydra strains. The procedure used is a modification of the lateral tissue grafting method used by Webster (1966).

Four different strains have been examined. They are 105, a wild type standard strain, mh-1 which shows an unusually strong budding capacity and produces many abnormal buds along its body column in positions outside of the normal budding zone, reg-16 which is defective in head regeneration after head amputation, and L4 which has a very low budding rate.

It was found that mh-1 has a singificantly higher head-activation and a significantly lower head-inhibition potential than 105. This suggests that the abnormal budding in mh-1 is caused by the imbalance between the head-activation and the head-inhibition potentials of this strain. In contrast, strains reg-16 and L4 were found to have significantly lower head-activation potentials and significantly higher head-inhibition potentials than 105. These strains, therefore, also show an imbalance of the two potentials, but in the opposite direction from mh-1, and this imbalance appears to be responsible for their morphogenetic deficiencies.

These observations suggest that (1) the head-activation and head-inhibition potentials play crucial roles in hydra head morphogenesis as proposed by Wolpert *et al.* (1974) and by Gierer and Meinhardt (1974), and that (2) an imbalance between the two potentials leads to morphogenetic abnormalities. At present, however, it is not clear why a similar imbalance leads to different abnormalities in reg-16 (defective head regeneration) and in L4 (defective budding rate).

Morphogenetic Potentials of Chimeric Hydra Strain

Nancy WANEK and Tsutomu SUGIYAMA

In order to determine which cell types in hydra are responsible for morphogenetic potentials, two types of chimeric hydra have been constructed from the standard wild type (105) and the regeneration-deficient strain (reg-16). Reg-16 has a greatly reduced head regenerative ability, and has a very low head-activation potential and a high head-inhibition potential

when compared to 105 (see the preceeding paper).

Hydra tissue consists of three self-renewing cell lineages: the ectodermal epithelial cell lineage, the endodermal epithelial cell lineage and the interstitial cell lineage (which includes interstitial stem cells and their differentiation products, nerve cells and nematocytes).

Chimeric strains consisting of epithelial cell lineages (both ectodermal and endodermal) from 105 and the interstitial cell lineage from reg-16, and vice versa, were prepared using standard methods for eliminating and reintroducing interstitial cells (Marcum and Campbell, 1978).

Chimeric strains consisting of ectodermal epithelial cells from 105 and endodermal epithelial cells from reg-16, and vice versa, were prepared by a newly-developed procedure (Wanek and Campbell, 1981). In this method, a ring of vitally stained tissue (ectoderm and endoderm) is grafted into an unmarked polyp. Differential movement of the ectodermal and endodermal layers at the graft junction results in chimeric regions which are visible as marked ectoderm overlying unmarked endoderm (or unmarked ectoderm overlying marked endoderm). These chimeric regions are then excised and allowed to regenerate into whole chimeric animals. (These chimeric strains probably contain a mixed population of interstitial cell lineages from both parental strains).

The head regenerative capacity of the epithelial cell/ interstitial cell and ectoderm/ endoderm chimeras was examined in detail and compared to that of 105 and reg-16. The [105 epithelial cell/ reg-16 interstitial cell] chimera shows normal regenerative capacity when compared to 105. In contrast, the [reg-16 epithelial cell/ 105 interstitial cell] chimera shows greatly reduced regenerative capacity comparable to reg-16.

The [reg-16 ect/ 105 end] chimera shows almost normal regenerative ability, whereas the [105 ect/ reg-16 end] chimera has regenerative ability which is significantly lower than 105, but slightly higher than reg-16.

These results indicate that the regenerative defect of reg-16 resides in its epithelial cells but not in the interstitial cell lineage, and that the endodermal epithelial cells have more influence than the ectodermal cells on head regeneration. Analysis of the head-activation and head-inhibition potentials of the chimeric strains is now in progress.

An SR-spiroplasma unable to Kill *Drosophila* Males

M. A. YAMADA, S. NAWA and T. K. WATANABE

NSR-spiroplasmas, originally isolated from the hemolymph of *Drosophila nebulosa* and transplanted to *D. melanogaster* females, usually kill the male progeny in the embryo stages. Among the NSR carrying *D. melanogaster* stocks, one (strain-A) changed to produce males in the progeny. Both males and females of this strain-A had the spiroplasmas in the hemolymph, which were transmitted transovarially into the next generation. This spiroplasma has neither male-killing ability nor any apparent deleterious effects on the fecundity and longevity of host flies. When the standard NSR females (strain-B) which had the NSR-spiroplasmas and no male progeny were back-crossed to the males of the strain-A for 4 generations, no males were produced in their progeny. When the spiroplasmas of strain-A were injected into the spiroplasma-free females of both strain-B and Oregon-R flies, they were transmitted transovarially without the male-killing effect. But when the spiroplasmas of strain-B were injected into the spiroplasma-free females of strain-A, they were transmitted and completely killed the male embryos.

The spiroplasmas of strain-A were not discriminated morphologically from the NSR-spiroplasmas of strain-B under the dark field microscope. Since the SR-spiroplasmas from various origins can be distinguished by forming clumps if the hemolymphs of flies carrying different SR-spiroplasmas are mixed on a slide glass, the spiroplasmas of strain-A were mixed with those of WSR (*D. willistoni* origin), HSR (*D. hydri* origin; non-male-killing spiroplasma) and the original NSR, respectively. Both spiroplasmas of WSR and HSR reacted with the spiroplasmas of strain-A but the NSR spiroplasmas did not. These results suggest that the fly itself of strain-A has not changed in the resistance to the SR-spiroplasma action but the NSR-spiroplasmas of strain-A has lost the male-killing ability.

Hatchability and Some Developmental Characters of Flies in Natural Population of *Drosophila melanogaster*

Kiyoshi MINATO

There were large differences in the oviposition rate, the hatchability of eggs, and the average pupation period and so on among various laboratory

stocks of the Oregon-R strain of *Drosophila melanogaster*. Therefore, to know which stock of the Oregon-R strain shows the similar characters to those of natural population, the wild flies were collected to investigate some characters to be compared with Oregon-R flies.

Sixteen fertlized female flies of *D. melanogaster* were collected at Misima and the same numbers of iso-female lines were established through brother-sister mating of each offspring. The flies were analyzed after three generations of culture in the laboratory with food of agar-cornmeal-yeast-sucrose.

The oviposition rates of the lines of wild flies were relatively low (the average of 5.5 eggs/female·day) compared to those of three lines of Oregon-R (each 7.3, 9.6 and 10.6 eggs/female·day), under present conditions. In the egg mortality, some lines of wild flies showed the rates of more than 10% with the average of 6.5%, which was higher compared with those of three lines of Oregon-R (each 1.8, 2.4 and 5.3%). In the larval mortality, the lines of wild flies also showed fairly higher rate (some showed more than 20% with the average of 12.2%) than those of Oregon-R (average, 2.4%). Thus, so far as iso-female lines were used, the wild flies appeared to be accompanied with rather high degree of lethality in both egg and larval periods. In the pupal mortality, there was little difference between the wild and Oregon-R flies (both 1–3%).

The average pupation periods of the lines of wild flies were widely ranged from 5.5 days to 7.3 days, while those of Oregon-R were equally near 6.1 days. Furthermore, it was remarkable that each different value of the former lines was maintained almost unchanged over at least ten generations.

Sex-specific Lethals of *Drosophila melanogaster*

Kugao OISHI and Takao K. WATANABE

All the sex-specific lethals so far reported are either on the *X* or on the second chromosome. A third chromosome (named #132) extracted from a natural population of Katsunuma in 1979 was found to be male-specific lethal when homozygous if mothers are heterozygous, and lethal for both females and males when mothers are homozygous. Two recessive genes were identified on the #132 chromosome, maleless (3)132 [*mle*(3)132, 3-25.8] which is responsible for the male-specific lethality irrespective of whether mothers are heterozygous or homozygous, and lethal (3) maternal 132 [1(3)*mat*132] which causes maternal effect lethality on the homozygous

progeny (thus it is a rescurable maternal effect lethal mutant). No further pursuit on the latter, which is located between *red* (3–53.6) and *e* (3–70.7), was attempted. Homozygous *mle*(3)132 males die at various larval stages but never pupariate. An extreme delay in development was observed: at 100 hr after egg laying when other genotypes were 3rd instar larvae, surviving homozygous males were mostly still in the 2nd instar and some even in the 1st instar stage. The lethality is exerted only on the single-*X* individuals regardless of the phenotypic sex, changes of which can be caused by various sex-transformation mutants such as *dsx*, *tra*, *tra*-2 and *tra*-2^{OTF}. No interactions with a second chromosome male-specific lethal, *mle*(2)27, was observed. Gynandromorph analysis showed that only about 5% of the pupative sex mosaics homozygous for *mle*(3)132 eclosed. Those mosaics which did appear were with very small *XO* patches in which structures themselves were reduced in size. These and other observations seem to indicate that the primary effect of *mle*(3)132 is the retardation in the rate of cell division. If the gene is acting, as in other male-specific lethals, to cancel the dosage compensation, Minute (*M*) effect (retardation in cell division rate as well as some morphological effects) may result which would also explain the lethality.

Transplantability of Teratocarcinomas Derived from 6 day Embryos of B10 H-2 Congenic Mice in Allogeneic Hosts

Takehiko NOGUCHI and Choji TAYA

We produced teratocarcinomas, OTT10A-5 and OTT10Sn-3 from 6 day embryos of strains, B10.A/SgSn(H-2^a) and B10/Sn(H-2^b), respectively. These teratocaricnomas obtained from B10 H-2 congenic mice allowed us to examine the effect of difference in H-2 haplotype on the transplantability of embryonal carcinoma cells.

Solid tumors of the teratoma lines were transplanted subcutaneously into syngeneic and allogeneic H-2 congenic mice. The result (summarized in Table 1) indicates that these teratocarcinomas could not grow beyond the H-2 barrier. When OTT10Sn (H-2^b) tumors were transplanted into B10.129(6M)/Sn(H-2^{bc}), some of them were accepted probably because of the similarity in H-2 haplotype between the grafts and the hosts.

Teratocarcinoma lines, 402A and OTT6050, or the derivative cell lines have been reported to grow beyond H-2 barrier. It is also well established

Table 1. Rejection of OTT10A and OTT10Sn tumors by allogeneic B10 H-2 congenic mice

Teratocarcinoma (H-2 haplotype)	Host strain (H-2 haplotype)	Transplantation site	Sensitivity to tumor
OTT10A	B10.A (H-2^a)	subcutaneous	15/15
(H-2^a)	B10.BR (H-2^k)		0/8
	B10/Sn (H-2^b)		0/5
	B10.129 (6M) (H-2^{bc})		0/3
OTT10A	B10.A (H-2^a)	intraperitoneal	5/5
(H-2^a)	B10.BR (H-2^k)		0/4
	B10/Sn (H-2^b)		0/5
OTT10Sn	B10/Sn (H-2^b)	subcutaneous	21/22
(H-2^b)	B10.129 (6M) (H-2^{bc})		8/15
	B10.A (H-2^a)		0/5
	B10.BR (H-2^k)		0/4

that murine embryonal carcinoma cells are H-2 antigen negative. Transplantation behavior of B10-derived teratocarcinomas was unexpectedly different from that of 129-derived teratocarcinomas. The H-2 specificity-dependent transplantation behavior of the B10 teratocarcinomas suggests that the charactor of the stem cells of these teratocarcinomas may be somewhat different from those of 129-derived teratocarcinomas, especially in respect to H-2 antigen expression.

Aggregation Chimeras between Embryonal Carcinoma Cells of a Testicular Teratocarcinoma (STT-2) and 8-cell Mouse Embryos

Kazunori HANAOKA and Takehiko NOGUCHI

Some embryonal carcinoma cells (EC cells) can be incorporated into blastocysts by aggregating with blastomeres of 8-cell embryos (Fujii and Martin, Develop. Biol. **74**: 239, 1980), or with 8-cell embryos themselves (Stewart, J. Embryol. Exp. Morph. **58**: 289–303, 1980).

We reinvestigated these results using teratocarcinoma cells with different origins. The teratoma lines used were STT-2, OTT10A-5 and OTT10Sn-3 which were derived from mice strains, 129/Sv-*ter*, B10.A/SgSn and B10/Sn respectively. Clonal cell lines were also used. A211, A323, $B_\beta 721$ and $B_\beta 826$ were all derived from OTT10A-5 (Nishimune *et al.*, Annual Rep. 30: 50, 1979). 10Sn-1 and 10Sn-2 were from OTT10Sn, and 311 from STT-2.

Stem cells of these teratoma lines were enriched by passaging every 2 to 3 days on feeder layer (3T3-A31, mitomycin C treated). Clumps of EC cells (10 to 20 cells) were aggregated with 8 to 12-cell stage embryos by essentially the same method as Stewart, except that aggregation was carried out in modified PBS containing phytohemagglutinin P. Presence of cells derived from EC cells was checked using glucose phosphate isomerase as a genetic marker. F_1 hybrid embryos between C3H and C57BL (Gpi-1^b) were the partners for EC cells of STT-2 and 311(Gpi-1^a), while F_1 embryos between Balb/c and DBA(Gpi-1^a) were the partners for EC cells of OTT10A, OTT10Sn and their derivative cell lines (Gpi-1^b).

EC cells of these teratocarcinoma lines could form aggregates with normal embryos, and about 80% of the aggregates developed into blastocysts within 48 hours. Electrophoretic analysis of Gpi-1 revealed that blastocysts developed from aggregates between EC cells of STT-2 or 311 and the partner embryos were chimeric, but we failed to get any evidence for the presence of EC-derived cells in blastocysts developed from cleaving embryos aggregated with EC cells of OTT10A, OTT10Sn and their derivative cell lines. The charactor of the black mice-derived EC cells might differ from those of STT-2. They probably failed to participate in the normal development and eventually died.

V. CYTOGENETICS

Segregation of F_2 Hybrids between Mauritius and Oceanian Type Black Rats

Tosihide H. YOSIDA

The Mauritius type black rats occurring in Mauritius island were characterized by having 42 chromosomes, among which extra 8 small acrocentrics having been included in the karyotype of the Oceanian type black rat. Comparative karyotype analyses between the Mauritius and Oceanian type black rats showed that the small extra acrocentrics found in the former black rat resulted from the Robertsonian fission of the metacentric pairs no. 14 and no. 18 occurring in the latter black rat with 38 chromosomes (Yosida *et al.* 1979, Chromosoma **75**: 51–62). From the laboratory matings between the Mauritius type and the Oceanian type black rats F_1 hybrids were obtained. They were characterized by 40 chromosomes, among them pairs no. 14 and no. 18 were heteromorphic each consisting of metacentric and acrocentrics (Yosida 1980, Proc. Jap. Acad. 55(B): 557–561). In mating between the F_1 hybrids, F_2 offspring were also obtained successfully.

The average litter size of the F_2 hybrids was 4.3 ± 1.7, slightly smaller than that of F_1 hybrids and their parental Mauritius and Oceanian type rats, but higher than those of F_2 hybrids between Oceania $\times$ Ceylon and Oceania $\times$ Asia. Among the 17 F_2 offspring of Mauritius $\times$ Oceania, karyotypes of 16 rats were availably analysed. If the no. 14 and no. 18 chromosomes in both F_1 parents are consisted of one metacentric and two acrocentrics, and if they segregate regularly in the meiotic division, F_2 rats with 9 different karyotypes are expected to be produced theoretically. The segregation ratio of the karyotypes in these 16 F_2 rats was not significantly different from the expected value.

Based on the present hybridization experiments the following conclusion was drown that; (1) the extra small acrocentrics included in the Mauritius black rats, which were derived from the Robertsonian fission of small metacentrics, inherited normally to offspring through all dividing processes of the mitosis and the meiosis. (2) the Mauritius type black rat is more closely related to the Oceanian type rat than the Ceylonese and Asian type ones.

Spontaneous Occurrence of a Mosaic Rat Carrying Translocation/Normal and Translocation/Inversion Pair no. 1 and Its Genetical Survey

Tosihide H. YOSIDA

Spontaneous occurrence of the 1/12 chromosome translocation in a female Lewis strain rat was previously reported (Yosida 1980, Proc. Jap. Acad. **56B**: 268–272). The no. 1 chromosome of the rat was heteromorphic for the translocation and its normal partner, and the no. 12 was also heteromorphic for a normal chromosome and a smaller partner by deletion. Among 17 offspring obtained by mating between the female with the translocation and a normal male, 9 rats were normal homomorphic, but the remaining 8 were heteromorphic. One female among the latter rats was characterized by mosaic in somatic cells consisting of two kinds of the karyotype; one of them was heteromorphic for pair no. 1 consisting of the 1/12 translocation and normal partner (t/+), while the other showed the heteromorphic pair no. 1 resulted from 1/12 translocation and the inversion pair no. 1 (t/i). The inversion chromosome was characterized by the submedian centromere, and its length was similar to the normal no. 1. This means that the submetacentric member might have been produced by pericentric inversion in the normal chromosome partner to the translocation pair no. 1. By G-banding staining it was confirmed that the submetacentric has been derived by the pericentric inversion.

The chromosome mosaic of the present rat was initially found in tail cultured cells. In tail and ear cultures, the frequency of t/+ and t/i cells was nearly identical, but in bone marrow that of the t/i cells was considerably higher than in that of the t/+ cells.

In the mating of the mosaic female rat with the normal male, 6 offspring (4♀: 2♂) were obtained. If the female germ cells would be mosaic as found in the somatic cells, the offspring with three cells types, (+/+), (+/t), and (+/i), are expected at ratio of 1: 2: 1. The 6 offspring showed the segregation ratio to be expected. Based on these results it can be said that the chromosome mosaicism of the female rat occurred in both somatic and germ cells, the two typed cells (+/t and i/t) were included almost equally in the female gonad, and they transmitted to offspring through regular meiosis. As the cause of occurrence of the mosaic rat it is considered that the 1/12 translocation should have first occurred in a Lewis rat, and the

pericentric inversion could have been taken in the normal chromosome partner in the heteromorphic pair no. 1. At the early stage of embryogenesis the inversion should have occurred in one zygote resulting in the establishment of mosaic rat with two cell types.

Segregation and Fecundity of Offspring from the 1/12 Translocation Heterozygotes of the Norway Rat

Tosihide H. YOSIDA

Translocation between pair no. 1 and 12 chromosomes in a female Lewis strain rat has been described in the previous paper. This rat had heteromorphic pair nos. 1 and 12 by translocation and deletion. By mating between the female translocation heterozygotes (t/+) and the normal male (+/+), 89 rats (♀ 46 and ♂ 43) were obtained. They segregated into 53 rats with the normal karyotype (+/+) and 36 with the translocation heterozygotes (t/+). Fifty nine offspring (♀ 33+♂ 26) obtained by the reciprocal mating segregated into the normal (+/+) and the heterozygotes (t/+) at almost the same frequency. The segregation ratio in total 148 rats was not significantly different from the expected value.

From the mating between female and male heterozygotes (t/+), 68 rats (♀ 33: ♂ 35) were obtained. They segregated into the three types, normal karyotype (+/+), heterozygotes (t/+) and the translocation homozygotes (t/t). The number of rats with translocation heterozygotes was significantly higher, but those with the translocation homozygotes was lower than that of the expected one. All 148 rats from 16 litters obtained by mating between translocation homozygotes (t/t) had always the 1/12 translocation homologous pair (t/t). Average litter size of the rats with transolcation was very high (9.2±3.2), and the sex ratio was the female 4.9 to male 4.3. In the control rats having the normal karyotype which were established from the translocation heterozygotes, litter size was 7.4±3.3 in average of 11 litters, with the same number of females and males. From these studies it can be said that the rats with 1/12 translocation have not any deriterious effect on ability of the reproduction. The rats with 1/12 translocation thus obtained was named the LET-stock.

Segregation and Fertility of the Norway Rats with Metacentric Pair no. 1 Derived by Pericentric Inversion

Tosihide H. YOSIDA

A female LEW (Lewis) strain rat with the chromosome mosaic consisting of two cell types was originally obtained in this laboratory (Yosida 1980, Proc. Jap. Acad. **56**: 322–327); from this rat offspring with the normal and the inversion pair no. 1 (+/i) were obtained. By use of these rats, the segregation and fertility of rats with the inversion pair no. 1 was examined by the following combinations of matings; females with inversion heteromorphic pair no. 1 and normal males (+/i♀×+/+♂), its reciprocal (+/+♀×+/i♂), and females and males with inversion hetermoorphic pair no. 1 (+/i♀×+/i♂). Eighteen offspring (9 females: 9 males) obtained by mating between the (i/+) females and the normal (+/+) males segregated into 7 normal (+/+) and 11 with the heteromorphic pair no. 1 (i/+). Thirty-two offspring (9 females: 23 males) obtained by the reciprocal matings (+/+ ♀×i/+ ♂) segregated into 13 normal (+/+) and 19 heteromorphic pair no. 1 (i/+). Fifty rats (♀ 23: ♂ 27) obtained by mating between the females and males with inversion heteromorphic pair no. 1 segregated into 19 normal (+/+), 22 inversion heteromorphic (i/+) and 9 inversion homomorphic (i/i). These segregation ratios were not significantly different from the expected ones.

Fertility of the rats with inversion pair no. 1 was almost normal in all matings, although it was slightly smaller in the case of matings between inversion heteromorphic and normal rats (6.3 ± 3.3 in average litter size), and also between inversion heteromorphic females and males (5.7 ± 2.3). Average litter size of the 16 litters obtained by matings between the inversion homomorphic females and males was 7.4 ± 2.8, which was not significantly different from the control. Based on the above study it can be said that in the Norway rat the individuals with inversion pair no. 1 seemed to exert no effect on the fertility so far as the breeding has been taken in the laboratory. In the case of the transolcation pair no. 1, the fertility was rather better than the control rats with the normal karyotype as described in the above paper of this report. If such karyotype rearrangements occurred in natural population, the rats with the altered karyotype might have taken over those with the normal karyotype. The Norway rats with the metacentric pair no. 1 resulted from the pericentric inversion were

named the LEM-stock.

Frequency of Sister Chromatid Exchanges in Lung Primary Cultures in the Indian Spiny Mouse, *Mus platythrix*

Tosihide H. YOSIDA and Toru INOUE[1)]

It has been shown that the karyotype of the Indian spiny mouse (*Mus platythrix*) is characterized by 12 acrocentric autosome pairs and XY sex chromosomes, and further that several pairs of the chromosome complement of this animal would be produced as a result of tandem fusion of certain acrocentric chromosomes in the house mouse (*M. musculus*) (Yosida 1979, Proc. Jap. Acad. **55(B)**: 270–274). The number of sister chromatid exchanges (SCEs) of the house mouse in the presence of 0.5 μg/ml BrdU is reported to be 8.5$\pm$0.4/cell in the second cell cycle (Kato 1977, Intern. Rev. Cytol. **49**: 55–97). Since there is no information on the SCE value for the Indian spiny mouse, studies on the SCEs of this animal were carried out under the treatment with several concentrations of BrdU.

Exponentially growing lung cultured cells were treated with BrdU at concentrations from 0.1 to 25.0 μg/ml in the dark for 24 to 36 hrs. The highest frequency of labeled cells with BrdU at the stage of the second cell cycle was found to occur at 30 hrs inoculation (72.2%). The number of SCEs increased gradually from 7.6$\pm$0.75 to 18.1$\pm$0.93 at the above concentrations, but the curve became flat in the samples under the dose of 0.25 to 1.0 μg/ml. As the occurrence of SCEs in the dose of 0.5 μg BrdU was considered as of spontaneous frequency in the house mouse (Kato 1977), that of the SCEs in the Indian spiny mouse seemed to be 11.1/cell.

Clear heterochromatic C-band has been shown in the centromeric regions in almost all chromosomes of the house mouse, whereas it was very small and not clear in the Indian spiny mouse. The small amount of the C-band heterochromatin in the Indian spiny mouse was considered as a cause of higher frequency of SCEs in this animal than in the house mouse.

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

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

Frequencies of the X and Y chromosome dissociation in the primary spermatocytes were examined with six *Mus musculus* subspecies, fifteen laboratory inbred mouse strains and their F_1 hybrids. Three different types (type I, II and III) in the mode of X-Y dissociation were identified. The type I was found in inter-subspecies hybrids (BALA/c × *molossinus*, *castaneus*, *urbanus* or *bactrianus*), where the X-Y dissociation was less than 20% in each subspecies but 60–80% in the F_1 hybrids. The type II was observed in DBA/2J and DDK strains, in which the dissociation frequency was about 30% in the homozygous condition and increased up to 50% in inter-strain hybrids (C57BL/6J×DBA/2J or DDK). The type III found in WB/Re strain also showed high dissociation frequency (51.7%) in the homozygous condition but the value reduced remarkably (51.7→10.9%) in (C57BL/6J×WB/Re)F_1. We suggested that some Asian wild mice would have the type II or III as well as the type I. *Mus musculus* species can be classified into two groups in terms of the X-Y dissociation frequency of the type I; (1) *brevirostris*, *domesticus* and inbred laboratory mouse strain, and (2) *molossinus*, *castaneus*, *urbanus* and *bactrianus*.

Multiple Sex Chromosome Mechanism (XXY) in the Filefish, *Stephanolepis cirrhifer*

Makoto MUROFUSHI,[3] Sin OIKAWA,[4] Shohei NISHIKAWA[5] and Toshide H. YOSIDA

It is already reported that the chromosome number of the filefish, *Stephanolepis cirrhifer*, was $2n=34$, and all of the chromosomes were acrocentric (Murofushi and Yosida 1979, Jap. Jour. Genet. **54**: 191–195).

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The sex chromosome, however, could not be identified because the specimens used were only females. The male and female specimens were newly obtained and their karyotypes were reexamined. The diploid chromosome number in all females was 34 as already described, while that in all males was 33, among them one large metacentric chromosome was always found in their karyotypes. Such large metacentric element was never found in the female specimens. If this species was characterized by the usual XY sex determing mechanism, the chromosome numbers in female and male would be the same. However, one chromosome was always less in the male than in the female specimens. These chromosome complements suggest that the male in this species has the X_1X_2Y sex determination mechanism, and the large metacentric chromosome should be the Y element.

To confirm the above finding, the spermatocytes were observed. In the secondary spermatocytes two typed cells, $n=16$ and $n=17$, were found. In cells with 16 chromosomes one metacentric was always included, while in those with 17 chromosomes such element was never observed. Based on these findings a conclusion was drown that this species has the X_1X_2Y sex chromosome complex in the male and the $X_1X_1X_2X_2$ in the female. Unfortunately we could not distinguish the X chromosomes from the autosomes in mitosis and meiosis, because they are not morphologically differentiated. Finding of the multiple sex chromosome seems to be important for the study on differentiation and evolution of the sex chromosomes in the fish, because it is regarded as that the morphological differentiation does not occur in this animal group.

Cytological Studies on Male Crossing Over in *Drosophila ananassae*

Muneo MATSUDA, Hirotami T. IMAI and Yoshiko N. TOBARI[1)]

Recently we have succeeded to obtain high quality of meiotic chromosome figures in *D. ananassae* by using the air drying methods improved by Imai *et al.* (Chromosoma **59**: 341–393, 1977). To determine the mechanism of male crossing over in *D. ananassae*, cytological studies were carried out using two different heterozygous males between a marker strain (*b se*; *bri ru*) and two wild strains, TNG and L8. TNG F_1 (*b se*; *bri ru*/ TNG) males are characterized by a high frequency (about half value of females) and L8 F_1 (*b se*; *bri ru*/ L8) males are characterized by an extremely low fre-

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quency (0–0.1%) of male recombination. We demonstrated the presence of chiasmata in TNG F_1 males and a positive correlation between chiasma frequency and genetic recombination value (average chiasma frequency: 0.63/bivalent in TNG F_1, 0.10/bivalent in L8 F_1). These results strongly suggest that male recombination in *D. ananassae* is meiotic in origin. In addition to chiasma, a new type of chromosome aberration characterized by two chromosome breakages occurring simultaneously at comparable sites along a homologous pair and disordered reunion of the breakage points was observed, particularly in the spermatocytes of TNG F_1 (9.2%). Because there was a parallelism between chiasma and chromosome aberration in distribution pattern, we proposed that the chromosomal aberration might be closely related to formation of the chiasma, and under the control of a common causative factor.

VI. MUTATION AND MUTAGENESIS IN ANIMALS

Rat-Liver S-9 Mediated Mutagenesis in Germ-Cells of the Silkworm by Benzo(a)pyrene

Akio MURAKAMI

Benzo(a)pyrene [BP] is an aromatic hydrocarbone that has been a well-known environmental agent. BP has also been a potent carcinogen in experimental animals and a mutagen which requires to be metabolically activated to the reactive ultimate form(s) in order to exert its mutagenic or carcinogenic activities. This aromatic compound was selected as a test chemical for the short-term carcinogenic compounds screening programme in the Ministry of Health and Welfare of Japan. Although BP was proved to be mutagenic in most test organisms, this carcinogenic polycyclic hydrocarbon has been tested for mutation induction in the silkworm with negative results for the egg-colour specific locus test. We tested BP for mutagenic effect by suspending it in 0.1% carboxylmethyl cellulose (CMC) solutions. BP was injected into pupal females. There was no significant increase for the specific locus mutation over controls. As we have already been reported in this Report that mutagenicity of a promutagenic polycyclic hydrocarbon Dimethylbenzanthracene (DMBA) was only detected in pupal oocytes of the silkworm when the parent compound was *in vitro* incubated with S-9 fractions from rat-liver homogenates before injection. It is very likely to forecast that BP could not be detected the mutation in silkworm test systems unless the metabolic activation system is supplemented. This report concerns the positive mutagenic activity of BP in pupal oocytes of the silkworm by the egg-colour specific locus test coupled with the *in vitro* mammalian metabolic activation system.

Several concentrations of BP dissolved in dimethylsulfoxide (DMSO) solution were incubated with the incubation mixture at 30°C for 20 min. The incubation mixture in 0.025 ml per capita was made up of a BP in 0.017 ml DMSO solution, 0.0166 ml S-9 fractions from livers of rats which were pretreated with polychlorinated Biphenyl (PCB) and 0.0067 ml cofactor systems containing NADPH, β-NADH, G-6-P, ATP, and others as described by Sugimura *et al.* (1976). After injection with BP without the S-9 treat-

ment, no detectable mutation frequency was still found in pupal oocytes after all. While, the result of the experiments with the S-9 treatment indicated that BP was clearly mutagenic to pupal oocytes. The increase of mutation frequencies was significant when compared to the control (e.g. 2.9×10^{-5} at *re* locus) without an apparent relationship to the exposure dose level used. The frequencies of *re* locus mutations detected were 46.8×10^{-5} in 75 μg/pupa, 61.2×10^{-5} in 100 μg/pupa and 46.0 in 125 μg/pupa. This inconsistency is to be expected with a limited yeild-rate for the mutagenic intermediates produced under the described experimental condition.

The present observation confirmed that the silkworm egg-colour specific locus test method coupled with the *in vitro* rat-liver metabolic activation system—the silkworm/rat-liver S-9 system—is useful to test the mutagenicity for promutagenic polycyclic hydrocarbones.

Specific Locus Mutations Induced in the Silkworm by Diethylnitrosamine

Akio MURAKAMI

Several dialkylnitrosamines are well-known mutagenic agents in various test organisms and potent carcinogenic agents in experimental animals. The dialkylnitrosamines are one of typical indirect mutagens which require metabolic activation to mutagenic derivatives. In the silkworm, Dimethylnitrosamine (DMNA), one of dialkylnitrosamines, has been tested for mutation production in germ-cells with negative results for egg-colour specific locus mutations and for dominant lethals. So far as we know, ethyl compounds for methansulphonates, nitrosoguanidines and others are more effective for the production of mutations in germ-cells of the silkworm than these methyl compounds. In the present short communication, we present data on the mutagenic effects of Diethylnitrosamine (DEN) in the silkworm, the results for which may be compared with that of DMNA.

Several different concentrations of DEN were made up in solution with 0.85% NaCl. They were tested by injection into the abdomen of pupal females and males. The injected pupae were then mated individually to *pe*: *re* marker stocks each for the specific locus tests. DEN treatments at high doses ranged from 1.5 to 2.0 μl per female pupa (or oocyte) produced a significant increase in egg-colour specific locus mutations compared to

controls, but no increase of the mutations was detected at low dose treatments. There was no detectable increase over controls for specific locus mutations in male pupae (or spermatozoa) even after high dose treatments with DEN as well as DMNA. In the dominant lethal experiments in parallel with the specific locus tests, DEN affected only pupal oocytes at the high dose experiment groups, but not affected spermatozoa. At up to near mortal concentrations for pupae, DMNA did not produce the dominant lethal mutations in any of the pupal germ-cells. This finding clearly indicates that premeiotic oocytes in the pupae presumably have the capacity to metabolize the dialkylnitrosamines to mutagenic intermediates, but mature sperm have not the capacity as well as other pupal tissues or organs regardless of the sexes. Namely the active metabolites of dialkylnitrosamines may be short-lived so that damage in the pupae would be only produced in target cells or oocytes that come into direct vicinity to the site of metabolic activation. From the present finding and others, it can be also said that the large amounts of mutagenic metabolites would be required to induce the specific locus mutations as well as dominant lethal mutations.

The failure to detect the mutational events with DMNA in pupal oocytes might be resulted from some un-explained reasons other than the metabolic activation. In the silkworm, differential mutagenic sensitivity to the dialkylnitrosamines, DMNA and DEN, is very similar feature to that of the monoalkyl methanesulphonates, MMS and EMS. In this connection, it is worthwhile to note here that methyl compounds, MMS, MNNG and DMNA are generally more toxic to silkworms than ethyl compounds, EMS, ENNG and DEN. Accordingly, it can be explained that the absence of mutagenicity to DMNA may be due to a lack of sufficient amounts of effective mutagenic intermediates for the induction of mutations because the dosage is restricted by the high mortality of the treated insects at the higher concentrations. It can not completely exclude a possibility that the lack of mutagenic activity have probably resulted from a repair of altered DNA before mutation detection.

Lack of Effects on the Mutagenicity with Procarbazine in Silkworms

Akio MURAKAMI

Procarbazine has been used as a carcinostatic agent. This chemical

agent is known to be as a promutagen and that is metabolically activated by oxidation to azoprocarbazine. Mutagenic tests with *Salmonella* and *Saccharomyces* for reversion or foward mutations did not show a positive response to Procarbazine regardless of whether they were performed with the S-9 fractions from rat livers, while this drug gave a positive response in mammalian cells in culture, in mammals and *Drosophila* for various genetic endpoints. In *Drosophila*, both sex-linked recessive lethal mutations and dominant lethals were induced by this carcinostatic agent in male post-meiotic germ-cells or spermatids. Thus, it can be expected that in the silkworm, procarbazine would be effective on the induction of recessive visible mutations and dominant lethal mutations. This communication reports the result of mutagenicity tests of Procarbazine·HCl in the silkworm.

Sample Procarbazine·HCl was the gift of Nippon Loche, Japan. The compound was dissolved in a 0.85% NaCl or physiological saline for the silkworm was tested at various concentrations up to 750 μg/capita. Each concentration of the compound was administered by the injection method into the abdomen of wild-type male larvae and both sex pupae. The recessive visible mutation was detected by the egg-colour specific locus method. The treated individuals were mated to non-treated opposite sexes of the marker stock homozygous for *pe* and *re* genes.

We found that Procarbazine was not effective for the induction of specific locus mutations in larval spermatocytes/spermatids, pupal spermatozoa and oocytes. There is no evidence that dominant lethal mutations at the reduction of embryonic hatchability were induced by this drug in any of these germ-cells.

The failure to detect the mutational events with Procarbazine in the silkworm is to be expected with a lack of effective metabolic activation systems. Accordingly, the mutagenicity test of the drug in the S-9 mix is in progress. Hydrazone of monomethyl hydrazine and formyl isopropyl-benzamid are biological active intermediates of procarbazine. It may be suggestive to note that mutagenicity of hydrazines is not yet detected in the silkworm test system. The induction of dominant lethals in *Drosophila* was observed only at the high dose of the drug (Biljleven, W.G.H. and E. Vogel, 1977). This indicates that large amounts of the chemical or its metabolites would be required to induce the specific locus mutations as well as dominant lethal mutations in the silkworm test system as well as *Drosophila*.

Dominant Lethal Mutations Induced by Some Indirect-Acting Mutagens in the Silkworm, *Bombyx Mori*

Akio MURAKAMI

The mutagenicity of chemical compounds with a special emphasis on the indirectly acting mutagens was studied by dominant lethal tests as the reduction of egg-hatchability in pupal pre-meiotic oocytes and sperm of the silkworm. Wild-type (C108 × Aojuku) F_1 hybrids at the mid-stage pupa were given a single dose of at least five different doses for each chemical and mated with the opposite sex moths of a different stock.

Among chemicals tested two mycotoxins, Aflatoxin B_1 and Sterigmatocystine, gave a positive response in the dominant lethal test for both oocytes and sperm as has been reported to be positive by the specific locus test. A minor tranquillizer Diazepam also gave a positive response for both germ-cells by the dominant lethal test, although it has been shown to be a negative response by the specific locus test. Chlorodiazepoxide, a diazepin derivative and a minor tranquillizer, was also mutagenic in oocytes of the silkworm. 2-Acetylaminofluorene, Caffeine, Theophylline, and *p*-Ethoxyacetanilide (or Phenacetine) were all mutagenic for oocytes by the dominant lethal test, but not for sperm regardless of suspiciously positive or negative in the specific locus test for both germ-cells. The nitrofurane compounds, Furylframide or AF-2 and Nitrofurazone, were not mutagenic in the induction of dominant lethals in pupal oocytes at up to near killing doses, while Panfurane, 3-Amino-6-[2-(5-nitro-2-furyl)vinyl]-1, 2, 4-triazine, was only mutagenic in the oocytes at the high doses ranged from 250 to 500 μg/pupa. It has been reported that Panfurane is mutagenic in the oocytes of the silkworm by the specific locus test at the low dose (ca. 5 μg/pupa) (Tazima, 1974: Ind. J. Genet. & Plant Breeding, 34A, 302–310). The shape of the dose-response curves on the induction of dominant lethals in pupae treated with these indirect mutagens as well as the positive control, Ethylmethane sulphonate (EMS) and *tris*[1-(2-Methylaziridinyl) phosphine oxide] (meTEPA) was almost the same and sigmoidal fashion.

From the present study and others, it may safely be said that chemicals having the ability to induce dominant lethal mutations tend to induce recessive visible mutations detected by the egg-colour specific locus method. In addition to accuracy of the dominant lethal test method, simplicity makes it possible to test the mutagenic activity in a large number of environ-

mental chemicals. Thus, it can be stated the dominant lethal test in the silkworm may be a useful short-term assay system for emvironmental mutagens. (This work was supported by in part a Grant-in-Aid for Cancer Research from the Ministry of Health and Welfare of Japan.)

VII. RADIATION GENETICS AND CHEMICAL MUTAGENESIS IN MICROORGANISMS AND PLANTS

Genetic Study on the Initiation of Sporulation in *Bacillus subtilis*

Yoshito SADAIE

Ten isogenic strains of different *spoO* loci have been constructed to study the mechanism of initiation of sporulation in *Bacillus subtilis*, which is the best studied microorganism for differentiation. Because sporulation is under the control of catabolite repression, we examined the ability of these *spoO* strains to use different carbon or nitrogen sources. They grew well on the minimal agar supplemented with different carbon sources including glucose, mannose, maltose, sorbitol, arabinose, fructose, glycerol, sucrose, or trehalose. *SpoO* mutants examined were not mutants concerning catabolite repression of sugar utilization. Catabolite repression working on sporulation appears to be very similar to that on histidine utilizing operon. *SpoO* mutants grew well in the minimal medium with histidine as sole carbon and nitrogen sources. The first enzyme of *Hut* operon, histidase, was examined in toluene treated cells and ten *spoO* mutants all showed higher activities suggesting derepression of *Hut* operon in *spoO* mutants.

The effects of *spoO* mutations on vegetative growth were examined with these mutants. They grew well at 37°C and 48°C in a complex sporulation medium or in a minimal medium. They were resistant to various kinds of antibiotics including MC, Rif, Nal, Km, CM, Amp, PenG, and EtBr, and mutable toward DNA damaging agents. *SpoO* mutations so far examined did not affect vegetative growth. Strains used were *spoOA34*, *spoOB136*, *spoOC9*, *spoOD8*, *spoOE81*, *spoOF221*, *spoOG14*, *spoOH17*, *spoOJ87*, and *spoOK141* mutants.

On the Process of Competent Cell Formation in *Bacillus subtilis*

Yoshito SADAIE and Tsuneo KADA

The process of competent cell formation for transformation has been studied with early stationary phase cells of *Bacillus subtilis* which had been

grown in an enriched Spizizen minimal medium and transferred to a second synthetic medium. The formation of competent cells took place around 50 min after transfer of the early stationary phase cells into the second medium in accordance with the onset of cell division of total population. Rifampicin, chloramphenicol, penicillin G and tunicamycin were strong inhibitors of competent cell formation as well as vegetative cell division. Competent cells formed were no longer sensitive to the above agents. An inhibitor of chromosomal replication, hydroxyphenylazouracil did not inhibit development of competency. A D-alanine requiring mutant strain developed competency even in the absence of D-alanine in the second medium. These results suggested that competent cells were formed with less cross-linked cell walls and by a factor(s) whose gene(s) was activated during cell division. Competent cell formation was completely blocked by 0.7M ethanol, which is a specific inhibitor of early events during sporulation. Competent cells were formed even in media which supported sporulation. Development of competency was also studied with the *spoO* mutants of ten different loci. Most *spoO* mutations reduced development of competency except for *spoOC*, *spoOG* and *spoOJ*.

These results suggest that there are common steps involved both in forespore septation and in competent cell formation.

Antimutagenic Effect of Cobaltous Chloride on *Bacillus subtilis* and *Escherichia coli* Mutator Strains Possessing Error-Prone DNA Replicating Enzymes

Tadashi INOUE, Masumi SHIZAKI, Masayuki KATO and Tsuneo KADA

We previously reported that cobaltous chloride markedly diminished the spontaneous mutation frequency to histidine prototrophy in a *Bacillus subtilis* mutator strain NIG1125 (*his mut*-1) whose mutator activity has been ascribed to its temperature-sensitive DNA replicating enzyme which is error-prone at the permissive temperature (Inoue *et al.* 1981 Mutation Res. **91**: 41). In order to confirm that the cobaltous chloride interferes with mutagenesis itself, we attempted to determine the effect of the metal compound on mutation *rate*, not on mutation *frequency*, by adopting the method originally developed by Matney (1955. J. Bacteriol. **69**: 191) which is based on the fact that bacterial populations growing on the surface of membrane

filters can be easily transfered from one solid medium to another without disturbing the spacial relationship among the members of the population. The experiments yielded the results that the treatment of the cells with 40 μg/ml of cobaltous chloride diminished the mutation rate from 2.33×10^{-5} mutation/cell/generation to 0.30×10^{-5} mutation/cell/generation. These results strongly indicated that the metal compound really interfered with mutagenesis itself acting on the error-prone DNA replicating enzyme.

To investigate further the antimutagenicity of the metal compound, we examined the effect on another mutator strain, *Escherichia coli* SG902 (*leu dnaE*^{ts}), which has similar mechanisms to *B. subtilis* NIG 1125 for its mutator activity. The spontaneous mutation to leucine prototrophy was also remarkably supressed in this strain by the metal compound. In addition, we found that the strain SG902 was far more sensitive to cobaltons chloride than its parental strain DG17 (*dnaE*$^{+}$). This fact suggests that the target of the metal compound is *dnaE* gene product (DNA polymerase III). This was also confirmed by the following two lines of evidence. First, high proportion (4/42) of spontaneously arisen cobaltous chloride-resistant clones from SG902 was non-mutator. Second, high proportion (3/24) of spontaneously arisen temperature-resistant clones was cobaltous chloride-resistant. These results indicate that temperature-sensitivity, cobaltous chloride-sensitivity and mutability are closely linked to each other in the strain SG902 and that antimutagenicity of the metal compound is expressed by some mechanisms which involve DNA polymerase III.

Antimutagenic Activities of Human Placental Extract on Radiation-Induced Mutations in Bacteria

Tsuneo KADA and Hajime MOCHIZUKI

It is interesting to know whether human cells possess factors antagonizing with radiation-induced cellular mutagenesis. We report here that the human placental extract is working as a strong antimutagen in UV- and gamma-ray-induced mutagenesis in bacteria.

Cells of *Escherichia coli* B/r *try* were grown overnight in broth, washed by phsophate buffer and exposed to 3.45 J/m^2 of UV or 10 kR of gamma-ray (^{137}Cs); 0.1 ml or 0.2 ml portion of the irradiated suspension together with 0.1 ml of the placental extract were then incorporated into 3 ml of

molten solf agar mixed well, and poured onto the semi-enriched minimum agar. At the same time, the irradiated suspension was diluted 10^{-5} or 10^{-6} and 0.1 ml portion of each diluted solution was over-layered on the MB agar by means of the 3 ml soft agar. Plates were incubated at 37°C for 3 days and the number of induced mutant colonies and that of surviving colonies were scored.

Both for irradiation with UV- and gamma-rays, the number of induced mutant colonies decreased remarkably, whereas the effects of the extract on the survival were not so significant. It was shown that the presence of the placental extract did not modify significantly the number of viable bacteria which were not treated with radiation but the number of induced Trp^+ colonies per plate decreased considerably. The placental extract itself was not mutagenic. We confirmed that the established Try^+ cells were not particularly sensitive to the placental extract either in the case of UV or gamma-ray induction. These results indicate that the human placental extract contain factor(s) antagonizing with UV- or gamma-ray-induced mutations in this bacterial system.

The antimutagenic actions of the placental extract was also confirmed in other bacterial systems where the frequencies of mutations were reduced remarkably by it. We are examining if the present placenta factor might be involved in proofing spontaneous or induced mutagenic errors that are produced in the course of embryogenesis. (in J. Rad. Res. **22**, 297, 1981)

Antimutagenic Factor in Leaves of Camelia Families

Tsuneo KADA and Kenji KANEKO

It was previously suggested that anitmutagenicity of cobaltous chloride may be explained by its proofing activity in error-prone replication of DNA by a modified DNA polymerase (Kada, T. and Kanematsu, N. 1978, Proc. Jap. Acad. **54**: 234; Inoue, T. *et al.* 1981, Mut. Res. **41**: 91). We recently found that leaves of the Camelia plants contain a similar factor.

Since our daily consumed green tea is produced from plants of the family *Camelia japonica*, its antimutagenicity was studied.

Fresh plant leaves were homogenized with an equal weight of cold water and the homogenate was centrifuged at about $1\times10^4\times g$. The sterile supernatant was collected and stocked at $-40\sim-80$°C. The antimutagenic potency was calculated in a similar way in which that of cobaltous

chloride was determined by Inoue *et al.* (1981). When *B. subtilis* cells of NIG1125 (*his met* mut-1) grew in semi-enriched agar containing cobaltous chloride at a concentration of 1 μg/ml, 821 His^+ mutants disappeared in the course of 2.8×10^7 cellular divisions taking place. It comes out that about 30 mutations are lost per 1×10^6 divisions due to presence of the antimutagen. Doing similar experiments, we could calculate the antimutagenicity level of samples. The specific activity of antimutagenicity represents a concentration (μg/ml) of cobaltous chloride whose antimutagenic activity is equivalent to that of the test sample. That of *Camelia japonica* ranged between 10 and 20.

Effects of β-irradiation from ^{3}H-Water on Transforming DNA of *Bacillus subtilis*

Tsuneo KADA, Hajime MOCHIZUKI, Tadashi INOUE and Yoshito SADAIE

In order to elucidate mechanisms and efficiencies of DNA inactivation of tritium, the bacterial transforming system was studied. DNA was extracted from cells of *B. subtilis* Marburg (arg^+), purified by the phenol method, dissolved in SSC buffer solutions containing different concentrations of ^{3}H-water ranging 0.001 μCi/ml to 10 μCi/ml. The solutions were then kept at 4°C for β-irradiation. After different periods, portions of the solutions were sampled, dialyzed, against cold SSC to eliminate radioactivity and exposed to competent cells of *B. subtilis* H17 arg^- to measure inactivation effects of the arginine marker by β-rays. We found that, when % inactivations were plotted against ^{3}H-dose (μCi/ml$\times$days), effects of β-irradiation increased drastically by decreasing ^{3}H concentrations. For example the D_{37} dose for 10 μCi/ml teratment was 18 μCi/ml$\times$day, wheras that of 0.1 μCi/ml treatment was about 0.4 μCi/ml$\times$day.

These results showed that the inactivation efficiency of β-irradiation of tritium became progressively higher by lowering concentrations of tritium water. It is indicated that the RBE of tritium irradiation may be very high (more than 100) at least in the case of in vitro DNA inactivation under the above experimental conditions (Related results in press in J. Rad. Res.)

Human Enzymes Functioning in Repair of DNA Damage Induced by Gamma-Irradiation

Tadashi INOUE and Tsuneo KADA

Ionizing radiation produces many kinds of lesion in DNA including strand breaks and modification of base as well as of sugar moieties. Living organisms can, however, repair most of these damages efficiently. Our systematic studies on the repair mechanisms operating on gamma-ray-induced DNA damages using permeabilized cells of *Bacillus subtilis* revealed that some repair processes are necessary prior to those involving DNA polymerase and DNA ligase. Further studies showed that there exist enzymes (primer activating enzymes) which convert some of the gamma-ray-induced lesions to effective priming sites (*i.e.*, 3′-OH terminals) for repair replication by DNA polymerase.

We recently demonstrated that the level of the primer-activating enzyme activity was lower in the extracts from ataxia-telangiectasia fibroblasts than those from normal fibroblasts (Inoue *et al.* 1981, Biochim. Biophys. Acta **655**: 49). Ataxia-telangiectasia is an autosomal recessive disease of man characterized by telangiectasia, neurological degeneration, immunological deficiency, predisposition to malignancy, and radiation hyper-sensitivity. It is therefore of interest to examine the relationship between these symptoms and defects in the primer-activating enzymes. Tissue culture cells, however, provide too little materials for extensive enzymatic characterization, so we have thus turn to human placenta to study further the physical and catalytic properties of human repair enzymes.

The primer activating enzyme activity in the placental extracts was separated into two fractions by means of DEAE-cellulose chromatography. Both fractions were further purified by affinity chromatography on gamma-irradiated DNA-cellulose. This procedure was found to be extremely effective for purification, giving an overall purification of 800-fold for both fractions. Preliminary characterization revealed that one of the primer activating enzyme fraction had a catalytic activity to enhance the priming activity of gamma-irradiated DNA without any effect on unirradiated DNA, and neither phosphatase nor exonuclease activity was detected in the preparation.

On the Expression of Waxy Starch Mutants Induced by Thermal Neutrons in Rice

Etsuo AMANO

Many waxy (*wx*) starch mutants have been induced and examined in rice, *Oryza sativa* var. Norin 8. When a chemical mutagen, ethyl methanesulfonate was used, half of the *wx* mutants induced were more or less intermediate between *wx* and normal non-waxy (*Wx*). To compare between the mutagenic treatments, *wx* mutants were induced by thermal neutrons of D_2O (deuterium water) facility of KUR (Kyoto University Reactor). In 1978, six *wx* like mutants were obtained from thermal neutron irradiation, which suppressed seedling height to 60% of the non-irradiation control. All mutants produced enough M_3 seeds for measurements of *wx*-indices. Visual examination of M_3 seeds revealed that only two of the mutants were full mutants and the rest four seemed to be very weakly waxy.

Two wave lengths photometry was used to measure the waxyness expression quantitatively. Hulled brown rice grain was crushed with pliers and put into a test tube, one grain for each tube and more than 100 grains for a plant. 3 ml of water was added to each tube and then autoclaved for 15 min at 1 atm pressure. Starch solutions were stirred while warming. After overnight cooling down to room temperature, supernatant fluids were measured photometrically after iodine staining. Concentration of the starch solution, or amylopectine, stained with iodine, was monitored by 430 nm blue light during the stained sample solution was diluted by water in the measuring cell. When transmittance of the 430 nm blue light increased to 50% by dilution, the transmittance of 660 nm red light was measured and registered by a digital printer as index value to express waxyness of the starch. The visually confirmed two full *wx* mutants were proved to be also full *wx* mutants photometrically. Other four mutants were very weakly waxy.

Example of intermediate *wx* mutants in rice induced by ionizing radiation has been reported, but such a high proportion as four intermediate mutants in six induced mutant lines could not be expected. These results and the presence of gene dosage effect of *Wx*-*wx* character in rice endosperm would suggest some kinds of specificity in the expression pathway for waxy starch in rice.

Induction of Waxy Starch Mutants in Rice

Etsuo AMANO

To investigate the relationship between mutagen treatment and characteristics of structural alterations in induced mutants, many waxy (*wx*) starch mutants have been induced in *Oryza sativa* var. Norin 8. Mutagenic treatments by ethyl methanesulfonate exhibited same high efficiency in mutation induction as the cases in maize. Presence of many intermediate phenotypes in the *wx* starch character might suggest that considerable proportion of these *wx* mutants might be induced as point or missence mutations. As another type of mutagenic treatment, irradiation of seeds by reactor thermal neutrons in the D_2O facility of KUR (Kyoto University Reactor) was successful to induce some waxy starch mutants. Some characters of these *wx* mutants were described elsewhere in this Annual Report.

In the present experiments, gamma-ray and a chemical agent, acridine orange, were tested to see activity to induce waxy starch mutations. M_1 plants were grown in plastic flats filled with ordinary soil, so densely that each M_1 plant could yield only a few panicles. From the seeds which were irradiated by 30 kR of ^{137}Cs gamma-ray, 106 panicles with one to nine fertile seeds and 252 panicles with ten or more seeds could be harvested. From 40 kR treatment, 959 and 1545 panicles respectively could be harvested. With 40 kR treatment, 1012 sterile panicles were also scored. As a chemical agent, acridine orange was selected expecting to induce some frame shift mutations by hypothetical mechanisms of insertion of the molecules into DNA double helix. Rice seeds were soaked into 2% water solution of the dye without any buffering salts, for 48 h at 27°C. Coleoptiles and some other embryonic tissues were stained in dark red color, but after some growth, young leaves seemed to be normal in apperance. The same planting method of M_1 was followed and yielded 213 panicles with one to nine seeds and 317 panicles with ten or more seeds.

Harvested materials were examined according to standard detection procedure for waxy starch mutants. After drying well the harvested materials, segregation of waxy starch mutant was surveyed on panicle bases. Seeds from a single panicle were hulled and endosperms were examined visually first. Opaque endosperms of any degree or other questionable grains were tested further by scraping off a small area of endosperm surface and staining by iodine reagent. Out of the two mutagen treatments, gamma-

ray and acridine orange dye, in the present experiments, 3392 fertille panicles were examined but no waxy starch mutant including detectable intermediate or leaky waxy mutant could be obtained. Reaction of rice to gamma-irradiation seemed to be very alike to maize in which viable mutant was difficult to induce by gamma-ray. Mutagenicity of acridine orange should be carefully examined further before omitting from mutagen list, because under the treatment condition used here, visible light activation of the dye-DNA complex might not be expected since embryo might be kept light-shielded by hulls.

Induction of Waxy Mutants in Maize by Gamma-Ray

Etsuo AMANO

To investigate the relationship between mutagenic treatments and induced genetic alterations, waxy (*wx*) mutants have induced and maintained for detailed analyses. Treatments by a chemical mutagen, ethyl methanesulfonate (EMS) have yielded many viable *wx* mutants. Germicidal ultraviolet light applied to pollen grain have also yielded three viable *wx* mutants. Among ionizing radiations so far tested, fast and thermal neutrons induced some *wx* mutants but viabilities of these mutants were very low. The most popular ionizing radiations, X- and gamma-rays have also been used, but the mutagenic activities for *wx* locus were very low. Only a few *wx* mutants were induced in some ten years of trials. All of them were not viable. To confirm the previous results and to obtain, if possible, viable *wx* mutants for further analyses, seed irradiation experiment was carried out.

Seeds of multiple dominant inbred line (C^1 *Sh Bz Wx*, *Y*)were irradiated by 20 kR of ^{137}Cs gamma-ray and planted in field. At flowering time, all the irradiated plants were detasseled. In order to obtain induced mutants with their inbred line genetic background, mixed pollen method was used to pollinate the treated ears. Male tassels were collected on the previous day from untreated control and a multiple recessive tester (*C sh bz wx*, *y*) separately. Tassels of each groupe were placed in a bucket of water on a large sheet of paper. On the day of pollination, pollen were shaked off from tassels onto the sheet of paper and sifted with a fine mesh sieve. Pollen of the two strains were then weighed and pollen of equal weight were mixed together in a large glass beaker. They were brought to the field in Petri

dishes. Pollen were placed on silks of the treated plants using a small brush. By this method, mutant F_1 kernels would be segregated in 1 : 3 ratio within the mutant sector. Among the non-*wx* kernels in the sector, half of the inbred line type kernels, or 1/4 of the total kernels, within the sector would be expected to be heterozygous for the mutant gene with inbred line background. From these heterozygotes, new *wx* mutant with the original inbred line background would be isolated in the next generation. F_1 hybrids with the tester line or with any contaminating pollen would be distinguished by out-growth due to heterosis. After harvesting and drying, kernels were examined visually on the female ear bases. Opaque endosperms were tested by iodine staining for waxyness. 530 ears carrying nine or less kernels and 2,568 ears with ten or more kernels were examined and three ears segregated *wx* kernels. The frequency on ear bases amounted to 0.1% which was comparable to previous results of X- and gamma-rays. In the present experiments, several *wx* kernels were segregated suggesting better survival than the previous cases. Three sh_1 and six non-C^I mutants were also detected. As a trend, radiations induced more mutations in distal loci than proximal ones and chemical mutagen, EMS, in reverse. The difference in this trend might be statistically insignificant, but reproducibility of the results seemed to be high after several years of experience.

Automated Analysis of Maize Pollen

Etsuo AMANO

Pollen grains are haploid gamete cells of uniform shape and size, and can be obtained and handled in large quantity. If appropriate genetic traits (ex. *Wx*-*wx* or *Ae*-*ae*) are used, they can be an excellent material for investigation of rare but important biological events like intracistronic recombinations or mutations induced by very low level of mutagens. This advantage will be further improved, if the laborious counting and examination can be made automatically. Objectivity of the observed results will be also increased.

For automation of pollen analysis, techniques of flow analysis and image analysis would be applicable. In image analysis, samples are fixed on a slide glass, and then each picture frame taken by a TV camera is scanned for analysis. In flow system, sample grains are suspended in solution and pass through a narrow detection area and signals generated by the detector

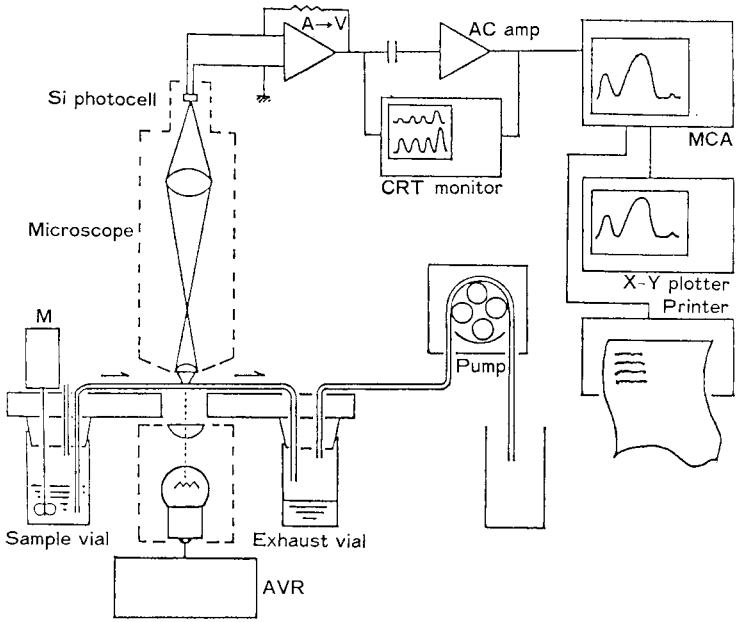

Fig. 1. Schematic diagram of instrumentation of the flow system.

are examined. Here, transportation system of the sample grains is important but fixation of samples on a slide glass is not necessary. Unfixed sample pollen grains can be analyzed repeatedly or analyzed by other method like ordinary visual analysis or by image analysis. Presently a flow analysis system with an optical detector was tested using maize pollen. The flow system used is shown schematically in Figure 1. Sample pollen grains were suspended in iodine solution and agitated by a small stirring blade. They were transported through a glass capillary (0.8 mm diameter) by gentle suction of air using a microtube pump. The capillary was fixed between a slide glass and a cover slip. Space around the capillary was filled with balsam. The capillary was positioned precisely in the light path of a microscope. Although the capillary used for maize pollen grain was quite large compared to diameter of a pollen grain, particles flowed at the center of the capillary when the suspension was transported at the rate of 2 ml/min or 4 ml/min. This centering of pollen grains in flow was an unexpected advantage of the present system and made focusing of pollen images on the

photocell easy. Interuptions of the light path by pollen grains were detected by a silicon photocell after optical magnification of about ten times. Electric pulses generated by the photocell were amplified and sent to a multichannel pulse height analyzer (MCA, Series 30 by CANBERRA). MCA used was capable of storage of data up to 10^6–1 counts in each of its 1024 channels. Each input pulse was assorted to corresponding channel according to its pulse height which would reflect the genetic character. The frequency distribution of pulse height can be displayed on a TV screen of the MCA. Graphic display and digital data including integrated data of region of interest (ROI) can be printed out by a X-Y plotter and a line printer. Extra care was taken to stabilize the light source to illuminate the capillary. Mechanical fixation, soldered leader wires, and an electronic voltage regulator were used.

At the flow rate of 2 ml/min, count rate of 500 pulses/sec (3×10^4 pulses/min) seemed to be optimal for pulse separation and scoring speed. In this concentration and speed of flow, 10^6 pollen grains may be analyzed in some half an hour. Staining and ratio of transmitting and reflecting light are very important to differentiate the pulse height generated by the sample pollen grain with different genetic traits. For good discrimination of the genetic traits, like *Wx-wx*, illumination of the sample grains must be improved.

Trial to Induce Waxy Starch Mutant in Diploid Wheat

Etsuo AMANO

Waxy (*wx*) starch mutants have been found in many crops like rice, maize, barley, sorghum and millets. These mutants have been picked up and maintained in the long agricultural history in eastern Asia. Recognition and preference for waxy starch might closely relate to the cooking habit of the people in this area. In rye which is diploid but did not become a main food crops in this area, and wheat or oats both of which are polyploid, no waxy starch mutant has been reported. However, starches of these rye, wheat and oats also consist of both amylose, straight chain molecules, and amylopectine, branched molecules, in the same normal proportion as rice, maize or barley. If genetic alterations which would disturb amylose synthesis pathway could be induced, it would become a low amylose waxy starch mutant. Possibilities of inducing waxy starch mutants in rye,

wheat and oats would be suggested by the fact that waxy starch mutants were found in barley which is closely related to these three crops. Another support for the possibilities would be the case of *Oryza glaberrima*, an African species of cultivated rice in which no waxy starch mutant had been reported. The first waxy starch mutant in this species was induced by a chemical mutagen, ethyl methanesulfonate (EMS). Breaking through the genetic barrier of polyploidy would not be impossible, if the techniques of synthesis of hexploid bread wheat are followed, waxy starch mutations being induced step by step in the constituting genomes. Encouraged by these examples, experiments to induce waxy starch mutation in wheat were carried out.

A diploid spcies of wheat, *Triticum monococcum*, was selected to use in the experiments. Preliminary experiments suggested that soaking treatment of seeds of barley or wheat should be made at lower temperature than rice or maize, the summer crops. After presoaking in distilled water, seeds were soaked in 0.1 M solution of EMS for 20 hours at 15°C. During the soaking treatments, seeds containing Ehrenmyer flasks were gently shaked in the water bath for uniform contact of seeds with the treatment solution. After rinsing and post-soaking in distilled water for 24 hours, seeds were sown in wooden flats. Seedlings were transplanted to field in November. On M_1 plants, many chlorophyll mutation stripes could be observed indicating that the treatments might be adequate. Two spikes were harvested from each of M_1 plant. Well dried endosperms were examined for segregation of possible waxy starch phenotype, on spike bases. The material used in the present experiments, *T. monococcum flavescens*, had relatively transluscent appearance in its endosperm. However, the transluscence was not enough, compared to rather transparent rice or maize, to distinguish opaque apperance of waxy starch phenotype by visual examination as the first screening procedure. Each opaque or questionable seeds were further tested by iodine staining for confirmation. Higher protein content in wheat should be also considered for the phenotypic expression. In the present experiment, 400 spikes harvested from the treatment by 0.1 M solution of EMS were examined but no waxy starch mutant was detected. In case of maize and rice, the mutant segregating spikes would be found, after optimal treatments, in about a half to one percent of the spikes examined. It would be suggested that grain to grain tests for iodine staining to more than some hundreds of spikes might be

required for induction and detection of the waxy starch mutant in the diploid wheat.

Effect of Tumor Promoter in Soybean

Taro FUJII

12-*O*-tetradecanoyl-phorbol-13-acetase (TPA) isolated from oil of the seeds of *Croton tiglium* is a very strong tumor promoter. Another indol alkaloid compound, teleocidine isolated from *Streptomyces mediocidicus* is also suspected to be tumor promoter. To examine the effects of these chemicals in higher plants, seeds of soybean T219 were treated and frequency of spot per leaf was investigated. (Chemicals were kindly supplied by Dr. H. Fujiki of the National Cancer Center Research Institute). Chemicals were disolved in DMSO and diluted to given concentrations. The amount of DMSO added for disolve (5 μg/ml) has no effect on mutation induction. Different concentration of chemicals, viz., 1, 5 and 10 μg/ml of TPA, and 1, 10 and 50 μg/ml of teleocidine were applied to dry seeds, Mutagenicity tests of these chemcials were repeated twice with similar concentrations, however, spotting frequency did not increase at least concentions tested. It is known that about 80% of the carcinogens induce mutagenic effects. Although both chemicals has a potency to promote of tumors in rats under the cooperation with initiating substance(s), no mutagenic activity has been reported in single treatment on Ames' spot test method. Experimental results with soybean seeds indicated that both chemicals has no mutagenic effect in plants.

Combination treatment of caffeine with TPA and teleocidine was carried out to see the effect of these chemicals on the caffeine mutagenesis. Single treatment of 0.02% caffeine induced about 10 spots per leaf (control value is about 1.5 per leaf). Unexpectedly, such large number of mutant spots induced by caffeine treatment decreased to about a half by mixture of 5 μg/ml of TPA or 50 μg/ml of teleocidine, respectively. Combination treatment of gamma-rays and TPA was also conducted to see the effect of chemical on the radiation induced mutational damage. However, no difference in spotting frequency was observed between single radiation treatment of 200 or 500 R and combination of chemical with radiations. Mechanism is not well explained at present, but some sort of repair process might be involved in the combination of two chemicals, and some hidden

action of promoters may act to caffeine mutagenesis but not to radiation mutagenesis.

VIII. POPULATION GENETICS (THEORETICAL)

Average Time until Fixation of a Mutant Allele in a Finite Population under Continued Mutation Pressure: Studies by Analytical, Numerical, and Pseudo-sampling Methods

Motoo KIMURA

We consider a single locus, and denote by A the wild-type allele and by A' the mutant allele that is produced irreversibly in each generation from A at the rate v. Let $1+s$, $1+h$, and 1 be respectively, the relative fitnesses of mutant homozygote $A'A'$, mutant heterozygote $A'A$, and wild-type homozygote AA. Then, it is shown, on the basis of the diffusion equation method, that the average time until fixation of the mutant allele (A') in a randomly mating population of effective size N_e, given that the initial frequency is p, is

$$\bar{T}(p)=4N_e\int_p^1 e^{-B(y)}y^{-V}dy\int_0^y\frac{e^{B(x)}x^{V-1}}{1-x}dx,$$

in which $B(x)=(S/2)x^2+Hx(1-x)$, $S=4N_es$, $H=4N_eh$, and $V=4N_ev$. Of particular interest are the cases in which the mutant allele is deleterious ($s=-s'$, $s'>0$). Three cases are considered; the mutant is: (i) completely dominant $s=h=-s'$, (ii) completely recessive $s=-s'$, $h=0$, and (iii) semidominant $s=-s'$, $h=-s'/2$, in which s' is the selection coefficient against the mutant homozygote. It is shown that the average time until fixation is shorter when the deleterious mutant allele is dominant than when it is recessive if $4N_ev$ is larger than 1. On the other hand, the situation is reversed if $4N_ev$ is smaller than 1. It is also shown that for a mutant allele for which $N_es'>10$, it takes such a long time until fixation that we can practically ignore the occurrence of random fixation of a deleterious allele under continued mutation pressure. To supplement the analytical treatment, extensive simulation experiments were performed by using a device called the pseudosampling variable, which can enormously accelerate the process of simulation by a computer. This method simulates the diffusion process itself rather than the binomial sampling process (in population genetics the diffusion model is usually regarded as an approximation

of the discrete binomial sampling process). For details, see Proc. Natl. Acad. Sci. USA 77, 522–526.

A Simple Method for Estimating Evolutionary Rates of Base Substitutions Through Comparative Studies of Nucleotide Sequences

Motoo KIMURA

Some simple formulae were obtained which enable us to estimate evolutionary distances in terms of the number of nucleotide substitutions (and, also, the evolutionary rates when the divergence times are known). In comparing a pair of nucleotide sequences, we distinguish two types of differences; if homologous sites are occupied by different nucleotide bases but both are purines or both pyrimidines, the difference is called type I (or "transition" type), while, if one of the two is a purine and the other is a pyrimidine, the difference is called type II (or "transversion" type). Letting P and Q be respectively the fractions of nucleotide sites showing type I and type II differences between two sequences compared, then the evolutionary distance per site is $K=-(1/2)\ln\{(1-2P-Q)\sqrt{1-2Q}\}$. The evolutionary rate per year is then given by $k=K/(2T)$, where T is the time since the divergence of the two sequences. If only the third codon positions are compared, the synonymous component of the evolutionary base substitutions per site is estimated by $K_S'=-(1/2)\ln(1-2P-Q)$. Also, formulae for standard errors were obtained. Some examples were worked out using reported globin sequences to show that synonymous substitutions occur at much higher rates than amino acid-altering substitutions in evolution. For details, see J. Mol. Evol. **16**, 111–120.

Genetic Variability and Effective Population Size When Local Extinction and Recolonization of Subpopulations are Frequent

Takeo MARUYAMA and Motoo KIMURA

If a population (species) consists of n haploid lines (subpopulations) which reproduce asexually and each of which is subject to random extinction and subsequent replacement, it is shown that, at equilibrium in which mutational production of new alleles and their random extinction balance each other, the genetic diversity (1 minus the sum of squares of allelic fre-

quencies) is given by $2N_e v/(1+2N_e v)$, where

$$N_e=\tilde{N}+n/(2\lambda)+n\tilde{N}v/\lambda,$$

in which $\tilde{N}$ is the harmonic mean of the population size per line, n is the number of lines (assumed to be large), λ is the rate of line extinction, and v is the mutation rate (assuming the infinite neutral allele model). In a diploid population (species) consisting of n colonies, if migration takes place between colonies at the rate m (the island model) in addition to extinction and recolonization of colonies, it is shown that effective population size is

$$N_e=\tilde{N}+n/[4(v+\lambda+m)]+n\tilde{N}(v+m)/(v+\lambda+m).$$

If the rate of colony extinction (λ) is much larger than the migration rate of individuals, the effective population size is greatly reduced compared with the case in which no colony extinctions occur (in which case $N_e=n\tilde{N}$). The stepping-stone type of recolonization scheme is also considered. Bearing of these results on the interpretation of the level of genetic variability at the enzyme level observed in natural populations is discussed from the standpoint of the neutral mutation-random drift hypothesis. (Proc. Natl. Acad. Sci. USA **77**: 6710–6714.)

Amino Acid Diversity of Immunoglobulins as a Product of Molecular Evolution

Tomoko OHTA

Based on population genetics theory of the evolution of multigene families, the sequence variability of the variable regions of immunoglobulins compiled by Kabat *et al.* (1976) has been analysed. An amino acid identity coefficient either within or between species is calculated separately for both the hypervariable and the framework regions. Under the somatic mutation hypothesis, the somatic component of amino acid diversity exists in addition to the germ line component and should contribute an amount of change between the hypervariable and framework regions that is independent of the time since the divergence of any two immunoglobulin gene families. The relationship between the identity coefficient of the hypervariable region and that of the framework region is shown to be not in accord with such prediction. The result indicates that the rate of evolutionary accumula-

tion of amino acid replacements in the hypervariable region is roughly three times more rapid than in the framework region and the hypervariability within a species is a necessary consequence of the high evolutionary rate. For details, see J. Mol. Evol. **15**, 29–35.

Linkage Disequilibrium between Amino Acid Sites in Immunoglobulin Genes and Other Multigene Families

Tomoko OHTA

Linkage disequilibrium between segregating amino acid sites in repeated genes which form a multigene family was investigated by using the population genetics theory. The degree of non-random association of amino acids is measured by the disequilibrium coefficient which is determined by the balance among various forces; unequal and equal crossing-over, mutation, random genetic drift and recombination which takes place between the two sites in question. Another measure of disequilibrium, 'standardized identity excess', represents excess probability of simultaneous identity at the two sites over that expected from random association of amino acids. Sequence data of variable region of immunoglobulins provide most interesting example of multigene family to apply the theory. Statistical analyses on identity excess for various groups and subgroups of variable region sequences of immunoglobulins suggest that a multigene family such as human κ or mouse κ gene family consists of several subfamilies between which recombination is limited. The analyses also indicate that the recombination may take place between any gene members in one subfamily. For details, see Genet. Res. Camb. **36**, 181–197.

Two-Locus Problems in Transmission Genetics of Mitochondria and Chloroplasts

Tomoko OHTA

A problem in transmission genetics was studied theoretically by regarding a cell as a population of organelle chromosomes. A hybrid cell of the two strains is assumed to consist initially of equal numbers of the two types of nucleoids, each containing an equal number of chromosomes. The two parent strains have different alleles at the two loci, and the two-locus dynamics of the hybrid cell line was analyzed. Within each nucleoid,

stochastic matching and gene conversion are assumed to occur between the chromosomes, and at a certain rate fusion and division are assumed to take place between the nucleoids within the cell. Under this model, the changes of average genotype frequencies at the two loci within a nucleoid and within a cell were formulated, and the final frequencies of recombinant chromosomes were obtained. Random segregation of nucleoids at cell division was incorporated into the analyses; in addition, the increase of homozygosity in the cell was formulated. The results may be useful for interpreting the observed data on organelle genetics of some species of algae and yeast. For details, see Genetics **96**, 543–555.

Composite Stepwise Mutation Model under the Neutral Mutation Hypothesis

Naoyuki TAKAHATA

As an extension of the conventional (Ohta-Kimura) stepwise mutation model, a new model is proposed. In this model, it is assumed that each charge state (electromorph) is represented by K alleles and that a mutation changes an allele either by one step in the charge space or to one of the other members of the identical electromorph. It is shown that the net genetic variability within a population is similar to that predicted by the infinite allele model rather than to that predicted by the stepwise mutation model, and the K-dependence of genetic variability is rather weak when $K \geqq 2$ and the effective population size is not much greater than the reciprocal of mutation rate. The results are compared with the recent observations at the xanthine dehydrogenase locus in *Drosophila pseudoobscura*. For details, see J. Mol. Evol. **15**, 13–20.

Polymorphism and Loss of Duplicate Gene Expression: A Theoretical Study with Application to Tetraploid Fish

Naoyuki TAKAHATA and Takeo MURUYAMA

We studied the fixation of null alleles at independent duplicate loci, assuming that wild-type active alleles mutate irreversibly to nonfunctional null alleles and that the population is finite and panmictic. Solving the two-dimensional Kolmogorov backward equation numerically, we obtained

the rate at which one of the active genes is lost and the amount of heterozygosity at specified times. Previously harmful genes, including recessive lethals, can be fixed at one of the duplicate loci, which would not happen with a single locus. Examination of data from several fish families showed that the rate of fixation of null alleles is too slow and the amount of heterozygosity too small to be compatible with complete recessivity at all loci. Our conclusion differs in this regard from that of Bailey *et al.* (Proc. Natl. Acad. Sci. USA **75**, 5575–5579). They also reported that the time taken for 50% of the loci to be fixed for null alleles is approximately $15N+v^{-3/4}$, in which N and v are the effective population size and mutation rate, when the alleles are completely recessive and the double homozygote is lethal. We found that the fixation rate depends not only on N, but also on Nv. For details, see Proc. Natl. Acad. Sci. USA **76,** 4521–4525.

IX. POPULATION GENETICS AND EVOLUTIONARY GENETICS (EXPERIMENTAL)

Molecular Evolution in Papova Viruses and Their Host Species, and in Bacteriophages

Takeo MARUYAMA and Eiichi SOEDA

Comparing homologous genes of three papova viral genomes, we showed the very close relative phylogeny among the viral species and their host species, and therefore the viral species seem to have evolved with their host organisms (Soeda *et al.* 1980). Additionally, the DNA-sequence data of bacteriophages $\Phi X174$ and G4 and their overlapping genes were examined for evolutionary patterns. It was made evident that overlapping genes have a quite different substitutional pattern with respect to the position of nucleotides in the codons than do non-overlapping sequences. Namely, in overlapping regions the third positions are usually substituted fastest, followed by the first positions, while the second positions are slowest in each gene, though different genes may have different rates of nucleotide substitution. With overlapping genes, this pattern does not apply, but rather is altered because of an interaction between the substitution rates in the two genes involved in an overlap. (Publication, Stadler Symp. **12**: 83–96.)

Stasipatric Speciation in *Drosophila*

Takao K. WATANABE and MASAOKI KAWANISHI

White (1968, Science **159**: 1065) proposed a model of speciation named "stasipatric speciation" from the karyological studies of a grasshopper species group. That is, a new species arose within the old species range and took over whatever area it could by eliminating the old species. This model can be applicable to some *Drosophila* species evolution if we take our hypothesis of interspecific mating preference (1979, Science **205**: 906) into account for the geographical distribution of some sibling species of *Drosophila*. Our hypothesis insists that a new species female rejects an old species male but the old species female accepts the new species male. Evolutionary sequence of the *D. takahashii* group can be speculated from our

hypothesis as follows; *D. pseudotakahashii* (New Guinea-Australia)→*D. trilutea* (Taiwan)→*D. lutescens* (Japan)→*D. takahashii* (India-Southeast Asia-Japan). We can suppose that the older species which had ever evolved in the center of the Southeast Asia having spread over to the present marginal areas such as Australia, Taiwan and Japan, then the newest species (*D. takahashii*) finally occurred in the center and is now spreading its distribution by eliminating the older species around the center. In another species group we can show the direction of species evolution as follows: *D. pseudoananassae* (from Thailand, Borneo to New Guinea, Australia)→ *D. malerkotliana* (from India to Borneo)→*D. bipectinata* (from India, Japan to Fiji, Australia)→*D. parabipectinata* (around Borneo). All the four species now coexist in Borneo which is the probable center of this group. The newest species (*D. parabipectinata*) had evolved recently in Borneo where already the other three older species existed, although it did not rid the older species yet. The reason why our hypothesis is favorable to infer the evolutionary change of the species distribution is as follows: (1) A new species can establish itself in a nonperipheral local colony previously occupied by the older species if she has an ability to discriminate and reject old species males. (2) Since the new species males are accepted by the old species females, old species population retreats its previous territory showing a hybrid zone when the new species adapts to the environment. Thus, asymmetrical mating preference between sibling species supports the stasipatric speciation model.

Directional Change in the Frequencies of Cosmopolitan Inversions of *D. melanogaster*

Yutaka INOUE and Takao K. WATANABE

Polymorphic inversions in several world populations of *D. melanogaster* were compared. Frequencies of two common cosmopolitan inversions on the second chromosome (*In*(2*L*)*t*, *In*(2*R*)*NS*) are listed in Table 1. The Old World and Australian populations generally showed the relation of *In*(2*L*)*t*>*In*(2*R*)*NS*, but the New World populations rather showed a *In*(2*R*)*NS*>*In*(2*L*)*t* relation.

A Japanese(Katsunuma) population has been surveyed for 16 years. It was clearly $2Lt>2RNS$ in the sixties but now is in $2Lt\approx2RNS$ due to a rapid decrease of *In*(2*L*)*t*. The relation of *In*(2*L*)*t*>2*R*(*NS*) was still

Table 1. Frequencies (%) of the second chromosomal inversions per arm of the several world populations

Population	No. of tested chromosomes	*2Lt*	*2RNS*	Reference
Japan (Katsunuma)	1200	31.7	22.8	The present study 1963–1969
Japan (Katsunuma)	2081	13.8	11.9	The present study 1970–1980
Korea	1720	9.8	7.5	Paik 1979
Papua New Guinea	52	53.8	42.3	Inoue and Watanabe 1980
Crete	64	6.3	1.6	Ashburner and Lemeunier 1976
Greece	32	4.7	0.9	Alahiotis 1977
Greece	426	2.3	0.9	Zachalopourou 1974
Israel	32	21.9	3.1	Ashburner and Lemeunier 1976
Egypt	1340	7.4	4.1	Mourad and Mallah 1960
South-Africa	90	18.8	0	Inoue and Watanabe 1980
Australia	68	26.5	16.2	Ashburner and Lemeunier 1976
Columbia	10	10.0	30.0	Ashburner and Lemeunier 1976
U.S.A.	1504	17.2	8.6	Warters 1944
U.S.A.	4323	13.0	16.0	Yang and Kojima 1972 Ashburner and Lemeunier 1976 Stalker 1976 Mettler *et al.* 1977

observed in other Japanese populations than Katsunuma. On the other hand, the past U.S. populations, about thirty years ago, had shown the relation of *In*(2*L*)*t*>*In*(2*R*)*NS* like the Old World populations, although the recent U.S. populations are in *In*(2*R*)*NS*>*In*(2*L*)*t*. Thus, it can be speculated that the original type of *In*(2*L*)*t*>*In*(2*R*)*NS* relation which was observed over the world has been changing to the new type of *In*(2*R*)*NS*> *In*(2*L*)*t* relation and that the U.S. population is an outrider.

Molecular Phylogenies of *Drosophila*: Protein Differences Detected by Two-dimensional Electrophoresis

Seido OHNISHI, Masaoki KAWANISHI and Takao K. WATANABE

Many abundant proteins (ca. 100) of *Drosophila* adults were investigated by an O'Farrell's technique (two-dimensional electrophoresis; 2DE). Eighteen species belonging to three taxonomically different groups (six species of the *D. melanogaster* subgroup, four of the *D. auraria* complex and eight of the *D. virilis* group) were used. The number of charge change

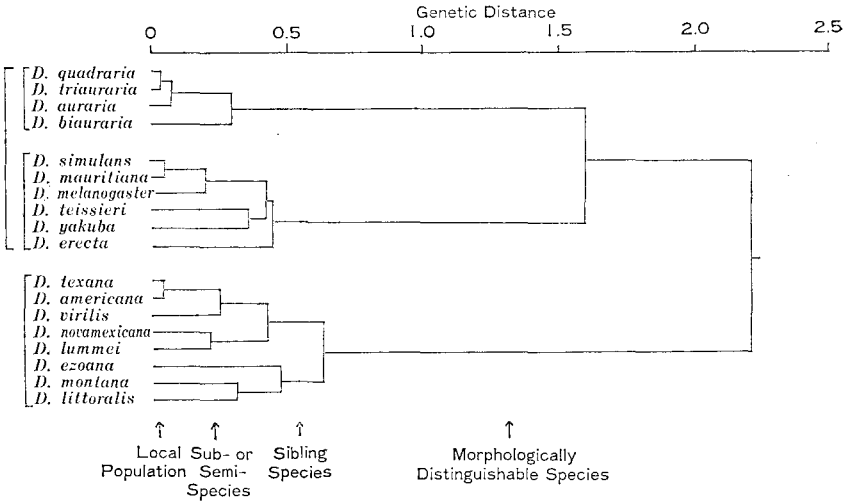

Fig. 1. The phylogenic tree of three *Drosophila* groups constructed from data on protein differences detected by two-dimensional electrophoresis. Arrows show the level of speciation according to allozymes' data of Ayala *et al.* (1974). Brackets at left margin indicate taxon in a conventional taxonomy.

Table 1. Correlation coefficients between genetic distances obtained by 2DE, SDE and the percent of successful mathings (PSM) in three taxonomically different groups

Group	d.f.	2DE vs. SDE	2DE vs. PSM	SDE vs. PSM
D. melanogaster subgroup	13	0.819**	−0.889**	−0.755**
D. auraria complex	14	0.504*	−0.734**	−0.720**
D. virilis group	8	0.782**	−0.900**	−0.715*

* Significant at 5% level.
** Significant at 1% level.

differences in the proteins was compared in all combinations of species pairs within the group (or complex). A dendrogram was constructed from genetic distances (Nei, M., Amer. Nat. **106**: 283, 1972) which were calculated from the above differences (Fig. 1). This phylogenic relationship was consistent with the results obtained from the percent of successful matings or conventional (one-dimensional) electrophoresis (SDE) as shown in Table 1, and also with the morphological and cytological phylogenies.

Therefore, if we consider that genetic variation within species estimated by 2DE is lower than that by SDE (Ohnishi *et al.*, Genetics, in press) and that many protein loci can be analyzed by a single gel in 2DE, this technique is a more useful tool for the taxonomical studies of genetically differentiated species.

Geographical and Ecological Variations of *Oryza perennis* at Six Isozyme Loci

Gerard SECOND and Hiroko MORISHIMA

In Asia, *Oryza perennis* is found mainly in the vicinity of rice fields, from West India to southern China and Indonesia, showing a range from perennial to annual ecotypes. In the line of previous investigations of *O. sativa*, the genetic diversity of *O. perennis* was surveyed at three isozyme loci, *Cat-A* (catalase), *Pgi-A* and *Pgi-B* (phosphoglucose isomerase), with regard to i) electrophoretic mobility and ii) heat stability (for *Pgi* only). About 300 strains were studied. In addition, the original data recorded by Drs. B. B. Shahi and C. Pai on three more loci, *Acp-1* (acid phosphatase), *Px-1* and *Px-2* (peroxidase), were reexamined with the same aim. The results are summarized as follows:

1) *Pgi* electromorphs were more numerous in *O. perennis* than in *O. sativa*. In all geographical areas and ecotypes of *O. perennis*, the most frequent electromorphs were those characterizing the Japonica type of *O. sativa*, although Indica cultivars are predominant in the distribution areas of *O. perennis*.

2) Similar bands of *Pgi* in different individuals rarely showed differential heat stability. The most frequent band was most thermo-resistant.

3) Two electromorphs only were found at the *Cat-A* locus. They were characteristic of the Indica and Japonica types of *O. sativa*, respectively, and showed a geographic cline clearly: The "Japonica" electromorph was frequent in southern China including Taiwan, and the "Indica" electromorph was frequent in other regions. A similar cline seemed to exist among the acid phosphatase electromorphs specified by the *Acp-1* locus.

4) All annual types of *O. perennis* so far studied lacked the "Indica" electromorph specified by the *Pgi-A* locus.

In interpreting these data, it must be taken into consideration i) that the current populations of *O. perennis* are relicts of larger populations that

should have existed before domestication by man, ii) that they have hybridized with *O. sativa* cultivars which might have originated in different geographical areas, and iii) that some of the present races of *O. perennis* may represent a return to wild type following hybridization between independently domesticated cultivars, as was observed in Africa. The data indicated a geographical pattern of variations in isozyme markers which are presumably neutral in seletion, among the populations of *O. perennis*. This pattern is related to the Indica-Japonica differentiation, but not to the present distribution of Indica and Japonica cultivars. The barriers to land migration of *O. perennis* like the Himalayan range and the ocean seem to account for the geographical pattern of variation. Thus far, no particular ecotype of *O. perennis* nor the populations in any particular geographical region, may be assigned to be the direct progenitor of *O. sativa*. Yet, the data are in agreement with the hypothesis of independent primary domestication of the Indica and Japonica types of *O. sativa*. An additional survey for a total of 25 isozyme loci including these six is under way.

Isozyme Polymorphism in Natural Populations of *Oryza perennis*

Chiang PAI and Hiroko MORISHIMA

Plants raised from the seeds of natural populations of wild rice, *Oryza perennis* Moench, collected in India and Thailand in 1979 (Morishima *et al.* 1980), were investigated with regard to peroxidase and acid phosphatase isozymes. The data were analyzed together with those for other *O. perennis* populations from different parts of the world so far observed. A total of 78 populations (60 Asian, 13 African, 4 Australian, and 1 American) were studied, and heterozygosity and gene diversity (as shown by $1-\Sigma x_i^2$, Nei 1975) were computed from the frequencies of alleles at the Px_1 and Acp_1 loci. It was found that:

1) Three codominant alleles, 0C, 2A, and 4A, are known at the Px_1 locus. Both 2A and 4A were frequent in Asian perennial populations while many of Asian annual populations were monomorphic with 2A. Allele 4A was predominant in African (perennial) and American (perennial) populations; allele 0C was fixed in the Australian (annual) populations.

2) At the Acp_1 locus, seven codominant alleles controlling the electrophoretic mobility of the AMC band-group are known, which were designated as −17, −9, −4, 4, 9, 12, and 24 according to the running distance

of band C (Pai *et al.* 1975). Indian perennial populations were polymorphic and generally showed high frequencies of alleles −4 and 9; Indian annual populations showed fixation of either −4 or 9. In Thailand, allele 9 was rare. Thailand perennial populations were polymorphic and showed a high frequency of allele −4; this allele was fixed in Thailand annual populations. African populations had fixed allele 4, while American as well as Australian populations had fixed allele 12.

3) The level of isozymic variability was generally higher in the perennial than in the annual type. Among Asian populations, the mean frequency of heterozygotes and mean gene diversity for the Px_1 locus were 0.201 and 0.273 for the perennial type, and 0.002 and 0.049 for the annual type, respectively. African populations, rhizomatous and strongly perennial, were rather monomorphic although they are almost completely outcrossed. Their heterozygosity was 0.036 and their gene diversity was 0.034 when a few populations which had coexisted with other *Oryza* species were excluded. This suggests that the African populations consisted of genetically identiacl clones propagating by rhizomes. The gene diversity at Px_1 and that at Acp_1 were intercorrelated among the populations examined, although the former locus was less polymorphic than the latter.

4) Almost all cultivars of *O. sativa* uniformly had allele 2A at the Px_1 locus and either allele −4 (Indica) or 9 (Japonica) at the Acp_1 locus. Wild populations, particularly those of perennial types, are more polymorphic. Geographic differences in the frequency of Acp_1 alleles were also detected.

X. HUMAN GENETICS

Hereditary Retinoblastoma: Host Resistance and Second Primary Tumors

Ei MATSUNAGA

Data for 26 cases of nonradiogenic osteosarcomas that developed in patients with retinoblastoma and in their close relatives were collected from the literature and analyzed. The distribution of unaffected and unilaterally and bilaterally affected cases of retinoblastoma agreed with the expectation that risk to osteosarcoma did not differ by the phenotypes of the gene carriers. This finding indicated not only that increased risk to second primary tumors was a pleiotropic effect of the retinoblastoma gene but also that host resistance to the gene effects was tissue-specific. The risk of gene carriers to nonradiogenic osteosarcoma was estimated at 1.2%, and the relative risk was estimated at 230. Liability of the gene carriers to osteosarcoma could be regarded as a multifactorial threshold character, and heritability was estimated at 71%. Results of analyses of ages at diagnosis suggested that nonradiogenic osteosarcoma developed slightly earlier in carriers of the retinoblastoma gene than in children not carrying the gene. These findings are discussed in the light of the host resistance model in which genes involved in tissue resistance must be suppressors to an error in differentiation (presumably a crucial event in malignant transformation) and radiogenic osteosarcoma in gene carriers is ascribed to the accumulation of induced mutations in that suppressor system. For details, see J. Natl. Cancer Inst., **65**: 47–51, 1980.

Retinoblastoma: Host Resistance and 13q- Chromosomal Deletion

Ei MATSUNAGA

Of genetic importance is the question as to whether the retinoblastoma gene is located on that segment of 13q, which when deleted, would develop the tumor at high frequency. While the question may eventually be answered by linkage studies of familial retinoblastoma using biochemical or chromosomal markers, we may take a different approach by comparing

the variation in expressivity or laterality among patients with hereditary retinoblastoma with that among patients with 13q-. This approach is valid provided that the host resistance that is largely controlled by genetic factors other than the major gene or deletion of 13q can be adjusted between the two groups of patients.

Data for 27 cases of retinoblastoma that developed in patients with 13q- were collected from the literature and analyzed. The distribution of unilateral and bilateral cases of retinoblastoma differed significantly from the expectation that the degree of expressivity does not differ between the retinoblastoma gene and deletion of 13q. The excess of unilateral cases among the patients with 13q-, which could not be accounted for by ascertainment bias, was attributed to somewhat lowered carcinogenic potential of deletion of 13q14 as compared with the retinoblastoma gene. It was argued that the retinoblastoma gene is probably not located on 13q, and perhaps 20% or more of the individuals with a deletion of 13q14 would not develop retinoblastoma. The normal allele at the retinoblastoma locus, the haplicon in the segment of 13q14, and the suppressor genes as defined by the host resistance model, may be all concerned, in their function additively and without dominance, with normal differentiation of the embryonic retinal cells. For details, see Hum. Genet. **56**: 53–58, 1980.

Ectopic Pregnancy and Myoma Uteri: Teratogenic Effects and Maternal Characteristics

Ei MATSUNAGA and Kohei SHIOTA

Environmental factors responsible for congenital malformations include a variety of physical, mechanical, biochemical, chemical, and biological agents which can affect the organogenesis of an embryo. Among these, little attention has been paid by teratologists to the potential teratogenic effect of spatial restrictions imposed by an altered micro-environment surrounding the developing embryo. The most clear-cut example of this is tubal pregnancy. Even if the tube does not rupture but distends, accommodating the growing conceptus with placenta formation, conditions in the wall of the tube, which are totally different from those in the uterus, must be adverse for the development of the embryo. The same could apply, to a lesser degree, to cases of pregnancy complicated by large myomas; small myomas are generally considered to be clinically insignificant.

Morphological data from 3,614 well-preserved human embryos derived from artificial termination of pregnancy, collected by Nishimura and his associates in Kyoto University, were used to determine whether ectopic implantation or enlarged myomas could enhance the prevalence of localized malformations of the embryo. The frequency of malformed embryos was 11.6% among 43 recovered from ectopic pregnancies, 6.2% among 97 from myomatous pregnancies, and 3.3% among 3,474 from normally implanted pregnancies not complicated by myomas. Unilateral amelia in the ectopic cases and caudal dysplasia in the myoma cases were significantly increased. Both malformations were quite unusual in the control group. It is argued that spatial restrictions could be a teratogenic agent in human embryos. Mothers of both ectopic and myoma cases were on average much older than mothers of the control specimens, and those with myomas had a higher frequency of previous pregnancy wastages. Ectopic pregnancy was found to be associated with lowered parity, previous ectopic pregnancy, and maternal smoking and drinking. These associations are discussed and interpreted in relation to etiology of each of the two conditions. For details, see Teratology **21**: 61–69, 1980.

Search for Maternal Factors Associated with Malformed Human Embryos: A Prospective Study

Ei MATSUNAGA and Kohei SHIOTA

Using data from 3,474 human embryos derived from induced abortion in the Nishimura collection, various maternal factors ascertained prior to morphologic examination of the specimens were analyzed in association with the frequency of malformed embryos. The overall frequency of malformed embryos was 3.3%; polydactyly, holoprosencephaly, exencephaly with or without myeloschisis, and cleft lip accounted for 88% of the total malformations. Mothers of low parity showed an increased frequency of malformed embryos. There were nine embryos from mothers with thyroid disorders, of which one was affected with exencephaly and myeloschisis and another with holoprosencephaly and polydactyly; this finding may support the relation of maternal hyperthyroidism with anencephaly suggested by previous case reports. The frequency of malformed embryos from mothers who drank alcohol was significantly lower than from those who did not.

Several possibilities were suggested to interpret this unexpected finding. No association was verified with maternal age, irregular menstrual cycles, irradiation, contraceptive methods, maternal occupation, and a smoking habit, or with parental consanguinity. For details, see Teratology **21**: 323–331, 1980.

Inactivation Centers in the Human X Chromosome

Yasuo NAKAGOME

According to Therman *et al.* (1974, 1979) and Summitt *et al.* (1978), there is only one inactivation center in the human X chromosome and without it an X chromosome or a segment thereof cannot be inactivated. This inactivation center has been proposed to be located in the proximal portion of the Xq and the presence of two Barr bodies in cases with duplication of this segment has been offered in support of this hypothesis.

In the present study, I compiled cases with a structurally abnormal X that had been reported in the literature and compared the involved segments of X and their replication patterns to test this hypothesis. These included 17 balanced and 26 unbalanced X-autosome translocations, each with inactivation of either a derivative X or a derivative of any of the autosomes [abb. der(X) and der(A)]. A further 52 cases with various structural rearrangements were studied.

When a particular segment of X remains active, this does not necessarily exclude the presence of an inactivation center(s) within it. On the other hand, the occurrence of inactivation, even in a single case or in a small proportion of cells, suffice to prove that the segment contains an inactivation center(s). In the present study, any segments of X which replicated late were taken to be inactivated.

In a case of X-autosome translocation, a pter→q112 segment was late-replicating. According to Therman *et al.* (Hum. Genet. 50: 59, 1979), X inactivation center lies near the border of the Q-dark and the adjoining bright band which presumably correspond to that of q13 and q21. The pter→q112 segment does not include this site although it is late replicating, i.e., the short arm contains an inactivation center(s). The presence of a probable case of i(Xp) is also in support of this conclusion. In an adjacent-2 segregant of a t(X; 6) (p21; p24) translocation, the X segment of der(6) replicated late in most of examined cells. It is obvious that distal half

(pter→p21) of the short arm of X has an inactivation center and it can suppress an autosome segment about 4 times larger. These findings do not necessarily preclude the existence of another inactivation center(s) in the proximal Xp. In a case of t(X; 14) (q13; q32) translocation, a der(14) replicated late as a whole in all 25 cells examined. Therefore, a q13→qter segment of X contains an inactivation cetner(s) and it can supress the autosomal segment of the derivative chromosome. It includes the proposed site of inactivation center by Therman et al. The shortest late-replicating segment of Xq reported to date involved q25 or 26→qter. Late replicating pattern, however, was detected in only 2 out of 37 and none in 273 informative metaphases analysed by the BUdR-plus-thymidine and simple BUdR protocols, respectively. Presumably, the segment was inactivated in a small number of cells, i.e., it contains an inactivation center(s).

It appears reasonable to assume that in addition to proximal Xq, both distal short arm (pter→p21) and distal Xq (q25 or 26→qter) also include an inactivation center(s). (For further details. see Nakagome, Y.: Amer. J. Hum. Genet., in press).

The "Loss" of Kinetochore from X Chromosomes of Aged Females

Yasuo NAKAGOME and Kazuso IINUMA[1)]

In 1974, Eiberg described a technique called Cd band which stained two small dark spots on both sides of a centromere (Kinetochore). Later, it was shown by Nakagome *et al.* that the presence of Cd-positive spots corresponded to that of a functioning centromere and that inactivation of the latter was associated with the loss of Cd spots (Clin. Genet. **9**: 621, 1976; Ann. Rep. Nat. Inst. Genet. **30**: 97, 1980). The results were confirmed by both Daniel (1979) and Maraschio *et al.* (1980). On the other hand, it has been known that the proportion of hypodiploid cells significantly increases in aged individuals. According to Fitzgerald and McEwen (1977), an X chromosome(s) of some elderly humans tends to separate prematurely at the centromere.

In the present study, it was attempted to know if X chromosomes of aged individuals lost function of their centromeres. Metaphase spreads were obtained by the standard leukocyte-culture technique, from two females both aged 81 years. Cd-band technique was that described by Eiberg

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(1974). In some of preparations, chromosomes were identified by DAPI staining and then stained with Giemsa.

In a few percent of metaphases, one of two X chromosomes assumed an appearance of two parallel bars without showing any constriction at the site of kinetochore. In a small number of cells, both Xs showed parallel appearance. The standard C-band technique (CBG) revealed small dark spot on each parallel bar at or near the site of inactivated kinetochore. On the other hand, Cd band revealed positive spot in none of the parallel bars (X).

It appears that in old women, an X chromosome(s) tends to lose Cd-band positive material which is associated with the loss of the function of a kinetochore.

High-resolution Banding. A Very Simple Procedure

Yasuo NAKAGOME and Takako MATSUBARA

The advent of banding techniques has made it possible to identify individual chromosomes or a part of it involved in various structural rearrangements. However, a still higher degree of resolution is urgently needed as each band contains hundreds of genes and it is reasonably expected that many of small structural rearrangements remain to be detected due to lack of resolving power of the present banding techniques.

Yunis (Science **191**: 1268, 1976) and Yunis *et al.* (Hum. Genet. **49**: 291, 1979) developed a technique of "high-resolution" banding which made it possible to increase the number of bands per haploid set from about 300 to 500–1000. However, it involves amethopterin black and later release by thymidine-rich medium. The entire process, including centrifugation, has to be done at 37°C with utmost cautiousness, and still results are not always satisfactory.

In the present communication, a technique of high-resolution banding is described. It is very easy to do, very effectual and reproducible.

Methods: Leukocytes were cultured for 72 hours by the standard technique. Either RPMI 1640 or Eagle's minimum-essential medium (15% fetal calf serum, half of it can be replaced with newborn calf serum) was used. One hour prior to the harvest, colcemid (0.025–0.03 microgram/ml) was added to the cultures. Thirty minutes later, acridine orange (20 microgram/ml) was further added (Chroma, dissolved in Hanks' solution).

Techniques of harvest was as usually we do except that hypotonic treatment was 15–20 min (0.075 M KCl) rather than 6–7 min in our standard technique. Air-dried slides were aged for about 10 days at room temperature (3 to 4 days at 60°C), treated with trypsin (0.05% in Hanks' balanced salt solution) for about 10 sec and then stained with Giemsa.

Mitotic index of the preparations thus obtained was somewhat less than that in control preparations without acridine orange. Chromosomes showed various extent of elongation, the total number of bands ranging from 300 per haploid set to about 700 or up. The majority of mitotic figures showed 450–500 bands/haploid set and roughly corresponded to what was obtained in our laboratory by the Yunis' method. Further details will be published elsewhere (MATSUBARA and NAKAGOME, in preparation).

Preservation of Whole Blood for Chromosome Analysis

Yasuo NAKAGOME, Tsunehiro YOKOCHI,[1] Takako MATSUBARA, and Fumio FUKUDA[1]

During the course of chromosome studies on parients with congenital abnormalities, it is sometimes necessary to start additional blood cultures. The initial culture is usually made by the standard technique, however, more and more banding techniques require the treatment of cells while they are in culture. Examples include DNA-replication study, the detection of sisterchromatid exchanges or fragile sites, and high-resolution banding. Additional cultures would also be needed upon the development of new banding techniques which may be useful in the definitive diagnosis of "inconclusive" cases and upon failure of the initial culture.

We now describe a technique which facilitates leukocyte cultures using blood samples kept in a freezer for months.

Up to 10 ml of heparinized blood was obtained from 12 normal individuals and 3 with various chromosome abnormalities. Blood (1 ml) was poured into several centrifuge tubes, the remaining blood was used for control cultures. To each of the tubes we then added 1 ml of culture medium (RPMI 1640) supplemented with 20% fetal calf serum (FCS), 50 mcg/ml Kanaymcin and 20% dimethyl sulfoxide (DMSO). DMSO had been autoclaved and stored in refrigerator until its addition to the medium. The tubes were kept in a freezer (−80°C) for up to 12 weeks.

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To start the cultures, the tubes were thawed in a 37°C water bath, 10 ml of culture medium containing 10–15% calf serum were added and the tubes were centrifuged (1,000 rpm, 5 min). The sediment was rinsed with the same amount of culture medium, transferred to a culture flask and 10 ml of culture medium containing 20% FCS, 50 mcg/ml Kanamycin and 0.3 ml of phytochemagglutinin were added. Then the flasks were placed in an incubator (37°C) for 72 hours. Chromosome preparations were made by the standard technique.

We froze 64 tubes from the 12 normal individuals and three tubes from the 3 individuals with chromosome abnormalities. Of 64 cultures from normal individuals, 56 (88%) were successful. In most of these, chromosome preparations contained numerous metaphases, many of which were suited for karyotyping. The mitotic indices of the successful cultures ranged from 1.7% to 11.9%, the average was 6.5% (control, 5.2±1.3%). The cultures were also successful in all 3 cases with chromosome abnormalities (For further details, see Nakagome *et al.* Cytogenet. Cell Genet. in press).

Two Cases of Down Syndrome with Unusual Karyotypes— A psu dic(21) (q22) and a rob (14q21q) in Cousins, and Maternally Transmitted Extra ring (21)

Takako MATSUBARA, Yasuo NAKAGOME, Kazuso IINUMA,[1]
Shigehiro OKA,[2] Nobuaki OGASAWARA,[3]
and Tsunehiro YOKOCHI[4]

Familial occurrence of Down syndrome is rare except for those caused by either translocation or mosaicism of one of the parents. A girl with typical Down syndrome had 46, XX, −21, +psu dic(21) (pter→cen→q22: q22→pter) karyotype. A maternal cousin was also Down syndrome with 46, XY, −14, +rob(14q21q) karyotype. It is noteworthy that two different types of structural rearrangement arose in a family both being *de novo*. According to Hagemeijer *et al.* (Hum. Genet. **38**: 15, 1977), the triplication of the 21q22 band is essential for the phenotype of Down syn-

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drome. The triplication of the most distal part of it, however, may not be necessary (Poissonnier *et al.* Ann. Genet. **19**: 69, 1976; and Raoul *et al.* Ann. Genet. **19**: 187, 1976). The psu dic(21) (q22) case was trisomic for no. 21 except for the most distal part of q22 band and showed typical features of Down syndrome in agreement with the literature.

The trisomy on account of the extra ring chromosome is also extremely rare (Cantu *et al.* Ann. Genet. **18**: 193, 1975). In most cases with a ring, one of the normal chromosome pair is substituted by the ring. A boy with typical Down syndrome had 47, XY, +r(21)(p11?q22) karyotype and elevated SOD-1 (the erythrocyte superoxide dismutase-1) activity. To our surprise, the mother showed 46, XX, r(21)(p11?q22) karyotype. She had normal phenotype with no sign common to the r(21) syndrome. SOD-1 activity was within normal limits. The analysis of chromosomal heteromorphism revealed a normal 21 chromosome in addition to the r(21) was transmitted from the mother to the son. The proposed SOD-1 locus is either in sub-band 21q22.1 (Sinet *et al.* Exp. Cell Res. **97**: 47, 1976) or in band 21q21q (Leshot *et al.* Hum. Genet. **57**: 220, 1981). SOD-1 activity and phenotype-karyotype correlation of the proband and the mother were compatible with the presence of break point close to the distal end of 21q22, i.e., the ring was, infact, r(21)(p11q22). (For further details, sse Matsubara *et al.* Jap. J. Hum. Genet. **26**: 55, 1981; and Matsubara *et al.* in preparation).

XI. BEHAVIORAL GENETICS AND ECOLOGICAL GENETICS

A Parasitic Wasp of *Drosophila*

Masaoki KAWANISHI and Takao K. WATANABE

One female of a *Trichopria* species (Diapriidae) was collected from a banana trap put in Katusnuma in 1978. This species name is still unknown but this wasp was found to oviposit in pupae of many *Drosophila* species. Ten *Drosophila* females were introduced into a vial with males and let them lay eggs for a day. When the eggs became to the late larvae (5 to 9 days after the egg laying), three wasp females aged 4 days with males were put into the vial and left for one day. Ten different species of *Drosophila* were examined for the host of the wasp. The numbers of *Drosophila* flies and wasps emerged from each vial were counted every day. Uninfected *Drosophila* flies developed in regular days and the wasp's development was delayed a few days (18.3 days for males and 20.5 days for females). The sex ratio of the wasps was distorted to female side significantly. The percent of wasp among the emerged host and parasites varied from 69% of *D. ananassae* vial to 18% of *D. auraria* vial. It was 61% for *D. melanogaster* and 37% for *D. simulans*, though they are closely related sibling species. This wasp did not lay eggs on larvae but on pupae exclusively and the size of pupae was not the determinative factor. The most probable factor seems to be the site *Drosophila* pupates. Highly infected *Drosophila* species tend to pupate remote from food such as the wall of vial. The wasp may prefer *Drosophila* pupae rather dry. The preference was examined from the view point of the coexistance of a sibling pair, *D. melanogaster* and *D. simulans*, in a cage population. Equal number of both species were introduced into four population cages. The parasitic wasps were contaminated into two cages and the other two cages were maintained without wasps (control). *D. simulans* flies were finally eliminated from the latter cages at 145 days as reported by many workers (see Kawanishi and Lee, 1978 Jap. J. Ecol. **28**, 213). However, the former cages have maintained *D. simulans* until the end of experiment (225 days). This suggested that the wasps preferably attacked *D. melanogaster* pupae pupating at remote sites from the food which resulted in the decrease of *D. melanogaster* to a good

balance of both *Drosophila* species in the cages. During the experiment numbers of *Drosophila* flies and wasps fluctuated alternately. The reason is probably as follows: When the wasp increases in number its food (*Drosophila* pupae) is limited, then the wasp's number reduces inevitably which allows *Drosophila* flies a chance of population growth. These results suggest the natural balance of animals not only between competing sibling species but also between food-chain-species.

Development of Resistance to Organophosphorous Insecticides in *Drosophila*

Yutaka INOUE

Drosophila melanogaster from a wineyard population showed a large variation in susceptibility to organophosphorous insecticides, Diazinon and Sumithion, though the average wild flies were more resistant to the insecticides than the laboratory flies probably due to an extensive use of these insecticides. The wild strains showed a significantly positive correlation in the susceptibilities to Diazinon and Sumithion ($r=0.294$, d.f.$=86$, $p<0.01$). This may be ascribed either to the application of both Diazinon and Sumithion in the field, or to the development of mutual cross-resistances. Flies cultured by the food containing Diazinon (30 ppm) or Sumithion (45 ppm) for 24 generations became resistant to each insecticide about threefold or twofold, respectively. The development of cross-resistance to the other insecticide was confirmed though it delayed 12 generations as shown in Table 1. Under the normal food these insecticide-resistant flies

Table 1. The development of Diazinon- and Sumithion-resistance after 12 and 24 generations of treatment

Population	Diazinon-resistance		Sumithion-resistance	
	12 gen.	24 gen.	12 gen.	24 gen.
Control	1.000±0.033	1.000±0.112	1.000±0.009	1.000±0.080
Diazinon treated (30 ppm)	1.276±0.024***	2.825±0.098***	0.969±0.013*	1.523±0.075***
Sumithion treated (45 ppm)	0.695±0.034***	1.088±0.116	1.019±0.007	1.921±0.059***

* Significant at 5% level.
*** Significant at 0.1% level.

showed significantly lower fecundity than the control (trade-off), although there were no differences in viability. An interesting phenomenon must be noted that flies from the insecticide-treated populations showed a significantly higher speed in development even in the normal food. It can be speculated that the use of insecticide selected *Drosophila* which can develop faster to get rid of insecticides in food.

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 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 (cf. Ann. Rep. **25**: 85). Careful attention has been paid to keep the same inbreeding coefficient in both selected lines.

The data for the seventh selected generation were obtained in this year. They indicated that the cumulative genetic gain for the average of males and females was 16.3, and the difference in avoidance between the DAR High line and the DAR Low line (High line minus Low line) was 25.8, while the difference in discrimination was −0.5. These results suggested that the selection for higher 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

Each full sib family of mice was divided into two groups; one was continuously reared under experimental conditions with noise treatment, while another was reared without noise treatment as a control. They were kept at 25°C with 12 hour light and 12 hour darkness. To the noise treatment group, noise (pink, 100 phon) was applied six times 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 were measured

with an automated Y-type maze apparatus at approximately 60 days old as well as their activity and body weight.

The results of the first generation obtained this year showed that there were significant differences in activities and body weights between the treated and control groups, that is, the noise reduced body weight and increased activities of mice. These results indicated that the effect of noise might arouse the emotionality of mice.

Niche Differentiation between Perennial and Annual Types within Populations of *Oryza perennis*

Hiroko MORISHIMA and Yoshio SANO

Thirty six populations of wild rice, *Oryza perennis* Moench, collected in India and Thailand, were studied on the relationship between perenniality and habitat condition. In general, the perennial type was found in deep swamps which could remain moist in the dry season and relatively stable throughout the year, while the annual type was found in shallow, temporary swamps which were parched in the dry season and more frequently disturbed by man. There was a tendency for the perennial type to be associated with perennial companion species, and for the annual type with annual companion species. Intermediate perennial-annual types were found in or around rice fields and did not seem to be fully adaptive in truly natural habitats (Morishima *et al.* 1980).

Two populations in which the perennial and annual types grew side by side were studied in some more details to look into microhabitat separation. Progeny lines from the collected seeds were tested in an experimental plot with automatic shortday control for morphological traits and zymograms of peroxidase, acid phosphatase, and phosphoglucose isomerase. In one population found in a swamp near Bhubaneswar, Orissa, India, the plants at the shallow-water periphery were an annual type, and those in the deep-water center were a perennial type. In the other population found in a roadside ditch near Chiangrai, Thailand, the plants collected from a strongly disturbed spot were annual while those distributed nearby were perennial to intermediate perennial-annual types, although habitat separation between them was not so clear as observed in the Bhubaneswar population.

In both populations, the annual plants so categorized by their low regenerating ability of excised stem segments flowered earlier and produced

more seeds of larger size giving a higher reproductive effort than the perennial plants. They also had other characteristics of the annual type, and appeared to be an *r*-strategist as compared with the perennial plants which tended to be a *K*-strategist, as discussed by Oka (1976). The genetic distances between the coexisting annual and perennial plant-groups, estimated from the data for 8 isozyme loci, were quite small and indicated their close relationship. They seem to have become differentiated in their present habitat in response to different modes of selection, as was pointed out in *Veronica peregrina* in vernal pools (Linhart 1976).

The perennial and annual types differ in niche preference. If disruptive selection is strong enough, sympatric differentiation may take place even within a single gene pool. Microhabitat separation would depend on how the impact of selection is localized and predictable. Major agents of *r*-selection for the annual type would be drought stress and disturbance in the dry season.

Effect of Experimental Soil Pollution on Weed Communities in Rice Fields

Hiroko MORISHIMA

Copper sulphate solution was applied to a concrete bed filled with field soil and planted to rice in order to study the effect of soil pollution on weed community. The application of copper was repeated several times since 1978, and the soil copper content measured in 1980 was 110 ppm. Two strains of barnyard grass, *Echinochloa crus-galli*, one being copper tolerant and the other non-tolerant, were sown in a 1:1 mixture in the 1978 season. Since then, rice was planted every year, but no weeding was made. An observation of the summer weeds in 1980 showed that barnyard grass dominated over other weed species in the copper plot while in the control plot, several weed species coexisted more equitably and the copper-tolerant strain of barnyard grass disappeared as replaced by the non-tolerant strain. The non-tolerant strain had a higher competitive ability than the tolerant strain (Morishima and Oka 1977). The trend of species diversity to decrease in response to pollution was also observed in the winter weed community. In winter, *Poa annua* was dominant in the copper plot. Total weed biomass per unit area tended to be greater in the copper-treated than in the control plot.

A herbicide, Propanil, has been applied successively in an experimental field since 1974. Soil samples were taken from the Propanil-treated and control (non-weeding) plots, and the plants raised from the soil-buried seeds were observed. In the treated plot, *Lindernia procumbens* obviously dominated over other species, but its domination was not distinct in the control plot. Total plant number as well as total biomass per unit area showed no significant differences between the treated and control plots.

The experiments thus indicated that soil pollution affects the structure of weed community resulting in a decrease of species diversity and dominance of certain species, although it does not affect the overall growth of weeds. This is in good agreement with the author's observations in copper polluted rice fields (Morishima and Oka 1977, 1980). The evolution of tolerance in the weed species grown in the experimental plots will be investigated later.

Studies on Pioneer Trees and the Pioneer Index: Comparison between the Tropics and the Temperate Zones

Kan-Ichi SAKAI and Shinya IYAMA

It is interesting in relation to ecological genetics of natural forest as well as tree breeding to investigate the characteristics of pioneer trees in a natural forest. In the present report, pioneering ability of tree species was numerically expressed by a pioneer index which was computed so as to make correlation between linear combination of component traits and pioneering ability maximum. Two indices were computed using 27 tree species in Hokkaido, Japan and 34 in Jave Island, Indonesia, each representing temperate and tropical forests, respectively. The following 14 traits were included in the calculation, each being scored 0 to 3 or 0 to 5: (1) light demand for germination, (2) germination speed, (3) dormancy, (4) germination percentage, (5) seed production, (6) seed fillingness, (7) annual productivity of seed, (8) flight ability of seed, (9) seed size, (10) flight apparatus of seed, (11) early seed production, (12) longevity, (13) initial growth and (14) wood decay. Four traits of largest contribution among the 14 traits were initial growth, early seed production, light demand for germination and annual productivity of seed for temperate forest and light demand, flight ability of seed, early seed production and flight apparatus for tropical ones. Light demand and early seed production were common in both cases, but

early growth and annual seed production were important for temperate forests and flight ability of seed for tropical ones. Seed dormancy did not seem to have an important role to the pioneering ability.

XII. APPLIED GENETICS

Nitrogen Fixing Activity of Bacterial Strains in Association with Rice Plants

Taro FUJII

In order to investigate the nitrogen fixing activity of bacteria in combination with rice plants, pure inoculation of nitrogen fixing bacterial strain into rice seedlings was conducted. Rice strain C5444 which showed high nitrogen fixing ability was used as a host plant. Six nitrogen fixing bacterial strains so far isolated from the rhizosphere soil of C5444 was used for the inoculation experiments. Pure culture of each bacterium was inoculated into the rhizosphere soil of germ free rice seedlings which were grown in a test tube filled with sterilized soil and reached two months old. Acetylene reducing activity was measured every two weeks after inoculation. No activity was observed in a seedling without inoculation. Plants inoculated with bacterial strains COC-8 and NG-13 showed appreciable acetylene reducing activity three months after inoculation. Plants inoculated with other four bacterial strains, however, did not show the activity during repeated measurements up to four months after inoculation (Table 1). From these results, the followings were concluded: Though various nitrogen fixing bacteria were existing in the rhizosphere soil of rice, only particular strains among them were able to manifest nitrogen fixing activity in association with rice roots, suggesting the importance of interaction between genotypes of bacteria and rice in regulation of "symbiotic" nitrogen fixation.

Table 1. Acetylene reducing activity of various nitrogen fixing bacteria inoculated into germ free rice rhizosphere*

Date of measurement	Control (no inoculation)	*Clostridium*	C0-C-8	NG-13	0-0-2	0-20	N-SG-47
May 6	0	0	1.5	0	0	0	0
May 14	0	0	3.6	2.3	0	0	0
May 23	0	0	8.4	2.8	0	0	0

* Acetylene reducing activity was expressed by the amount of C_2H_2 produced (nmol/h/plant).

Fruther Evidence for "One Locus Sporo-gametophytic Interaction" Model for F_1 Sterility Genes between *Oryza sativa* and *O. glaberrima*

Yoshio SANO

As reported previously (Ann. Rep. 30: 113), isogenic F_1-sterile lines having the genetic background of *O. sativa* and *O. glaberrima* parent were isolated from B_8F_2 plants, respectively. Experiments with those lines indicated that the F_1 sterility between the two species could be explained by a "one locus sporo-gametophytic interaction" hypothesis, which assumes that the parental species has S^aS^a and SS, respectively, and when a S gene is present in the maternal tissue, gametes with S^a deteriorate. To test this hypothesis, an experiment was carried out as follows:

An isogenic line having gene S_3 (from *O. glaberrima*, W025) in the genetic background of Taichung 65 (*O. sativa*) was crossed with another isogenic line of Taichung 65 having the glutinous gene (*wx*). The glutinous *vs.* non-glutinous character of pollen grains is testable by iodine reaction, and heterozygotes for the locus (+/*wx*) are expected to produce the two kinds of pollen grains in a 1: 1 ratio. In the F_1 plants between S_3/S_3^a and +/*wx*, however, pollen grains with *wx* were found to be eliminated by gametic selection. Out of 3,780 pollen grains observed, only 128 were glutinous. This indicates that S_3 and *wx* were closely linked and the *wx* gene was eliminated together with the S_3^a gene. In the selfed progeny, heterozygotes with S_3—*wx*/S_3^a—+ were obtained. As expected, the heterozygotes showed only 107 non-glutinous pollen grains out of 2,845 observed. The recombination value between S_3 and *wx* was calculated from the data to be 0.035.

The experimental result strongly supports the hypothesis that the F_1 sterility is controlled by sporo-gametophytic interaction of alleles at a certain locus. The data so far obtained indicate that there are a number of such F_1-sterility gene loci between *O. sativa* and *O. glaberrima*. Possibly, such allelic changes have been accumulated during the porcess of species differentiation. Their fixation will be rapid because of strong gametic selection, which would also affect the genic content of the chromosomes which carry such sterility genes. This type of sterility genes may be regarded as "selfish" genes.

The Direction of Pollen Flow between Two Cultivated Rice Species, *Oryza sativa* and *O. glaberrima*

Yoshio SANO

Oryza glaberrima is endemic in West Africa, while *O. sativa* is of tropical Asian origin. Prior to the introduction of *O. sativa* into Africa, the cultivation of *O. glaberrima* was well estabilshed (Porteres 1950). *O. sativa*, after being introduced, spread across the West African rice zone with selection for adaptability. At present, the two rice species are often grown in mixture in farmers' fields. They can be distinguished only by a few distinct characteristics like short and tough ligules and few secondary panicle branches of *O. glaberrima* which make contrast with long ligules and many secondary panicle branches of *O. sativa*. Although their F_1 plants are highly pollen-sterile, backcrossing is possible with the pollen grains of either parent. During the 1977 study-tour in West Africa, I have observed a number of F_1 plants in rice fields where the two species were planted in mixture. A basic problem that needs to be solved is whether or not introgression between the two species has played a role in their adaptation to the growing conditions in West Africa. I herewith report the result of an experiment on the extent of natural hybridization and which parent contributes its pollen in natural hybridization.

The seeds of coexisting plants of the two species, sampled from an upland and a lowland field in northern Nigeria, were used for the present experiment. The material consisted of two pairs of a *sativa* and a *glaberrima* strain. In 1978, each pair of strains was planted in a 1:1 mixture, in a concrete bed with automatic shortday control, with a spacing of 10×10 cm. They flowered from late July to early August and their flowering periods overlapped one another. Their seeds were harvested randomly from about 20 plants of each strain. In 1980, about 800 plants per strain were tested for the percentage of F_1 plants between the two species, which were easily distinguished by the occurrence of long awns, pollen sterility, and an intermediate ligule shape. Hybrid plants were observed in both strains of *O. glaberrima* with a relatively high frequency (8.9% in G_1 and 1.6% in G_2). In contrast, no hybrid was found in either strain of *O. sativa*.

The result of this experiment indicates that when introgression takes place, pollen flow occurs from *O. sativa* to *O. glaberrima*. This might be due to differences in flowering behavior and in the amount of pollen prod-

uced. If the hybrids were backcrossed successively by *O. sativa*, the resulting plants would have genes of *O. sativa* in the cytoplasm of *O. glaberrima*. Crossing experiments to detect cytoplasmic differences are in progress.

Mendelian Segregation Analysis for Three Isozyme Loci in Rice Cultivars

Gerard SECOND and Hiroko MORISHIMA

Three F_2 populations involving five parental strains of *Oryza sativa* were analysed at the germination stage for the segregation of two contrasting electromorphs which were presumed to be specified by the *Pgi*-A, *Pgi*-B, and *Cat*-A loci, respectively. Two of the crosses also involved glutinous and non-glutinous parents. As shown in Table 1, the observed segregation patterns were in good accordance with the presumed genic systems giving 1:2:1 ratios. In one of the crosses, a distorted ratio was observed for the

Table 1. F_2 segregation for *Pgi* and *Cat* electromorphs

Locus & Cross		P_1 type	F_1 type	P_2 type	χ^2
Pgi-A	1	54	88	46	1.45
	2	39	65	39	1.18
	Total	93	153	85	2.27
Pgi-B	1	52	88	48	.94
	2	31	79	31	2.05
	3	8	22	12	.93
	Total	91	189	91	.13
Cat-A	1	34	55	29	.97
	2	44	74	20	9.07*
	3	9	12	8	.93
	Total	87	141	57	6.34*
wx	1	75	204		.52
	2	72	255		1.55
	Total	147	459		.17

* χ^2 for 1:2:1 ratio, significant at 5% level.

Cross 1: Acc. 130 × Acc. 221—(*Pgi*: $A_1 B_2$; *Cat*: A_1; Non-waxy) × (*Pgi*: $A_2 B_1$; *Cat*: A_2; Waxy),

2: Acc. 143 × Acc. 221—*Ibid.*

3: T65 × C5444—(*Pgi*: A_2B_1; *Cat*: A_2) × (*Pgi*: A_2B_2; *Cat*: A_1).

Cat-A locus, which is not unusual in crosses between rice cultivars. No linkage relationship was found between any of the four loci investigated.

An Introgressed Electromorph Found in an Isogenic Line of *Oryza sativa* with a Sterility Gene from *O. glaberrima*

Gerard SECOND and Yoshio SANO

Two isogenic lines of *O. sativa* with a sterility gene introduced from a strain of *O. glaberrima* were analysed for phosphoglucose isomerase (PGI) loci at the germination stage. The isogenic lines had been isolated from B_8F_2 populations using Acc. 108, an Indica strain of *O. sativa* as the reccurent parent (Sano, Chu, and Oka, Jap. J. Genetics **54**: 121–132). The parents, Acc. 108 and W025, and the F_1 plants between the isogenic lines and Acc. 108 were examined. According to Second and Trouslot (ORSTOM, TD 120, 1980), the genic interpretations for *Pgi* zymograms of Acc. 108 and W025 were A^1/A^1 B^2/B^2 and A^3/A^3 B^1/B^1, respectively. One of the isogenic lines was like the *O. sativa* parent but the other had A^3 and B^2 alleles in homozygous state, suggesting that A^3 had been introgressed from *O. glaberrima*. The zymogram observed in this isogenic line is unique since the combination of genes A^3 and B^2 has never been found in any rice strain. The A^1 and A^3 electromorphs exhibit only a small difference in their PGI mobilities. The F_1 hybrids between the isogenic line and Acc. 108 showed a band located in between A^1 and A^3 and slightly broader than the parental bands. It may be presumed that *Pgi-A* locus is located on the chromosome on which a sterility gene from *O. glaberrima* is also located. Recombination between them was detected in the F_2. Further investigation of the F_2 population is in progress.

Simulation Experiment on the Mass Selection for a Quantitative Character When the Effect of Genes is not Equal

Shinya IYAMA

Computer simulation was performed in order to visualize the effect of mass selection on the change of gene frequency when the effects of genes affecting a quantitative character were not equal. The results showed that the magnitude of additive effect of the genes and the joint effect of dominance and gene frequency had important roles in the response of the individual

genes to the selection. When the genes had not equal effect, selection for the genes having small effect was delayed compared with the genes of large effect. Similarly. when selected from the low gene frequency together with non-recessive genes, recessive genes stayed at the initial level of low frequency longer than other genes. Since those genes of slow response to selection stayed at low gene frequency longer, they were likely to be under serious risk of being lost from a population due to random genetic drift when the population was relatively small. It was suggested from the results that population size should be chosen carefully to avoid the loss of genes, especially ofr those genes as of small effect or recessive.

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ABSTRACTS OF DIARY FOR 1980

January	14	157th Biological Symposium
	21	264th Meeting of Misima Geneticists' Club
	24	265th Meeting of Misima Geneticists' Club
February	2	266th Meeting of Misima Geneticists' Club
March	14	158th Biological Symposium
	17	159th Biological Sumposium
	27	160th Biological Symposium
	28	267th Meeting of Misima Geneticists' Club
April	15	268th Meeting of Misima Geneticists' Club
May	12	269th Meeting of Misima Geneticists' Club
	20	161st Biological Symposium
June	28	162nd Biological Symposium
July	17	163rd Biological Symposium
	31	164th Biological Symposium
August	13	165th Biological Symposium
September	1	166th Biological Symposium
	6	167th Biological Symposium
	27	168th Biological Symposium
October	14	169th Biological Symposium
November	12	170th Biological Symposium
		171st Biological Symposium
	13	172nd Biological Symposium
	28	173rd Biological Symposium
December	18	174th Biological Symposium

FOREIGN VISITORS IN 1980

January	14	CHAPEVILLE, François, Universite Paris VII, France
	14	TSUGITA, S., European Molecular Biology Laboratory, West Germany
	31–Feb. 15	NEUNGTON, Neelobol, Mahidol University, Thailand
February	21	TORREY, J. G., Harvard University, U.S.A.
March	12–14	STICH, F. H., B.C. Cancer Research Center, Canada
	17	GEORGIEV, G. P., Institute of Molecular Biology, U.S.S.R.
	21–Apr. 9	CROW, James F., University of Wisconsin, U.S.A.
	20	LEGAY, J. M., Universite de Lyon, France
	27	NIERHAUS, Knud, Max-Planck-Institut für Molekulare Genetik, West Germany
April	1–3	INOUE, M., State University of New York, U.S.A.
	9	ANDERSON, E. S., Central Public Health Laboratory, England
	14–15	NAORA, H., Australian National University, Australia
May	1–Aug. 6, '81	SECOND, Gerard, Office de la Recherche Scientifique et Technique Outer-mer, France
	1–Apr. 30, '82	ACHERMANN, Joseph, Universität Zürich, Switzerland
	4–May 12, '82	KECK, Wolfgang, Max-Planck-Institut fur Virusforschung, West Germany
	12–Jul. 11	ZAW, Khin Maung, Bio-Medical Research Center, Burma
	16	ALTUKHOV, Yu. P., Institute of General Genetics, U.S.S.R.
June	28	David, Jean R., Laboratoire de Biologie et

		Genetique Evolution, France
July	4–5	OHTSUBO, E., State University of New York, U.S.A.
	15–Sept. 15	WU, Hsin-Kan, Institute of Botany, Academia Sinica, Taiwan
	16	LEVAN, Gören, University of Gothenburg, Sweden
	30	SHIBATANI, A., Commonwealth Scientific and Industrial Research Organization, Australia
August	1–2	RASMUSSEN, Søren Wilken, Carlsberg Laboratory, Denmark
	2	GARAL, Jean-Pierre, Universite Claude Bernard Lyon-1, France
	1	FESTING, Michael F. W., Medical Research Council Laboratory Animals Centre, England HEDRICH, Hans. J., Zentralinstitut fur Versuchstiere, West Germany KOFFMAN, Harold A., National Institutes of Health, U.S.A. MOBRAATEN, Larry, The Jackson Laboratory, U.S.A. MOUTIER, R., Centre de Selection et d'Elevage des Animaux de Laboratoire C.N.R.S., France RADZIKOWSKI, Cz., Polish Academy of Sciences, Poland
	9	KRISHNAMURTHY, N. B., University of Mysore, India CHOWDAIAH, B. N., Bangalore University, India
	9–10	CHEN, P. S., Universität Zürich, Switzerland
	13–14	DÜBENDORFER, A., Universität Zürich, Switezrland
September	1–2	De SERRES, F. J., Michael D. Shelby, National Institute of Environmental Health Sciences, U.S.A. MENDELSOHN, M. L., Laurence Livermore Laboratory, U.S.A.

	5–7	SHREFFLER, Donald C., Washington University, U.S.A.
	13	JIANG, Tong-Qing, South-West Agr. Coll., China
	18–Sept. 16, '81	WANEK, Nancy, University of California, U.S.A.
	29	PAUN, Yen-Xang, South China Inst. of Tropical Crops. China
October	2–Mar. 31, '81	PAI, Chian, Nat. Chung-Hsing University, Taiwan
	14–15	MOSCONA, A. A., University of Chicago, U.S.A.
	16	HERZENBERG, L. A., Stanford University, U.S.A.
	20	FRANKEL, O. H. and W. J. PEACOCK, Commonwealth Scientific and Industrial Research Organization, Australia
	27	HSIEH, Sung-Ching, Taiwan Agr. Res. Inst., Taiwan
		PERNES, J., C.N.R.S., France
	28	XU, Yuntian, Inst. Crop Germplasm Resources, China
November	6	POULSEN, E., National Food Institute, Denmark
	12	De NAVA, C. C., Universidad Nacional Autonomade Mexico, Mexico
	12–13	GILES, Norman H., University of Georgia, U.S.A.
	13–14	OHNO, S., City of Hope National Medical Center, U.S.A.
	18	RILEY, Ralph, Agricultural Research Council, England
	27	SANDBERG, A. A., Roswell Park Institute, U.S.A.
December	18–19	MARKERT, C. L., Yale University, U.S.A.

AUTHOR INDEX

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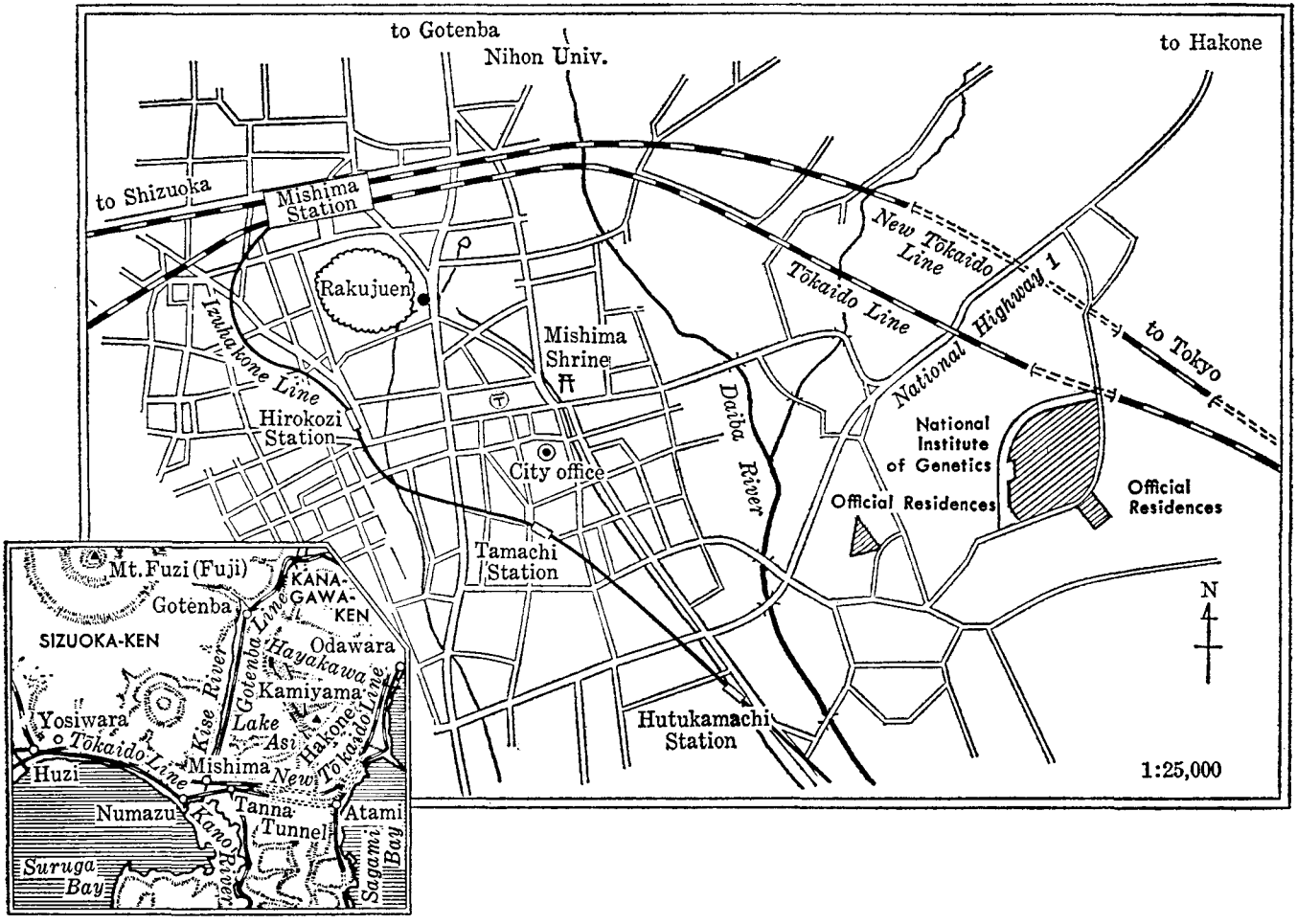
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Tōkaido Line
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Huzi
Numazu
Tanna Tunnel
Atami
Kano River
Suruga Bay
Sagami Bay