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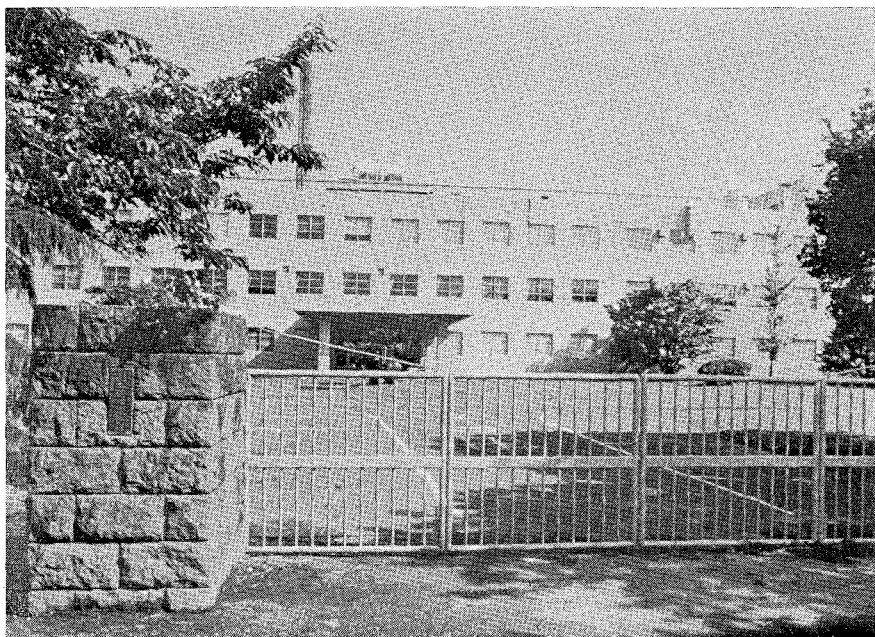
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No. 27, 1976



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## GENERAL STATEMENT

During the past 27 years since the foundation of the Institute, a large number of scientific papers have been published by our staffs and various prizes have been awarded to them from several learned societies. This year an Order of Cultural Merits, one of the most glorious awards in Japan, was conferred upon Dr. Motoo Kimura, the Head of the Department of Population Genetics. As a pioneer of the population genetics in this country, Dr. Kimura has contributed a number of valuable theoretical papers. His article on neutral theory in evolution which was proposed on his penetrated insight into the random modification of genetic code has been highly evaluated in and outside Japan and won the great honor for the first time in this new field of genetics.

I realize that it is not easy to accomplish distinguished research works, but taking this opportunity, I would like to request earnestly to our staff members to follow him in contributing excellent papers of full originality. Hopefully, they should keep in mind that the government has been investing a huge amount of money on their studies.

Genetics, being a nucleus of biological sciences, has developed at surprisingly quick tempo. Among others it is of great importance to recognize that recombination of DNA molecules has become technically feasible between different species. The desire to create new superior organisms by combining genes from different species had long been a subject of biology, and gave birth to the science of genetics. I cannot help exciting when I think over that man has just developed a technique for realizing his long cherished desire. Needless to say this technique offers a very promising prospect for practical application. I should like, however, to emphasize the importance of this technique in the field of pure genetic researches. For instance, mechanisms of gene action can be analyzed at biochemical level if genes of higher organisms could be cloned by connecting them with bacterial plasmids. This will undoubtedly bring about in near future a revolution in the methodology of genetic studies. Though there are many problems to be solved by that time, we realize that genetic studies have entered in a new era.

The Institute held two international meetings in the fiscal year of 1976: one was a symposium on "Molecular Evolution and Polymorphism" as

the Second Taniguchi International Symposium on Biophysics organized by Dr. M. Kimura, and the other was East Asian Training Course on Mutagenicity Testing of Chemicals organized by Dr. Tsuneo Kada. The former symposium, being participated by reputed scientists from several countries, discussed strategies for further advancement of genetic studies. The latter aimed to provide senior scientists of East Asian countries with a balanced comprehensive overview of chemical mutagenesis and mutagenicity testing of chemicals. There were 14 participants and 22 lecturers from six countries. I am convinced that both meetings will prove to be significant for the future development of our science.

As regular annual events, the Institute was opened to the public on April 17th, having as many visitors as we had last year. Public lectures were delivered on November 13th at the lecture hall of the National Science Museum in Tokyo. Speakers and topics were: Dr. Hideho Suzuki, Department of Microbial Genetics, "Regulation and flagella formation in bacteria," and Dr. Chozo Oshima, the Head of the Department of Physiological Genetics, "Insect bioclocks." Both gave deep impression to the audience.

The purchasing program of the neighboring area has been concluded, and there will be accommodated buildings for Genetic Stocks Center. The old annex building has completely been remodelled for low level radioisotope laboratories.

*Y. Tajima*

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## PROJECTS OF RESEARCH FOR 1976

### Department of Morphological Genetics

- Genetic studies of the silkworm (TAZIMA and ONIMARU)
- Radiation and chemical mutagenesis in the silkworm (TAZIMA and ONIMARU)
- Cytogenetics in the silkworm (MURAKAMI and IMAI)
- Studies on recombination in the silkworm (MURAKAMI)
- 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)

### Department of Cytogenetics

- Studies on chromosomal evolution in rodents (YOSIDA)
- CHROMOSOME study on experimental tumors (YOSIDA)
- Experimental breeding and genetics of mice, rats and other wild rodents (YOSIDA and MORIWAKI)
- Cytogenetical study on sister chromatid exchange in mammalian cells (KATO)
- Cytogenetical and immunological studies on mouse plasma cell tumor (MORIWAKI)
- Biochemical studies on serum transferrin variations in rodents (MORIWAKI)
- Immunogenetical study on the histocompatibility antigens of wild rodents (MORIWAKI)
- Cytogenetical study of ants (IMAI)

### Department of Physiological Genetics

- Behavior genetics of *Drosophila* (OSHIMA and WATANABE)
- Analysis of deleterious and inversion chromosomes in natural populations of *Drosophila melanogaster* (WATANABE)
- Studies on fitness of *Drosophila* under controlled environment (OSHIMA and WATANABE)
- Genetic studies on the effects of adverse environments on *Drosophila* flies (OSHIMA and WATANABE)

**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)  
Effects of exogenous DNA on plant seed formation (ENDO)  
Genetic analysis of developmental mechanisms in hydra (SUGIYAMA and FUJISAWA)

**Department of Applied Genetics**

- Quantitative genetic studies in poultry (KAWAHARA and FUJISHIMA)  
Genetics studies in wild populations of Japanese quails (KAWAHARA)  
Theoretical studies on breeding techniques (IYAMA)  
Behavioral genetic studies in mice (FUJISHIMA)  
Genetic studies in natural stands of forest tree species (IYAMA)  
Simulation studies on artificial selection (IYAMA)  
Evolutionary studies in wild and cultivated rice species (OKA and MORISHIMA)  
Ecological genetic studies in some grass species (MORISHIMA)  
Genic analysis for isozyme variations in rice (ENDO and OKA)  
Genetic effects of environmental pollution on plants (IYAMA, MORISHIMA and OKA)

**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)

Mechanisms of recombination repair (SADAIE and KADA)

Mutation and differentiation studies of plant tissue culture (AMANO and KADA)

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 and SHIOTA)

Genetic studies on retinoblastoma (MATSUNAGA)

Clinical cytogenetic studies of congenital abnormalities in man (NAKAGOME and IINUMA)

Molecular cytogenetic studies of human chromosomes (NAKAGOME)

Studies on human chromosome variants (IINUMA, MATSUNAGA and NAKAGOME)

#### Department of Microbial Genetics

Genetic regulatory mechanism of DNA replication in *E. coli* (HIROTA and NISHIMURA, and YASUDA)

Genetic regulatory mechanism of cellular division in *E. coli* (HIROTA)

Molecular genetics on DNA replication (YASUDA and HIROTA)

Molecular mechanisms of cell division in *E. coli* (SUZUKI, NISHIMURA and HIROTA)

Genetics of bacterial cell envelope (HIROTA, SUZUKI, and NISHIMURA)

Molecular genetics on the regulatory mechanism of flagella formation (HIROTA and SUZUKI)

Synthetic bacterial plasmid (YASUDA)

#### Department of Population Genetics

Theoretical studies of population genetics (KIMURA, MARUYAMA and OHTA)

Studies on molecular evolution from the standpoint of population genetics (KIMURA and OHTA)

Theoretical studies on the evolution of multigene family (OHTA)

Mathematical studies on the genetics of structured populations (MARUYAMA)

Experimental studies on protein polymorphism in *Drosophila* (YAMAZAKI)

Statistical studies on protein polymorphisms in natural populations (MARUYAMA and YAMAZAKI)

Department of Molecular Genetics

Studies on the chemical structure of genome of viruses containing double-stranded RNA (MIURA, SUGIURA, SHIMOTOHNO)

Studies on the interaction between RNA polymerase and template nucleic acid (MIURA, SUGIURA, SHIMOTOHNO, and SOEDA)

Studies on structure and function of messenger RNA (MIURA, SHIMOTOHNO)

Genetical and enzymological studies on *E. coli* RNA polymerase (SUGIURA)

Genetic Stocks Center

Studies and conservation of germplasm resources in rice and wheat species (FUJII, SANO and OKA)

On the sex expression in monoecious plants (FUJII)

Specificity of mutagen tolerance in higher plants (FUJII)

Exploitation of genetic ability of nitrogen fixation in Graminae (FUJII, SANO, IYAMA and HIROTA)

Studies on teratocarcinogenesis in mice (NOGUCHI)

# RESEARCHES CARRIED OUT IN 1976

## I. MOLECULAR GENETICS

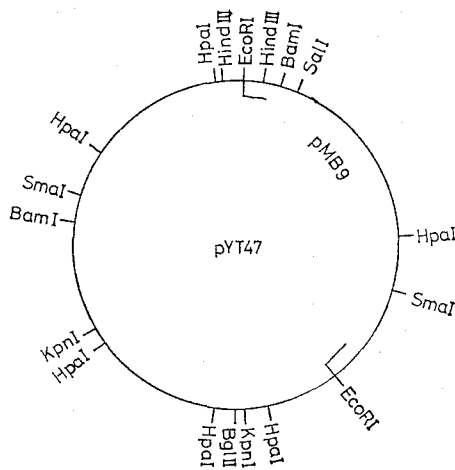
### Cloning of *dnaA* Gene in *Escherichia coli*

Yutaka TAKEDA and Yukinori HIROTA

It has been shown that the gene product of *dnaA* is required in the initiation step of *E. coli* chromosome replication. We report here successful cloning of the *dnaA* gene.

*E. coli* DNA was prepared from W3110 and fragmented by digestion with *EcoRI*. The DNA fragments were linked with a ColEI vector, pMB9 or pCRI, by T4 DNA ligase. The  $\text{CaCl}_2$ -treated cells of CRT46*recA* (temperature sensitive *dnaA* mutant) were transformed with the ligation mixtures and the transformants which became temperature and drug resistant were selected. From these transformants, two plasmids were isolated: one synthesized by ligation with pMB9 and the other with pCRI, and, referred to as pYT47 and pYT48, respectively.

The DNA of pYT47 was cleaved into two fragments by *EcoRI* digestion,





producing the linear molecule of pMB9 and the fragment originated from *E. coli* DNA. The DNA of pYT48 was cleaved similarly, but produced pCR1 instead of pMB9. The *E. coli* DNA fragments of these plasmids were identical with each other, as shown by the restriction mapping (Fig. 1) and heteroduplex analyses. Molecular weight of the *E. coli* DNA fragment obtained from either plasmid was *ca.*  $5.5 \times 10^6$  daltons. These plasmids were confirmed to contain *dnaA* by transformation, but contained no known genes around *dnaA*, such as *tna*, *asn*, *bglA*, *rbs*, and *uncA*. About 10 copies of the plasmid per chromosome were found in the wild type cells. They were able to replicate in the presence of chloramphenicol, as known in the ColE1 replicon.

### Construction and Characterization of an F-ColE1 Hybrid Plasmid

Yutaka TAKEDA, Seiichi YASUDA and Yukinori HIROTA

We have constructed an F-ColE1 hybrid plasmid by means of *in vitro* recombination between pVH51, a mini-ColE1 plasmid, and pSC138, a mini-F-ampicillin plasmid.

This plasmid, named pSY111, can replicate under conditions where the replication function of F or ColE1 is inhibited. For example, pSY111 was maintained stably in male strains or in the presence of acridine orange, although replication of F factor is known to be inhibited under these conditions. pSY111 could also replicate in a *polA1* strain or a *polA12* strain at high temperature, although ColE1 could not. When both F and ColE1 replicon were inhibited in an Hfr *polA12* strain at high temperature, this plasmid could not replicate autonomously.

We have examined the compatibility properties of pSY111. This plasmid was fully compatible with *Flac* and ColE1 as examined by transformation frequency and segregation kinetics. These results are different from that reported by Cabello *et al.* (Cabello, F., K. Timmis & S. N. Cohen. 1975 *Nature* **259**: 285), who observed that pSC134, a pSC101-ColE1 hybrid plasmid, was incompatible with both pSC101 and ColE1. The discrepancy could be due to the difference in the parental plasmids used to construct the hybrid plasmids. Cabello *et al.* have suggested that their results support the model of negative control of the initiation of DNA replication. However, the nature of DNA replication of pSY111 could not be explained by the negative control model. We conclude, therefore, that the model is

not applicable to all replicon and the detailed experiments are needed to obtain the generalized model of the plasmid replication.

### **Cloning and Mapping of the Replication Origin of *Escherichia coli***

Seiichi YASUDA and Yukinori HIROTA

The replication origin of *Escherichia coli* has been cloned on a non-replicating DNA fragment coding for ampicillin resistance. This recombinant DNA, named pSY211, replicates depending on the presence of the replication origin and can be recovered as a closed circular plasmid DNA of 10.7 Mdal. A restriction map has been constructed. *EcoRI* cleaves pSY211 into two fragments: One is the ampicillin fragment of 4.5 Mdal and the other is a chromosomal fragment of 6 Mdal, which contains the origin. The 6 Mdal *EcoRI* fragment has four *BamHI* sites, three *HindIII* sites and one *XhoI* site. A mutant of pSY211 has been isolated which is deleted of the *BamHI* fragments of the chromosomal fragment. In *recA* hosts pSY211 is lost at a high frequency. In *recA*<sup>+</sup> hosts pSY211 is integrated into the chromosome due to nucleotide-sequence homology between pSY211 and the replication origin of the *E. coli* chromosome. The integration site has been mapped. We conclude that the replication origin is located at a site between *uncA* and *rbsK*, at about 83 min on the genetic map of *E. coli*.

### **The 5'-Terminal Structure of Bacterial Messenger RNA**

Kin-ichiro MIURA and Fumio IMAMOTO

The 5'-terminal methylated, blocked structure has now been detected in eukaryotic mRNAs commonly since our discovery of this structure in mRNA of silkworm cytoplasmic polyhedrosis virus (Annual Rep. No. 25 (1974) p. 12). In order to clarify whether this modified structure is present in prokaryotic mRNA or not, the 5'-terminal structure was studied on mRNAs transcribed from tryptophan operon in *Escherichia coli* by labelling with [<sup>32</sup>P] phosphate and [methyl <sup>3</sup>H] methionine. Total RNA was extracted from the cell and fractionated in glycerol density gradient. The fractions containing mRNAs were digested with ribonuclease T<sub>2</sub> and then with *Penicillium* nuclease P<sub>1</sub>. The blocked structure NpppN was sought in the enzymatic digests by chromatographic procedures. Any blocked

structure was not found. Instead, nucleoside triphosphate pppN and diphosphate ppN were detected as the 5'-terminal nucleotides of *E. coli* mRNA.

When the [<sup>3</sup>H] methylated RNAs were fractionated by electrophoresis in polyacrylamide gel, any methylation was not detected in mRNA fractions. Ribosomal RNAs, including their precursor RNAs, and transfer RNAs were methylated.

### Mechanism of Formation of the Modified Structure at the 5'-Terminus of mRNA of Reovirus

Kunitada SHIMOTOHNO and Kin-ichiro MIURA

At the 5'-terminus of mRNA of reovirus, the modified structure including the 7-methylguanosine blocking through 5'-5'-pyrophosphate linkages and ribose-methylation at 2'-position of the first nucleotide was found (Annual Rep. No. 26 (1975) p. 12). It was expressed as m<sup>7</sup>G<sup>5'</sup>pppGm-C-C- ----. Reovirus, which contains double-stranded RNA as a genome and enzymes including RNA polymerase, phosphohydrolase and methylases, is able to synthesize mRNA carrying the 5'-terminal modification *in vitro* in the presence of a methyl-donor S-adenosylmethionine (SAM). The process of the formation of the modified structure at the 5'-terminus of this mRNA was studied and compared with the case of silkworm cytoplasmic polyhedrosis virus (CP virus) (Annual Rep. No. 26 (1975) p. 14).

The limited RNA synthesis *in vitro* was carried out with the pretreated reovirus particle in the presence of [<sup>3</sup>H methyl]SAM by controlling the amount of substrates NTP and the reaction temperature. Oligonucleotides thus produced were separated by DEAE cellulose-urea column chromatography in order of size. The rates of methylation at the 5'-terminal part in these oligonucleotides were analysed. The following conclusion was derived from these experiments: at first the confronting nucleotide structure G<sup>5'</sup>ppp<sup>5'</sup>G is constructed, then one of guanine residues is methylated at the 7 position, forming m<sup>7</sup>GpppG, and methylation at the 2' position in ribose of the other G occurs at the same time to the starting of chain elongation of RNA. The latter 2'-methylation has been performed until elongation of one or two nucleotides in the present case of reovirus, whereas three or four nucleotides elongation is required for the completion of methylation of CP virus.

In order to confirm the steps of methylation the confronting nucleotide structure without methyl groups,  $G^{5'}ppp^{5'}G$  and  $G^{5'}ppp^{5'}A$ , were prepared by chemical synthesis and added to the reaction mixture of RNA synthesis. GpppG can be a substrate for the methylase associated to reovirus, but GpppA can not. This is not conflict with the 5'-terminal structure of reovirus mRNA,  $m^7GpppGm$ . The situation in CP virus is opposite: CP virus methylates GpppA, but not GpppG. The methylation enzyme in reovirus as that in CP virus seems to recognize the substrate structure strictly and to methylate after the blocked structure is constructed. In fact, GTP in the reaction mixture was not methylated. The methyl group incorporated into GpppG by reovirus was found only in 7-position of one of guanine bases, but not in 2'-position of ribose. Here, again it is clear that methylation at the 7-position of the blocking quanosine residue is carried out at first, and the ribose moiety in the G-residue is not methylated before one or two nucleotides link to it.

#### **Modified Structure at the 5'-Terminus of Low Molecular Weight RNA in Nucleus of Animal Cell**

Kunitada SHIMOTOHNO, Toshiyuki URUSHIBARA and Kin-ichiro MIURA

In the research on the gene structure and its expression of the double-stranded RNA-containing virus, especially silkworm cytoplasmic polyhedrosis virus (CPV), a novel structure was discovered at the 5'-terminus of the messenger RNA strand. The internucleotide linkages (linkages between pentose and phosphate in a backbone chain) in a nucleic acid molecule are repetition of the 5'-3' linkage, but 7-methylguanylic acid combines to the 5'-terminal nucleotide of the mRNA strand in a confronting state between 5' and 5' of the two nucleotides, inserting two pyrophosphate linkages as follows:  $m^7G^{5'}ppp^{5'}Am-G-U-$ . The similar structure has been found thereafter for many kinds of messenger RNA (mRNA) of cells as well as viruses of eukaryote organisms. Excepting some instances, the modified structure of the 5'-terminus of mRNA seems to be common for eukaryotic organisms, and it is expressed generally as follows:  $m^7G^{5'}pppN(m)-N(m)-N-$ . The presence of such a modified structure was surveyed for other cellular RNAs.

In order to detect small amount of a modified structure in a long RNA molecule, an efficient labelling with radioisotopes is required. The culture

of the baby hamster kidney (BHK) cell in the logarithmic phase was added with [ $^3\text{H}$ ] methionine as a methyl donor and [ $^{32}\text{P}$ ] orthophosphate. It was incubated further at  $37^\circ\text{C}$  for 12 hours. After cells were disrupted by homogenizing in a hypotonic buffer, nucleus and cytoplasm were separated. RNA extracted by phenol treatment was purified by DNase and fractionated in a glycerol density gradient.

The 5'-5' confronting nucleotide structure was explored as following: At first an RNA preparation was digested with ribonuclease  $\text{T}_1$ , which hydrolyzes RNA into 3'-nucleotides except the special places containing 2'-0-methyl nucleoside and pyrophosphate linkage as the 5'-5' confronting structure. The digest was analyzed by DEAE cellulose column chromatography using 7 M urea solvent. Two kinds of oligonucleotide containing modified structure were detected for nuclear small RNA. These components were desalted and then digested with *Penicillium* nuclease  $\text{P}_1$ , which cleaves internucleotide bonds to yield 5'-monophosphate regardless of 2'-0-methyl ribose residue. The nuclease  $\text{P}_1$  digest was further incubated with phosphomonoesterase to leave only the 5'-5' confronting nucleotide structure as it is. The confronting nucleotide component was then isolated by column chromatography using anion exchange resin Bio Rad AG-1.

The confronting nucleotide structure was identified by cleaving it with tobacco phosphodiesterase, which splits pyrophosphate linkages but not the normal 5'-3' internucleotide linkages. The resulting products were analyzed.

Based on these results, the 5'-terminal modified structure of the nuclear low molecular weight RNA was concluded as  $\text{m}_3^{2,2,7}\text{G}^{5'}\text{pppAm}$ .

In the RNase  $\text{T}_2$  digest of the RNA, there were two oligonucleotides which were resistant to this enzyme. From both the oligonucleotides the same confronting nucleotide structure was detected. As the second oligonucleotide is one nucleotide longer than the first one according to the DEAE-urea chromatography, these are judged as  $\text{m}_3^{2,2,7}\text{G}^{5'}\text{pppAm-Np}$  and  $\text{m}_3^{2,2,7}\text{G}^{5'}\text{pppAm-Nm-Np}$ , respectively. Such a confronting nucleotide structure was detected only in the nuclear low molecular weight RNA fraction (4S-10S) either in the normal BHK cell or the adeno-transformed BAT cell. These RNA fraction was further separated by polyacrylamide-gel electrophoresis. The 5'-5' confronting nucleotide structure was found only in the 5.7S RNA as  $\text{m}_3^{2,2,7}\text{G}^{5'}\text{pppAm-N}\cdots$  and in the 5.9S RNA as  $\text{m}_3^{2,2,7}\text{G}^{5'}\text{pppAm-Nm-N}\cdots$ .

A part of this work was published in Proc. Japan Acad. **52**, 563-566

(1976).

### **Research on the Enzyme Cleaving the 5'-Terminal Methylated Blocked Structure of Messenger RNA**

Kin-ichiro MIURA and Kunitada SHIMOTOHNO

A new phosphodiesterase was isolated from tobacco cell culture in Sugimura's laboratory (National Cancer Center, Tokyo), where the formation and degradation of poly (ADP-Ribose) are studied (H. Shinshi, M. Miwa, K. Kato, M. Noguchi, T. Matsushima & T. Sugimura (1976) *Biochemistry* **15**, 2185-2190). The enzyme hydrolyses phosphodiester linkages in NDP, NTP, inorganic pyrophosphate, cyclic nucleotides,  $\text{NAD}^+$  and poly(ADP-Ribose), but it does not cleave backbone of DNA and RNA. The tobacco phosphodiesterase seems to be specific for pyrophosphate linkage except for cyclic phosphate bonding.

Our recent studies show that the tobacco phosphodiesterase can delete 5'-terminal blocked structure in a eukaryotic mRNA and low molecular weight RNA without splitting a main chain in an RNA molecule. For instance, when [ $^3\text{H}$  methyl] labeled mRNA of CPV (cytoplasmic polyhedrosis virus) was incubated with tobacco phosphodiesterase, only [ $^3\text{H}$  methyl]  $\text{pm}^7\text{G}$  was released into an acid-soluble fraction as a labeled material. The rest of the radioactivity in CPV mRNA was remained in an RNA chain in an acid-insoluble fraction. Since the CPV mRNA carries the structure  $\text{m}^7\text{G}^5'\text{ppp}^5'\text{Am-G}\cdots$  at the 5'-terminus, tobacco phosphodiesterase splits only pyrophosphate linkages in the terminal blocked structure. During incubation with tobacco phosphodiesterase the chain length of RNA did not change. Thus, tobacco phosphodiesterase can be used for detection or identification of the blocked terminal structure containing pyrophosphate linkages in a nucleic acid molecule.

This work was collaborated with Drs. Masanao Miwa, Hideaki Shinshi and Takashi Sugimura, and a part of it was published in *FEBS Lett.* **65**, 254-257 (1976).

**Enzymatic Removal of the 5'-Terminal Methylated  
Blocked Structure of Tobacco Mosaic Virus RNA  
and its Effects on Infectivity and  
Reconstitution with Coat Protein**

Kin-ichiro MIURA and Kunitada SHIMOTOHNO

The 5'-terminus of tobacco mosaic virus (TMV) RNA, long thought to be an unphosphorylated A residue (Sugiyama, Y. and Fraenkel-Conrat, H. (1963) *Biochemistry* **2**, 332-334; Fraenkel-Conrat, H. and Fowlks, E. (1972) *Biochemistry* **11**, 1733-1736), was recently identified as  $m^7G^5'ppp^5'Gp$  (Zimmern, D. (1975) *Nucleic Acids Res.* **2**, 1189-1201; Keith, J. and Fraenkel-Conrat, H. (1975) *FEBS Lett.* **57**, 31-33). Elimination of the 3'-terminal nucleoside of TMV RNA by a chemical procedure (periodate oxidation and subsequent cleavage by aniline) was reported to cause marked loss of infectivity (Steinschneider, A. and Fraenkel-Conrat, H. (1966) *Biochemistry* **5**, 2735-2743). However, this chemical procedure probably eliminated both the 5'- and 3'-ends of TMV RNA, so it is uncertain which elimination reaction was responsible for loss of infectivity. On the other hand, the assembly reaction of TMV RNA with TMV protein has been thought to start at the 5' end and proceed to the 3' end along the RNA chain (Ohno, T., Nozu, Y. and Okada, Y. (1971) *Virology* **44**, 510-516; Butler, P. J. G. and Klug, A. (1971) *Nature New Biology* **229**, 47-50; Thouvenel, J. C., Guilley, H., Stussi, C. and Hirth, L. (1971) *FEBS Lett.* **16**, 204-206). Now it is a problem whether the odd structure at the 5'-terminus is related to the assembly reaction.

These considerations prompted us to investigate the removal of the 5'-terminal blocked structure of TMV RNA and its effects on infectivity and assembly reaction. Recently a novel phosphodiesterase was purified from cultured tobacco cells (Shinshi, H., Miwa, M., Kato, K., Noguchi, M., Matsushima, T. and Sugimura, T. (1976) *Biochemistry* **15**, 2185-2190). As shown in the preceding report, this enzyme releases  $pm^7G$  from the 5'-terminal blocked structure of cytoplasmic polyhedrosis virus mRNA, but does not attack the polynucleotide chain of the mRNA. We used this enzyme to delete the 5'-terminal modified structure from a TMV RNA molecule.

The infectivity of TMV RNA which had been treated with tobacco phosphodiesterase was assayed on tobacco plants as free RNA or after recon-

stitution with TMV protein. TMV RNA lost its infectivity almost completely on removal of the 5'-terminal blocked structure. This is the first demonstration that the blocking structure of virus RNA is essential for infectivity.

In order to see the ability of reconstitution of TMV particle after treatment of TMV RNA with tobacco phosphodiesterase, the enzyme-treated RNA was incubated with TMV protein under the conditions for the reconstitution reaction, and analyzed by sucrose density gradient centrifugation. The results show that TMV RNA lacking the 5'-terminal blocked structure assembled with coat protein as well as native TMV RNA. Considering with other observations on the reconstitution process, the present result supports the idea that assembly does not start from the 5' end of TMV RNA.

This work was collaborated with Drs. Takeshi Ohno, Yoshimi Okada, University of Tokyo, and Hideaki Shinshi, Masanao Miwa and Takashi Sugimura, National Cancer Institute. The contents were published in *FEBS Lett.* **67**, 209-213 (1976).

### **Role of the 5'-Terminal Modified Structure of mRNA in Protein Synthesis**

Kunitada SHIMOTOHNO and Kin-ichiro MIURA

Since our discovery of the methylated blocked structure—the confronting nucleotide structure—at the 5'-terminus of the mRNA strand of CPV,  $m^7G^{5'}pppAm-$  (Furuichi, Y. and Miura, K., *Nature*, **253**: 375 (1975); Miura, K., Furuichi, Y., Shimotohno, K., Urushibara, T. and Sugiura, M., *Proc. 10th FEBS Meeting*, 95-108 (1975)), the presence of similar modified structures generally in eukaryotic mRNA's has been suggested (Review by Griffin, B., *Nature*, **255**: 9 (1975)). Although there are some exceptional cases, the confronting structure has been considered to function in protein synthesis. Some experiments have already supported this possibility (Both, G. W., Banerjee, A. K. and Shatkin, A. J., *Proc. Nat. Acad. Sci. U. S. A.*, **72**: 1189 (1975)), though there are some conflicting data (Review by Griffin, B., *Nature*, **263**: 188 (1976)).

Recently it was shown that a pyrophosphatase purified from tobacco cell culture specifically splits 7-methylguanylic acid and phosphate at the pyrophosphate linkages from the 5'-terminus of eukaryotic mRNA without



any scission at other parts of the RNA molecule (Shinshi, H., Miwa, M., Sugimura, T., Shimotohno, K. and Miura, K., *FEBS Letters*, **65**: 254 (1976); Ohno, T., Okada, Y., Shimotohno, K., Miura, K., Shinshi, H., Miwa, M. and Sugimura, T., *FEBS Letters*, **67**: 209 (1976)). Using this enzyme, we have explored the function of the confronting nucleotide structure at the 5'-terminus of mRNA.

The mRNA's of CPV, TMV (viral RNA) and globin were treated with tobacco phosphodiesterase, and their template activities for peptide synthesis were compared with that of untreated mRNA in the cell-free protein synthesizing system of wheat germ. In every case, enzyme-treated mRNA in which the confronting nucleotide structure at the 5'-terminus had been completely deleted lost the ability to incorporate [<sup>3</sup>H]leucine into the acid-insoluble peptide fraction.

The requirement for 7-methylguanylic acid in the confronting nucleotide structure at the 5'-terminus of mRNA was confirmed by the following experiments. When 7-methylguanylic acid (5'-phosphate) was added to the reaction mixture, the protein synthesis capability of native mRNA was inhibited markedly. The inhibitory effect of pm<sup>7</sup>G was highly specific among analogous derivatives of guanylic acid.

In every case, analysis of the product proteins by gel electrophoresis showed that the synthesis of proteins was inhibited equally for every kind of protein.

The initiation complex for protein synthesis of CPV mRNA and TMV RNA was not obtained on using tobacco phosphodiesterase-treated mRNA. Formation of the initiation complex of intact mRNA was also inhibited strongly by addition of pm<sup>7</sup>G.

These experiments show unambiguously that the 7-methylguanylic acid blocking structure at the 5'-terminus of eukaryotic mRNA is necessary for protein synthesis, especially for the step of formation of the initiation complex. This is common for three different kinds of mRNA. When m<sup>7</sup>G at the 5'-terminus is removed by  $\beta$ -elimination after oxidation of the free ribose moiety, the 3'-terminal nucleotide is also deleted. As the tobacco phosphodiesterase can remove only 5'-terminal modification, the effect of deletion of this structure can be clearly tested by the use of this enzyme.

There is a possibility that the confronting nucleotide structure is necessary to ensure the stability of mRNA, protecting it from exonucleolytic degradation. This was tested by incubating the CPV mRNA, with or without

pretreatment with tobacco phosphodiesterase, in wheat germ extract. Both the S-30 fraction (soluble fraction after centrifugation at 30,000 *g*) and S-100 fraction (soluble fraction at 100,000 *g*) were used for this experiment. The intact CPV mRNA was stable on incubation for 3 min, whereas the tobacco phosphodiesterase-treated mRNA was broken down within 1 min. Similar results were obtained for S-30 and S-100 fractions, and on adding pm<sup>7</sup>G. These results clearly show that the confronting nucleotide structure functions to stabilize the mRNA, preventing its degradation.

### A Procedure for the Isolation of Temperature Sensitive RNA Polymerase Mutants of *E. coli*

Masahiro SUGIURA and Koichi YOSHINAGA

DNA-dependent RNA polymerase of *E. coli* consists of four subunits:  $\alpha$ ,  $\beta$ ,  $\beta'$  and  $\sigma$ . As an approach toward understanding the precise role of each subunit in RNA polymerase during transcription, we undertook isolation of temperature sensitive (ts) mutants with altered RNA polymerases which expressed their temperature sensitivity in the usual *in vitro* reaction systems.

Ts lethal mutants derived from *E. coli* K12 strain PA3092 after mutagenesis with nitrosoguanidine were obtained from Dr. Hirota. All cells were grown in L-broth supplemented with 50  $\mu\text{g}/\text{ml}$  thymine.

The rates of uridine incorporation at 30°C and 43°C were measured for all the ts mutants. Cells were grown at 30°C in 2 ml medium for 2–5 hr until an exponential phase was reached. 0.5 ml of the culture was removed and incubated for 2 min at 30°C with [<sup>3</sup>H]uridine (0.2  $\mu\text{Ci}$ , 41.5 Ci/mmol). Rest of the culture was immediately transferred to 43°C. After incubation at 43°C for 16 min, 0.5 ml of the culture was removed and incubated for 2 min at 43°C with [<sup>3</sup>H]uridine as above. Pulse-labeled cells were precipitated with 2 ml cold 10% TCA containing 1 mM uridine for over 30 min and the precipitates were collected on Whatman GF/F filters and the radioactivities were counted. The ratio of the rate of uridine incorporation at 43°C to that at 30°C was obtained for each ts mutant. The parent strain had the ratio of 1–2. 280 strains which were low in these ratios (less than 0.5) were selected from 1200 ts mutants.

Toluene-treated cells of these strains were prepared according to Peterson *et al.* (1971, *J. Bact.* 107: 585) with modifications. Cells were grown in

5 ml medium to a density of  $5 \times 10^8$  cells/ml. The cells were chilled quickly, harvested by centrifugation and suspended in 0.5 ml of ice-cold 0.05 M Tris (pH 7.4) — 0.1 M KCl. The cold suspension was made 1% (v/v) in toluene and shaken 5 times by a vortex for 5 sec every 3 min. The suspension was kept at 0° during 3 min-intervals. After centrifugation, the cell pellet and the wall of tube were rinsed with the cold above buffer and resuspended in 0.2 ml of the same buffer. For RNA synthesis assay, reaction mixtures contained (0.2 ml) 0.04 M Tris (pH 7.9 at 25°C), 8 mM  $MgCl_2$ , 0.1 mM DTT, 0.1 mM EDTA, 0.1 M KCl, 2 mM spermidine, 0.8 mM  $K_2HPO_4$ , 0.4 mM each of ATP, CTP, GTP and [ $^3H$ ]UTP (10  $\mu Ci/\mu mol$ ) and 20  $\mu l$  toluene-treated cells. Incubations were for 10 min at 30°C and 43°C and acid-insoluble radioactivities were counted. Spermidine stimulated the incorporation of UTP. The ratio of UTP incorporation at 43°C to that at 30° was obtained for each selected mutant. The parent strain had the ratio of about 0.6. 50 strains low in these ratios (less than 0.4) were selected.

Crude RNA polymerase preparations from these 50 mutants were obtained by the simplified procedure as follows. Cells were grown in 300 ml medium at 30°C till late log-phase and harvested by centrifugation. 0.5 g of frozen cells were disrupted by sonication in 2 ml of 0.05 M Tris-HCl (pH 7.9), 0.01 M  $MgCl_2$ , 0.1 M KCl, 0.1 mM DTT, 0.1 mM EDTA, 10% glycerol and 20  $\mu g/ml$  DNase I, and centrifuged at 100,000 g for 1 hr. To the supernatant added an equal volume of saturated  $(NH_4)_2SO_4$  solution. The resultant precipitate was collected by centrifugation and washed with 8 ml of 45% saturated  $(NH_4)_2SO_4$  solution made in 0.01 M Tris-HCl (pH 7.9), 0.1 mM DTT, 0.1 mM EDTA and 10% glycerol (buffer D). The washed precipitate was dissolved in buffer D so that its conductivity was less than that of buffer D+0.1 M KCl (9 m $\Omega$  at 0°) and applied onto a 1 ml DEAE-cellulose column (Whatman DE-52) equilibrated with buffer D+0.1 M KCl. The column was washed with 8 ml buffer D+0.1 M KCl followed by 1 ml of buffer D+0.3 M KCl. The RNA polymerase was eluted with the next 1 ml of buffer D+0.3 M KCl. Reaction mixtures for RNA polymerase assay contained (0.2 ml) 0.04 M Tris-HCl (pH 7.9 at 25°C), 8 mM  $MgCl_2$ , 0.1 mM DTT, 0.1 mM EDTA, 0.2 mM each of ATP, CTP, GTP and [ $^3H$ ]UTP (10  $\mu Ci/\mu mol$ ), 0.2 M KCl, 0.4 mM  $K_2HPO_4$ , 100  $\mu g$  BSA, 5  $\mu g$  T<sub>4</sub>-DNA and 30  $\mu l$  enzyme preparation (approximately 1 OD<sub>280</sub>/ml). Incubations were for 10 min at 30°C and 43°C, and acid-insoluble radioactivities were counted. The ratio of RNA polymerase activity at 43°C to that at 30°

was obtained for each preparation and was about 0.5 for the parent enzyme. We obtained 4 RNA polymerase mutants from 1200 original ts strains. One of them, called JE10092, was identified as a ts  $\beta'$  subunit mutant (Sugiura *et al.* 1977 BBRC 76: 739, YOSHINAGA & SUGIURA 1977 BBA 479: 172).

## II. MICROBIAL GENETICS

### Mutants of *Escherichia coli* Altered in Penicillin Binding Proteins

Yukinori HIROTA, Yukinobu NISHIMURA and Hideho SUZUKI

Five thousand mutants of *Escherichia coli* thermosensitive in growth were isolated from independent origin and stocked as the source of mutations with which the mechanisms of cell division were investigated. Five hundred of these mutants were examined with respect to the defect in penicillin binding proteins (PBP), which were numbered as PBP-1, PBP-2, etc., according to Spratt and Pardee (1975 Nature **254**: 516). In the course of this screening for PBP mutations, we found a number of mutants defective in PBP-1 and these mutants fell into two categories. This led to the conclusion that the PBP-1 contained two different gene products designated as PBP-1a and PBP-1b. Mutations leading to the defects in these PBPs were referred to as *ponA* and *ponB* (penicillin binding protein *one A* or *B*), respectively. Consequently, we found eleven mutants defective in PBP-1a, four in PBP-1b, three in PBP-2, one in PBP-3 and three in PBP-4. In addition, we found a mutant defective in activity of D-alanine carboxypeptidase Ia (Matsubashi *et al.* in preparation). This enzyme is an identical entity with PBP-5/6 (Spratt and Strominger, 1976 J. Bacteriol. **127**: 660). By using these mutants, the map position of the mutations for each PBP was determined: *ponA* (PBP-1a) 73.5 min, *ponB* (PBP-1b) 3.3 min, *rodA* (PBP-2) 14.4 min, *ftsI* (PBP-3) 1.8 min, *dacB* (PBP-4) 68 min and *dacA* (PBP-5/6) 13.7 min.

One of the mutants for PBP-1a carried thermosensitive defect in penicillin binding ability of 1a (1a-ts); *i.e.*, the PBP-1a did not bind penicillin at 42°, but did at 30°. All of the *ponA* mutations examined, including this thermosensitive defect, were separated from the defect in thermosensitive growth of the mutants and cotransduced with *aroB* at a frequency of 80–90%.

Among four mutants of PBP-1b, three showed no penicillin binding band for PBP-1b and one showed faster migration of the electrophoretic bands of PBP-1b. The PBP-1b gave three electrophoretic bands in the wild type and in this mutant. All of the mutation in PBP-1b (*ponB*) were separated

from thermosensitive mutation in growth and cotransduced with *tonA* at a frequency of about 82%. The mutants which lost the PBP-1b were hypersensitive to  $\beta$ -lactams. The hypersensitivity was strictly linked with the loss of the band of PBP-1b.

The trials to construct the double mutant defective in both PBP-1a and PBP-1b were unsuccessful. This result suggested the lethality in the *ponA ponB* double mutant. The mutation *ponA*<sup>ts</sup> (1a-ts) was introduced into the *ponB* mutant by a cross. Although both of the parental strains were thermoresistant, 19% of the recombinants became thermosensitive and lysed at the restrictive temperature. The thermosensitive loss of the binding ability of PBP-1a paralleled with the thermosensitive growth in the double mutant. Therefore, it is concluded that the PBP-1a and PBP-1b may share a common part of the reaction required for cell elongation so that the loss of the function of PBP-1b may be bypassed by PBP-1a.

The mutations in PBP-2 and PBP-3 were linked with thermosensitivity in colony formation. The mutants defective in PBP-2 formed ovoid cells and those in PBP-3 showed filamentation at the restrictive temperature. These results agree with the previous works of Spratt (1975 Proc. N. A. S. U.S. 72: 2999).

The PBP-4 and PBP-5/6 were identified as D-alanine carboxypeptidase Ib and Ia, respectively (Matsuhashi *et al.* 1977 Proc. N.A.S. U.S. 74: 2976, Matsuhashi *et al.* in preparation). The mutations, *dacB* (PBP-4) and *dacA* (PBP-5/6) were cotransducible with *argG* and *leuS*, respectively, at a frequency of about 87%. The double mutant lacking both enzyme activities grew normally at 40° as well as at 30°, indicating that these enzymes were dispensable for cell growth and division.

The extensive studies on the isolation and characterization of the PBP mutants allowed us to determine the chromosomal location of all the genes for the PBPs. This opens the way to construct multiple mutants defective in various PBPs and to dissect the roles of the PBPs in cell growth and division.

### Synthetic Cole1 Plasmids Carrying Genes of Cell Division in *Escherichia coli*

Yukinobu NISHIMURA, Yutaka TAKEDA, Akiko NISHIMURA,  
Hideho SUZUKI and Yukinori HIROTA

To clarify the functions of the genes involved in the processes of cell division, the elucidation of the gene products and their properties must be

of crucial importance. The amplification of the gene involved in cell division may facilitate the identification and biochemical characterization of the gene product. Clarke and Carbon prepared a collection (1976, Cell 9: 91) of *Escherichia coli* strains which harbored ColE1 plasmids carrying small random segments of the *E. coli* chromosome. This collection was utilized to search the plasmid carrying the genes for the process of cell division and for the murein-lipoprotein.

The 2000 strains in the collection were screened for the correction of the following genetic defects: *par* (partition of daughter nuclei), *fts* (cell division), *rod* (determination of a cell shape) and *lpo* (synthesis of the murein-lipoprotein). We found plasmids carrying *E. coli* chromosomal segments containing *ftsB*<sup>+</sup>, *ftsE*<sup>+</sup>, *ftsI*<sup>+</sup>, *ftsM*<sup>+</sup> and *parA*<sup>+</sup>. However, none was found to transfer *ftsA*<sup>+</sup>, *ftsC*<sup>+</sup>, *ftsF*<sup>+</sup>, *ftsG*<sup>+</sup>, *ftsJ*<sup>+</sup>, *ftsK*<sup>+</sup>, *ftsL*<sup>+</sup>, *parB*<sup>+</sup>, *rod*<sup>+</sup> and *lpo*<sup>+</sup>. Among the *fts* mutations, *ftsI* was identified as a mutation affecting penicillin-binding protein (PBP) 3 (according to the nomenclature by Spratt 1975, Proc. N. A. S. U.S. 72: 2999). The PBP was examined in the strains carrying the plasmid which corrected the *ftsI* defect. One of the strains carrying pLC26-6 was found to overproduce the PBP-3 by about 10-fold.

Meanwhile, it was found that the gene for PBP-1a (*ponA*) was cotransducible with *aroB* and the defect in PBP-1b (*ponB*) was strictly correlated to the bacterial phenotype of hypersensitivity to cephaloridine (Suzuki *et al.*, in this Ann. Rep. p 27). These findings led us to search the synthetic plasmids carrying the genes for PBP-1a and for PBP-1b by examining the correction of *aroB* and of hypersensitivity to cephaloridine. One strain carrying the plasmid pLC29-47 which corrected the *aroB* mutation was found to overproduce the PBP-1a and four strains carrying the plasmid; pLC4-43, pLC4-44, pLC19-19 and pLC26-43, which corrected hypersensitivity to cephaloridine, overproduced the PBP-1b.

### Penicillin Binding Proteins Involved in Cell Elongation

Hideho SUZUKI, Yukinobu NISHIMURA and Yukinori HIROTA

At least six penicillin binding proteins (PBP) exist in *Escherichia coli* and they are numbered as PBP-1, PBP-2, etc., in the order of their molecular weights (Spratt and Pardee, 1975 Nature 254: 516). Among them, PBP-1, -2 and -3 were inferred to be involved in cell elongation, cell shape determina-

tion and cell division, respectively (Spratt, 1975 Proc. N.A.S. U.S. 72: 2999).

We found that the PBP-1 contained two components designated as 1a and 1b and isolated mutants defective in each component. The defective mutations of 1a and 1b were mapped at separate loci on the chromosome. The defect either in 1a or in 1b, *per se*, did not render the cell thermosensitive in growth. All the mutants defective in PBP-1b became hypersensitive to  $\beta$ -lactam antibiotics. The minimal inhibitory concentration of cephaloridine and cephalothin was in the range of 0.1–0.2  $\mu\text{g}/\text{ml}$  for the PBP-1b mutant, while it was 2–4  $\mu\text{g}/\text{ml}$  for the wild type (Table 1). The affinities of PBP-1a and -1b for these antibiotics were determined by competition experiments.

Table 1. The binding of cephalosporins to the penicillin binding proteins

	Concn. required to reduce [ <sup>14</sup> C]penicillin-binding by 50% ( $\mu\text{g}/\text{ml}$ )*				Minimal inhibitory concn. ( $\mu\text{g}/\text{ml}$ )			
	PBC-1a	-1b	-2	-3	Mutant 1a <sup>-</sup>	Isogenic wild type	Mutant 1b <sup>-</sup>	Isogenic wild type
Cephaloridine	0.11	2.7	45	3.4	2	2	0.1	2
Cephalothin	0.04	20	30	1.0	5	4	0.2	3

\* Determined by competition experiments.

As shown in Table 1, PBP-1a showed a conspicuously high affinity for these antibiotics. Cephalothin induced filamentation of wild type cells at 5–10  $\mu\text{g}/\text{ml}$ . At this concentration range, cephalothin binds to PBP-1a and -3 but does not to PBP-1b (Table 1). On the other hand, cephalothin was effective at a concentration far below that required to bind with PBP-1b, in the mutant defective in PBP-1b. From these observations, it was inferred that the biochemical reactions for cell elongation can proceed normally in the presence of either PBP-1a or -1b and cell elongation in the PBP-1b mutant may be sustained by PBP-1a which is very sensitive to the  $\beta$ -lactams, and therefore, cell elongation becomes hypersensitive to these antibiotics. These interpretations were compatible with the genetic analyses of the 1a/1b mutant.

The double mutant carrying the thermosensitive defect in PBP-1a (1a-ts) and no binding ability of PBP-1b showed thermosensitive growth, lysing at the restrictive temperature. The parental strain carrying either the 1a-ts or the 1b mutation was thermoresistant.

Murein-synthetic activity of the mutant defective in PBP-1a or -1b was



measured *in vitro* by incorporation of radioactive N-acetylmuramyl pentapeptide. The activity for murein synthesis *in vitro* was decreased to about 1/10 in the mutant defective in PBP-1b, although the lipid-linked precursor was formed in a considerable amount. Thus, PBP-1b is involved in the reaction at the final stage of murein synthesis for cell elongation. The cells of the mutant defective in PBP-1b were able to propagate normally. Presumably PBP-1a took over the reaction required for cell elongation, as a substitute for PBP-1b.

### Differential Solubilization of Membrane Proteins of *Escherichia coli* with Detergents

Masao YAMADA, Hideho SUZUKI and Yukinori HIROTA

Recently O'Farrell devised a high resolving technique for separation of proteins by combination of isoelectric focusing and SDS-gel electrophoresis (two dimensional electrophoresis) (O'Farrell, P. H. 1975, J. Biol. Chem. **250**: 4007). He applied it to resolving of the whole cell extract of *E. coli*, and about 1,100 protein spots were displayed on a 12×16 cm plate. This technique is a powerful tool for studying the correlation of genes to gene products and the regulation of protein synthesis at a cellular level. We adopted the procedure to study membrane proteins.

Envelope fraction of *E. coli* was obtained by differential centrifugation after disruption of the cells by a sonic disintegrator, solubilized with 0.5–2% various detergents at 30–37°C, and then analyzed by the two dimensional electrophoresis. The number of spots of proteins resolved on the electrophoretogram depended on the detergent used, and increased in the following order: sodium deoxycholate, Triton X-100, Brij 35, Brij 58, sodium dodecyl sulfate (SDS), sodium N-lauroyl sarcosinate (Sarkosyl). Major proteins in the outer membrane were not extracted with any detergent without heat treatment. Sarkosyl and SDS almost completely solubilized only the inner membrane while Triton X-100 solubilized it partially. These results are not compatible with previous observation by Filip *et al.* (1973, J. Bacteriol. **115**: 717) in which Sarkosyl solubilized the inner membrane selectively but Triton X-100 and SDS solubilized both the inner and outer membranes.

Some proteins altered their isoelectric point by heating in the presence of detergent resulting in appearance of multiple spots along the isoelectric focusing dimension. So, a method to obtain the highest resolution for the

separation of membrane proteins is as follows: First, envelope was solubilized with 2% Sarkosyl and then Sarkosyl-insoluble fraction was solubilized with 2% SDS at 100°C for 2 min. Each fraction was analyzed separately by essentially the same procedure reported by O'Farrell. Using this technique, at least 250 and 50 proteins were found to exist in the inner and outer membrane of *E. coli*, respectively.

### Isolation of Specialized Transducing Bacteriophage $\lambda$ Carrying *leuA-ftsI-murEFC-dll-ftsA* Region of *Escherichia coli* Chromosome

Masao YAMADA, Seiichi YASUDA and Yukinori HIROTA

Thermosensitive mutants of cell division of *E. coli* K-12 were isolated in our laboratory whose penicillin binding component 3 could not bind [<sup>14</sup>C]-benzylpenicillin. The mutation, designated as *ftsI*, was mapped at about 1.8 min of *E. coli* chromosome (Nishimura, Y. *et al.* 1977, Plasmid **1**: 67). Around this locus, many other genes concerning cell wall synthesis and septum formation have been mapped. They include *ftsA*, temperature-sensitive filament formation (Richard, M., and Y. Hirota 1973, J. Bacteriol. **116**: 314), *murCEF* and *ddl* which are involved in the formation of nucleotide sugar intermediate of peptidoglycan synthesis (Wijsman, H. J. W. 1972, Genet. Res. **20**: 65), and *mra*, a gene cluster coding the enzymes of peptidoglycan synthesis (Miyakawa, T. *et al.* 1972, J. Bacteriol. **112**: 950).

In order to determine the precise map in this region and to study on the regulatory mechanism of these genes, we have isolated phage  $\lambda$  carrying this region.

Strain JE5631 (*ftsI*) was transformed to temperature-resistant with UV-lysate of strain JE6600 (*leuA*,  $\Delta$  (*pro-lac*),  $\Delta$  (*gal-uvrB*), *thi*) with  $\lambda$ *pleuA i*<sup>21</sup> nin 5 integrated at a secondary attachment site in *leu* operon. Among about 200 temperature-resistant colonies isolated, 22 colonies gave high frequency transducing phages to *ftsI* mutant upon UV-induction. Lambda phages from the primary heterogenotes were classified into 5 groups according to the transducing ability to other loci. One phage transduced *leuA* and *ftsI* (class I), 3 phages transduced *leuA*, *ftsI* and *murE* (class II), 16 phages transduced *leuA*, *ftsI*, *murE* and *murF* (class III), 1 phage transduced *leuA*, *ftsI*, *murE*, *murF* and *murC* (class IV), and 1 phage transduced *leuA*, *ftsI*, *murE*, *murF*, *murC*, *ddl* and *ftsA* (class V). No phage that transduced other combination of these genes was obtained. One of the transducing phages

belonging to class III was a plaque-former and others were defectives. From the results, it is concluded that the order of genes on the *E. coli* chromosome is *leu-ftsI-murE-murF-murC-(ddl-ftsA)*.

### III. BIOCHEMICAL GENETICS AND IMMUNOGENETICS

#### A Comparison of Heat Sensitivity of Serum Esterase(Es-1) in Different Inbred Mice, *Mus musculus*

Tadashi AOTSUKA<sup>1</sup> and Kazuo MORIWAKI

Recent studies of *Drosophila* by Bernstein *et al.* (Proc. Nat. Acad. Sci. **70**: 3928, 1973) and Singh *et al.* (Proc. Nat. Acad. Sci. **71**: 1080, 1974. Genetics **80**: 673, 1975) have given the experimental verifications of hidden genetic variability in electrophoretic isozyme classes by a heat inactivation technique. Such new physicochemical variabilities of isozymes would supply more informations about the genetic divergence occurred in the process of species formation. The present paper presents the results of heat inactivation experiments of serum esterase (Es-1) in laboratory inbred strains of house mouse (*Mus musculus*). Two alleles (Es-1a, Es-1b) which produce isozymes having different electrophoretic mobilities were detected at the Es-1 locus from the inbred strains (Popp and Popp, J. Hered. **53**: 111, 1962. Petras and Biddle, Can. J. Genet. Cytol. **9**: 704, 1967).

Total of 21 inbred strains were analysed in the present investigation (Table 1). Blood was obtained by inserting a heparinized capillary tube into the suborbital canthal sinus. After centrifugation, the serum was pre-treated at various temperatures (0–70°C) for 7 minutes before subjected to electrophoresis or the colorimetric assay of esterase activity.

Table 1 summarizes the results. The faster migrating isozyme (Es-1a)

Table 1. Types of heat sensitivity of Es-1 in 21 inbred mice, *Mus musculus*.  
Alleles fixed in each strain are also represented

Inbred strains	Allele	Heat sensitivity*
C57BL/10Sn, C57BL/6J, B10. BR, B10. D2		
NZB/Es-1a, HTI	a	Stable
C3H/HeJ, BALB/c, DDY, DDN, 129/J		
AKR/J, SM/J, CBA/CaHN.T-6, DBA/2J	b	Sensitive
A/J, SWR/J, SJL/J, HTG, PL, NZB/Es-1b		

\* The determination of the types of heat sensitivity was based on residual activity at 58°C.

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was more heat-stable than the slower migrating isozyme (Es-1b). At 58°C, the Es-1a retained 60% of its total activity, whereas the Es-1b lost more than 80% of the total activity.  $T_{1/2}$  (Temperature at which one-half of the total activity is lost) of the sensitive Es-1b was lower than that of the stable Es-1a by about 2.5°C. Further investigations are now in progress to answer the question whether the difference in the heat sensitivities between two types of Es-1 isozymes is due to the genetic alteration only on the Es-1 locus.

### Distribution of *Hbb* (Hemoglobin beta-chain) Alleles in Japanese Wild Mice, *Mus musculus molossinus*

Mitsuru MINEZAWA<sup>1)</sup>, Kazuo MORIWAKI and Kyoji KONDO<sup>1)</sup>

In wild mouse populations of Western hemisphere and also in laboratory strains, *Hbb*<sup>a</sup> and *Hbb*<sup>s</sup> alleles are common and *Hbb*<sup>p</sup> allele is rare. Only an AU/Ss strain carries the allele *Hbb*<sup>p</sup> (Petras 1969, Can. J. Genet. Cytol. **11**: 497-513; Staats 1976, Cancer Res. **36**: 4333-4377). In contrast, *Hbb*<sup>p</sup> and *Hbb*<sup>d</sup> alleles were observed commonly in Asian wild mice and several inbred strains originated in Japanese mice (*Mus musculus molossinus*, pet mice) possess *Hbb*<sup>p</sup> allele (Gilman 1974, Ann. N. Y. Acad. Sci. **241**: 416-433; Watanabe *et al.* 1976, Japan J. Genet. **51**: 377-383).

But, the three hemoglobin beta-chain alleles, *Hbb*<sup>p</sup>, *Hbb*<sup>d</sup> and *Hbb*<sup>s</sup>, were observed in several populations of Japanese wild mice, *Mus musculus molossinus* (Minezawa *et al.* 1976, this report **26**: 23-25).

Therefore, 259 mice were collected from 13 geographical regions in Japan with the object of doing a survey on the alleles. Hemolytic samples, untreated and treated with iodoacetic acid, were separated by starch gel electrophoresis. The numbers of examined mice and allelic frequencies at the *Hbb* locus in each region are demonstrated in Table 1. In Japan, on the whole, the *Hbb*<sup>p</sup> and *Hbb*<sup>d</sup> alleles were common and the *Hbb*<sup>s</sup> allele was rare. The frequencies of *Hbb*<sup>p</sup> and *Hbb*<sup>d</sup> *in toto* were 62.3 and 36.9%, respectively, and interregional differences were also observed. The *Hbb*<sup>p</sup> allele predominated or fixed in Shikoku and Mainland facing the Pacific Ocean, namely, San-yo, Kinki, Tokai and Kanto. The *Hbb*<sup>d</sup> allele predominated in Hokkaido, Okinawa and Oosumi Isls. The frequencies of the

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Table 1. Allelic frequency at hemolobin beta-chain (*Hbb*) locus in Japanese wild mice population

Localities collected	No. of animals examined	Allelic frequencies of <i>Hbb</i> (%)		
		p	d	s
Okinawa	4		100.0	
Amami Isls.	9	61.1	38.9	
Oosumi Isls.	7	21.4	78.6	
Kyushu	42	38.1	53.3	3.6
Shikoku	12	100.0		
San-in	23	56.5	41.3	2.2
San-yo	15	93.3	6.7	
Kinki	15	86.7	13.3	
Tokai	44	97.7	2.3	
Hokuriku	33	43.9	56.1	
Kanto	13	100.0		
Tohoku	23	63.0	37.0	
Hokkaido	19	7.9	92.1	
Total of all localities	259	62.3	36.9	0.8

two alleles in the other regions, Kyushu, San-in, Hokuriku and Tohoku, were intermediate between the two region groupes mentioned above. The *Hbb<sup>s</sup>* allele was found in 4 mice in a heterozygous state. The frequency was only 0.8% *in toto*.

From the results, it could be concluded that the Japanese wild mice, *Mus musculus molossinus*, was constructed originally of *Hbb<sup>p</sup>* and *Hbb<sup>d</sup>* alleles and that *Hbb<sup>s</sup>* was not an original allele, but intruded into the ancestral Japanese population of *Hbb<sup>p</sup>* and *Hbb<sup>d</sup>*. In both Western and Asian wild mice, *Hbb<sup>d</sup>* is a basic allele, whereas *Hbb<sup>p</sup>* allele is a characteristic marker for Asian wild mice. The origin of *Hbb<sup>s</sup>* allele remains unexplained but it is worth noting that the allele is common in Japanese fancy mice and laboratory strains (Mizuno *et al.* 1977, Exp. Anim. 26: 43-49).

### Suppression of Erythrocytic H-2 Expression in F<sub>1</sub> Hybrid between Two Mouse Subspecies, *Mus musculus domesticus* and *molossinus*

KAZUO MORIWAKI and TOSHIHIKO SHIROISHI

In general mouse histocompatibility genes are considered to be expressed

in the codominant fashion on most adult tissues. However, in the  $F_1$  hybrid between two kinds of mouse subspecies, the European feral mouse *Mus musculus domesticus* from which most of the present laboratory mouse probably originated and the Japanese feral mouse *Mus musculus molossinus*, expression of H-2 antigen on erythrocytes is shown to be markedly suppressed in the present study.

This phenomenon was first observed in a hemagglutination test for H-2.5 antigenic specificity of the  $F_1$  hybrid between the B10.A laboratory strain and the Mol Sg line derived from *M.m. molossinus*. Maximal antiserum dilution to give a definitely positive hemagglutination was 256 in B10.A, <8 in B10.D2, <8 in Mol Sg, 256 in (B10.A×B10.D2) $F_1$  and <8 in (B10.A×Mol Sg) $F_1$ . Thus, expression of H-2.5 specificity on erythrocytes is obviously suppressed in (B10.A×Mol Sg) $F_1$ , whereas never in (B10.A×B10.D2) $F_1$ .

These results have been further confirmed by the quantitative absorption of anti H-2.5 and anti H-2.23 sera with erythrocytes to be examined. After absorption, the residual antibody activity in the serum which is inversely proportional to the degree of H-2 expression, was assayed by the cytotoxicity method using  $^{51}\text{Cr}$ -labelled lymphocytes. Table 1 demonstrates the apparently reduced expression of H-2.5 (public) and H-2.23 (private) specificities on the erythrocytes of (B10.A×Mol Sg) $F_1$  hybrid quantitatively. No suppressed expression of H-2 antigens was observed in spleen cells of this intersubspecies hybrid. Mechanism of this type of suppression remains open.

Table 1. Suppression of H-2 antigen expression in  $F_1$  hybrid between *M. m. domesticus* and *M.m. molossinus*

Cells examined	H-2 antigenic specificities tested	Relative absorbing capacity (%)				
		Mouse strains and their hybrids employed				
		Mol-SgI	B10.A	B10.D2	B10.A × Mol-SgI	B10.A × B10.D2
RBC	K5	0	64.4	0	20.6	54.3
	K23	8.6	62.1	14.6	33.5	56.1
Spleen cells	K5	85.7	94.9	2.3	77.3	80.6
	K23	43.0	95.6	30.0	74.9	78.7

Mol SgI: *Mus musculus molossinus* line

B10.A, B10.D2: B10 congenic strains probably developed from *Mus musculus domesticus*.

### The Effect of K-region in H-2 Complex on Expression of H-2.5 Antigen on Erythrocytes

Toshihiko SHIROISHI and Kazuo MORIWAKI

Some H-2 antigens are detectable by cytotoxicity much more readily than by hemagglutination (Demant *et al.* 1971). It is also known that the C57BL/10J erythrocytes show a poor expression of H-2.5 antigen, although spleen cells and other cell types express it strongly. However it remains unanswered whether the presence or absence of the H-2 antigens on erythrocytes is controlled by the H-2 complex itself, or by the other genotype.

We made an attempt to answer this question and to identify the fine region controlling the expression of H-2 antigens on erythrocytes by using various B10 congenic strains.

Following strains were used: C57BL/10J (H-2<sup>b</sup>) (abbreviated symbol B10), B10.A (H-2<sup>a</sup>), B10.A(5R) (H-2<sup>i5</sup>), B10.A(2R) (H-2<sup>h2</sup>), B10.BR (H-2<sup>k</sup>), and B10.D2 (H-2<sup>d</sup>).

Alloantiserum, D-5bAF, supplied from NIH, which mainly directed H-2.5, was employed. Erythrocytes and spleen cells were examined for H-2.5 by the quantitative absorption and subsequent cytotoxicity test.

The results are summarized in Table 1.

Table 1. Quantitative absorption test with erythrocytes and spleen cell of various B10 congenic strains

B10 congenic strains	H-2 haplotype	Parental H-2 haplotype	H-2 complex							Absorption capacity (%) for H-2.5	
			K	IA	IB	IC	S	G	D	Erythrocyte	Spleen cell
B10.BR	k		k	k	k	k	k	k	k	88.6	64.3
B10.A	a	k/d	k	k	k	d	d	d	d	90.3	76.0
B10.A(2R)	h2	a/b	k	k	k	d	d	?	d	62.8	81.1
B10	b		b	b	b	b	b	b	b	20.9	79.5
B10.(5R)	i5	b/a	b	b	b	b	d	d	d	9.0	82.1
B10.D2	d		d	d	d	d	d	d	d	0.0	6.5

These data indicates the followings:

- 1) The degree of an expression of the H-2.5 antigen on erythrocytes is different among these various strains, but that on spleen cells is not.
- 2) The strains having a k-haplotype in H-2K, IA, and IB regions,



showed a strong expression of the H-2.5 antigen on erythrocytes, while those with a b-haplotype in these regions expressed it poorly.

All these data suggest that the degree of erythrocytic expression of the H-2.5 antigen is controlled by an allele within the H-2 complex, probably in K and neighboring regions.

#### IV. DEVELOPMENTAL AND SOMATIC CELL GENETICS

##### *In Vitro* Analysis of Tissue-Specific Defects in rudimentary Embryonic Cells of *Drosophila melanogaster*

Yukiaki KURODA

In the series of experiments on tissue-specific defects of cells from developmentally lethal embryos in *Drosophila melanogaster*, some defects in differentiation of muscle cells, imaginal disc cells and nerve cells have been found in cultured *deep orange* embryonic cells (Kuroda, *Nature* **252**, 40, 1974; *Develop. Growth Different.* **19**, 57, 1977). A similar defect in nerve cells has been found in cultured *fused* embryonic cells.

In the present work, tissue-specific defects of rudimentary<sup>39k</sup> (*r*<sup>39k</sup>) embryonic cells were investigated in culture. The *r*<sup>39k</sup> gene is located at 54.5 on the X chromosome. When the lethal effective phase was examined on 926 embryos collected from matings of *r*<sup>39k</sup>/*r*<sup>39k</sup> females and *r*<sup>39k</sup>/Y males, 4.0% died before gastrulation, 9.3% died by abnormal gastrulation, 13.2% died after head and trunk segmentation, 14.8% died after sac-like midgut formation, 40.7% died after entrance of air into trachea, 10.3% died after the stage of active movement and 5.7% died immediately after hatching.

When cells from *r*<sup>39k</sup> embryos which developed beyond gastrulation were cultured, the pulsation and syncytium formation of muscle cells, the proliferation of fibroblastic cells, the sphere formation of prospective imaginal disc cells, the extension and branching of nerve fibers of nerve cells and the droplet secretion on nerve fibers were normally observed as the same manner as those in wild-type embryonic cells.

On the other hand, epithelial cells grew and increased in size, but the brown chitinous pigmentation which was usually observed in wild-type embryonic epithelial cells was not observed in *r*<sup>39k</sup> epithelial cells.

##### Muscle and Cartilage Differentiation in Cultured Mesenchymal Cells from Embryonic Quail Limb-Buds

Yukiaki KURODA and Etsuya MATSUTANI

Mesenchymal cells in avian embryonic limb-buds differentiate into muscle

or cartilage in normal development. They are used for analyzing the mechanisms by which a common precursor cell is determined to differentiate into two phenotypically different types of cells. In the present study various culture conditions under which mesenchymal cells differentiated into muscle or cartilage were examined.

Limb-bud mesenchymal cells from 4 day (Stage 20-22) quail embryos were dissociated into single cells by treatment with EDTA and trypsin at 37°C after removal of the epidermis by cold incubation in trypsin solution. The cells were cultured in 35-mm petri dishes in various media supplemented with 15% fetal calf serum.

The growth of cells was largely dependent on the number of cells inoculated initially. When  $4 \times 10^5$  cells in petri dishes were cultured for 6 days in Medium No. 199 with serum, the number of cells increased as many as 4.4 times. When the number of inoculated cells decreased by half, no cell growth was found in the same incubation period. When the number of inoculated cells decreased to one-fourth, the number of cells decreased to one-tenth during incubation period of 4 days. The growth rates of cells were best in Medium No. 199 with serum, and then in Eagle's MEM with serum. No cell growth was found in Ham's Medium F12 with serum.

The differentiation of muscle cells and the formation of cartilage nodules were markedly accelerated by covering the cell layer in petri dishes with a cellophane membrane. With a cellophane membrane the differentiation of muscle cells was well progressed in Medium No. 199 with serum or Eagle's MEM with serum. The formation of cartilage nodules was accelerated in Ham's Medium F12 and Medium No. 199, each supplemented with serum.

### **Induction of 8-Azaguanine-Resistant Mutations by Sulfite in Cultured Embryonic Human Diploid Cells**

Yukiaki KURODA

Bisulfite is used as a preservative in wines, juices, foods and pharmaceutical products. It interacts with nucleic acid components and produces gene mutations in bacteriophage lambda, T4, *Escherichia coli* and yeast. In the present experiment the mutagenic effect of bisulfite on 8-azaguanine (8AG)-resistant mutations in cultured embryonic human diploid cells was examined.

Bisulfite reduced the colony-forming activity of cells to 65% and 0% of that of untreated cells at concentrations of  $10^{-2}$  M and  $10^{-1}$  M, respectively. The  $D_0$  value calculated from a concentration-survival curve for bisulfite was  $6.5 \times 10^{-2}$  M.

Triplicate inocula of  $10^5$  cells were treated with bisulfite at various concentrations for 2 hours, cultured in normal medium for a mutation expression time of 48 hours, and then selected with 30  $\mu$ g/ml of 8AG. The frequency of 8AG-resistant mutations increased as an increase in the concentrations of bisulfite employed for treatment. At a concentration of  $10^{-2}$  M of bisulfite the induced mutation frequency increased three times that in untreated control cultures.

The effect of mutation expression times on the frequency of induced mutations was examined. Among mutation expression times of 0, 24, 48, 72, 96 and 120 hours, a mutation expression time of 72 hours was most effective in inducing mutations by bisulfite. It was found that the number of cells in population increased 1.4 times during this mutation expression time.

### **Dose-Rate Effects of Ethyl Methanesulfonate on Survival and Mutation Induction in Cultured Chinese Hamster Cells**

Yukiaki KURODA and Katsura SUGIURA

In the field of radiation biology, the dose-rate effects on cell survivals and the induction of mutations have been extensively studied. On the other hand, a few reports have been made on the dose-rate effects of chemicals on cell survivals and the induction of mutations. In the present experiment the dose-rate effects of ethyl methanesulfonate (EMS) on the survival and the induction of mutations in Chinese hamster cells were examined.

The dose-rate effects of EMS on cell survivals were determined by the colony-forming activity of cells. Cells were treated with EMS at various concentrations for 1, 2, 3, 4, 4.5, 8, 16 and 20 hours, and then incubated in normal medium for 7 or 8 days. The most effective exposure time of EMS in reducing the survival fraction of cells was 4 hours. At shorter or longer exposure times than 4 hours EMS was less effective in reducing cell survivals. A threshold or minimum concentration of EMS giving a surviving fraction of 0.5 was found to be 0.1 mg/ml. No further decrease against this

concentration giving a surviving fraction of 0.5 may be shown even if cells might be exposed for infinite time.

Cells were treated with EMS at various concentrations for 1, 2, 4, 8, 16 and 20 hours, incubated in normal medium for 48 hours, and then selected in medium containing 0.1  $\mu\text{g/ml}$  6-thioguanine (6TG) for 10 days. An exposure time of 4 hours produced lower frequency of 6TG-resistant mutations than shorter or longer exposure times of EMS giving the same surviving fraction of cells. An exposure time of 20 hours produced the highest induced mutation frequency, suggesting that it may be useful as more sensitive procedure for detecting the mutagenic action of the chemicals using cultured Chinese hamster cells.

### **Selection and Characterization of a 5-Iodouridine-Resistant Variant in Cultured Syrian Hamster Cells**

Yukaiaki KURODA, Akiko YOKOYAMA and Tsuneo KADA

A variant cell line was obtained from Syrian hamster BHK 21 (C-13) cells after twice treatments with ethyl methanesulfonate and cyclic and continuous selections with  $10^{-3}$  M 5-iodouridine for 5 months. The colony-forming activity, growth rates in the presence of 5-iodouridine, 5-iododeoxyuridine (IUdR) and 5-bromodeoxyuridine (BUdR), distribution of chromosome numbers, uptake of  $^3\text{H}$ -thymidine ( $^3\text{H}$ -TdR) and cell surface properties of the variant cell line were compared with those of the original BHK cell line.

The variant cells were highly resistant to 5-iodouridine and showed cross-resistances to IUdR and BUdR. They grew more slowly and showed a short lag phase in normal medium. They showed a higher growth rate and a shorter doubling time in the presence of 5-iodouridine than in its absence.

The variant cells were more severely affected by short-term treatment with irradiated 5-iodouridine than the original BHK cells. The variant cells also showed less uptake of  $^3\text{H}$ -TdR into the cold TCA-insoluble fractions than the original cells and differed from the latter in morphology and mutual cohesion. This may be the first observation on the marked cross-resistances of the variant cell line to 5-iodouridine, IUdR and BUdR in cultured mammalian cells. For details, see *Japan. J. Genetics* 52: 133-147, 1977.

### Effects of *SR* and *da* on the Viability of Hybrid Males from *Drosophila melanogaster* × *D. simulans* Cross

Takao K. WATANABE and Masa-Aki YAMADA

The *NSR*-spirochetes injected to *D. simulans* killed sons in the embryonic stages and the *SR* condition was established in this species (*simulans-NSR*) by transmission of the spirochetes from mother to daughter through generations.

Hybrid progenies from normal *simulans* mothers and *melanogaster* fathers (control cross) were mostly (99.7%) males as expected, while those from *simulans-NSR* mothers, though the number was very small (2.0 per cross, when control was 37.0), were 35% males and 65% females. All these males and females carried *SR*-spirochetes when their hemolymph was examined. Since *NSR*-spirochetes usually killed males in the embryonic stages in *melanogaster* as well as in *simulans*, the appearance of adult males suggests that the hybrid males are somewhat resistant to the male-killing effect of *SR* agent.

A slight resistance to *NSR* killing effect on the hybrid males was recognized in the cross of *melanogaster* attached-X (*yf*:=) females × *simulans* males. Normal (*yf*:=) females produced 99.9% hybrid sons, but *SR* (*yf*:=-*NSR*) females did not have any adult offspring. Many larvae and pupae died on the wall of vials in the latter cross, and some (eye pigmentation stage) were proved to be males from their sex combs and genitalia.

The homozygous *da* (*daughterless*, 2-39 of *melanogaster*) females produce only sons regardless of the husband genotype, since the daughters die in the embryonic stages. When the *da/da* females were crossed with *simulans* males, all hybrid progenies died as larvae. Salivary gland chromosomes proved them to be males. Thus, sons were killed by the hybrid effect and daughters were killed by the *da* gene. For details, see Japan, J. Genetics 52: 9-14 (1977).

### Characterization of a Regeneration Deficient Strain of Hydra

Tsutomu SUGIYAMA and Toshitaka FUJISAWA

A mutant strain of *Hydra magnipapillata* (reg-16) with regeneration deficiency was characterized in detail.

The number of the tentacles regenerated per animal was used as a

measure of the regeneration capacity. Wild type strains generally had about 6 tentacles per animal and after amputation of the head and the foot, they regenerated practically the same number of the tentacles within 5 to 6 days. In contrast, reg-16 regenerated only about 10% of the original tentacles during the same period of time. The tentacle regeneration, however, was substantially enhanced when the regenerating fragment was subdivided longitudinally.

Lateral tissue implants that induced head formation in wild type hydra either regressed or induced foot formation in reg-16 polyps. This suggests that the regeneration deficiency in reg-16 is due to a defective polarity gradient.

A chimera strain was produced by introducing reg-16 interstitial cells into nf-1 strain (see next report). The chimera regenerated normally, suggesting that the defect of reg-16 is not located in the interstitial cells or nerve cells but probably in the epithelial cells.

### **Isolation and Characterization of an Interstitial Cell Deficient Strain of Hydra**

Tsutomu SUGIYAMA and Toshitaka FUJISAWA

A mutant strain of *Hydra magnipapillata* (nf-1) was isolated that contained no interstitial cells and their derivatives, nerve cells and nematocytes. This strain appears spontaneously in a mass culture of a strain (sf-1) which appears normal in every respect except in one: it occasionally gives rise to nf-1. Although nf-1 is unable to capture food by itself, it can grow and multiply when force-fed. Thus nf-1 is a suitable strain to study the roles of interstitial cells and their derivatives, especially the roles of nerve cells in morphogenesis.

Nf-1 regenerated normally retaining its original polarity when the head or the foot or both were amputated. The polarity gradient levels in nf-1 and its parental strain, sf-1, when examined by lateral grafting experiment, showed no significant difference.

A chimera strain was produced by repopulating interstitial cells from a wild type strain into nf-1. The comparison of growth rates between the wild type, sf-1, nf-1 and the chimera strain showed that the chimera grew as fast as the wild type and faster than sf-1 and nf-1.

These results suggest that interstitial cells and their derivatives play

very limited role in morphogenesis, but that nerve cells may play some "fine tuning" roles in hydra development which are basically established by epithelial cells.

### **Characterization of a Nematocyte Deficient Strain of Hydra**

Toshitaka FUJISAWA and Tsutomu SUGIYAMA

Nematocytes are hydra's stinging cells and they are located mainly in the tentacles where they are used primarily to capture and paralyze prey. *Hydra magnipapillata* has four types of nematocytes (stenotele, holotrichous isorhiza, atrichous isorhiza and desmoneme) which together account for one third to one fourth of the total body cells.

The nematocyte deficient strains can be isolated by sexual crosses. One such strain, *nem-4*, has virtually no stenoteles in its tentacles, but contains them at a normal level in the body column. And the stenotele nematocytes of *nem-4* turns over normally.

Grafting experiments between the *nem-4* head region and the wild type body column (and vice versa) showed that the wild type stenotele nematocytes can migrate into the *nem-4* tentacles but the *nem-4* stenotele nematocytes cannot move into the wild type tentacles. These observations suggest that the stenotele nematocytes of *nem-4* are produced normally by differentiation from the interstitial cells in the body column, but that they are somehow prevented from migrating into the tentacles in this strain.

### **The Relationship of Mitotic Activity of Primordial Germ Cells to Testicular Teratocarcinogenesis in Strain 129 Mice**

Takehiko NOGUCHI and Leroy C. STEVENS\*

Teratomas are tumors which have several unusual and interesting characters. They are usually composed of many different kinds of tissues. Teratomas are most commonly found in gonads. Spontaneous testicular teratomas are seldom found in laboratory mice except in the inbred strain 129. At present several substrains of 129 showing different teratoma incidences have been developed by introducing some mutant genes onto the genetic background of original 129. Testicular teratomas derive from primordial germ cells (PGC) in embryonic testes. Very small fractions of

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these PGC begin to develop, presumably through a process akin to parthenogenesis. Many non-genetic as well as genetic factors (or conditions) influencing the teratocarcinogenesis have been known. But little is known how these factors (or conditions) stimulate or inhibit the parthenogenesis-like activation of PGC.

In order to find a clue to this problem, developmental biological approach was made. Several experimental evidences indicate that the activation of PGC may occur during the developmental stages ranging from 12 days to 16 days of gestation. During this period the sex cord of male genital ridges develops into seminiferous tubules. PGC become mitotically very quiet a couple of days after they are enclosed into seminiferous tubules. As PGC under mitosis can easily be distinguished from the other cells, the mitotic rate of PGC was chosen as a marker of PGC differentiation during the period in question. The mitotic rate of PGC in fetal testes was determined with hematoxylin-eosin stained paraffin sections for several substrains of 129 mice that differ in incidence of testicular teratomas. If the mitotic rate is expressed by the number of mitoting PGC per testis, it peaked at 13 days of gestation and then decreased precipitously. At 15 to 16 days when early teratomas start to appear, the mitotic rate of PGC became very low. Testes of 129/Sv-ter which has the maximum incidence (33%) of teratomas, however, quite often contained larger number of PGC under mitosis at this late stage than usual. Probability for testes to have teratomas was several times higher in testes showing unusually high level of mitosis of PGC than in those showing normal level of mitosis. The incidence of testes containing unusually larger number of PGC under mitosis at the late stage was far less in the other substrains of 129 which have much lower incidences of teratomas.

When 12-1/2 day male genital ridges of 129×A/He are grafted to scrotal testes, they develop into testes and most of them have teratomas. When they are grafted to cryptorchid testes or to kidneys, the incidence becomes lower, although they develop into testes. In kidneys few of them has teratomas. In grafts in scrotal testes, the time when PGC became mitotically quiescent was considerably delayed. When 12-1/2 day genital ridges were grafted for 4 days in these adult sites, the mitotic rate of PGC was the highest in scrotal testes and was the lowest in kidneys. The delay in entering in a mitotically quiescent stage of PGC is well correlated with the high incidence of testicular teratomas.

## V. CYTOGENETICS

### Karyotypes of Four *Myotis* Bats in Japan

Masashi HARADA and Toshide H. YOSIDA

Cytotaxonomical investigations of four *Myotis* bats collected in Japan were carried out by conventional, G- and C-band staining techniques. The four species all had  $2n=44$  and their karyotypes were, excepting one chromosome pair, identical to each other. The only difference in their karyotypes was found in the morphology of the chromosome no. 5. A minute acrocentric (A) was observed in *M. nattereri* and a polymorphic (A) and ( $M^h$ ) which is a minute metacentric with a totally heterochromatic short arm was found in *M. hosonoi*. In *M. frater kaguyae* the pair no. 5 was a small submetacentric with a totally heterochromatic long arm ( $SM^h$ ). Polymorphic ( $SM^h$ ) and (M), which is a small metacentric induced from ( $SM^h$ ) by pericentric inversion, were seen in *M. macrodactylus*. Such a morphological differentiation of the no. 5 was interpreted by assuming the inversion and also duplicating growth of the constitutive heterochromatin. Their evolutionary pathway in genus *Myotis*, thus, is assumed to be as follows: (A)→( $M^h$ )→( $SM^h$ )→(M). This assumption was supported by the geographical evidence that the species with the (A) type of the pair no. 5 widely distributed throughout the world but the others are restricted to Asia ( $M^h$  type) and Japan in particular ( $SM^h$  and M types). Based on the differentiation of the chromosome no. 5, it is suggested that *M. nattereri* and *M. hosonoi* are phylogenetically closely related, while *M. f. kaguya* and *M. macrodactylus* are of another kin relationship.

### Geographical Distribution of No. 18 Chromosome Polymorphism in the Japanese Feral Mouse, *Mus musculus molossinus*

Kazuo MORIWAKI and Mitsuru MINEZAWA\*

Chromosome C-band patterns of the Japanese feral mice, *Mus musculus molossinus*, have been already observed by Dev *et al.* (Chromosoma 53: 335, 1975) who revealed that their bands in Nos. 1, 2 and 4 chromosome were larger than those of *M.m. domesticus* and those in Nos. 13 to 17 were smaller.

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We collected *molossinus* mice from various localities in Japan and examined the chromosome G- and C-band patterns. Characteristic features in their C-band patterns were almost similar as those reported by Dev *et al.* Moreover, an interesting C-band polymorphism in No. 18 chromosome has been found out. There were three types of the C-band pattern in the No. 18 chromosome; positive band (+), large positive band (+<sup>1</sup>) and positive band in the middle of the arm (+<sup>m</sup>). Results obtained from ten individuals are summarized in Table 1.

Table 1. C-band pattern polymorphism in No. 18 chromosomes of *Mus musculus molossinus*

Sample No.	Place of collection	C-band of No. 18 chromosome
1	Teine, Hokkaido	+ <sup>1</sup> /+ <sup>1</sup>
2	Johoji, Northeastern Mainland	+ <sup>1</sup> /+ <sup>1</sup>
3	Gotenba, Middle Mainland	+ <sup>1</sup> /+ <sup>1</sup>
4	Anjyo, Middle Mainland	+ <sup>m</sup> /+ <sup>m</sup>
5	Kanazawa, Middle Mainland	+ <sup>m</sup> /+ <sup>m</sup>
6	Mine, Southwestern Mainland	+ <sup>m</sup> /+ <sup>m</sup>
7	Hakozaki, Kyushu Island	+/+
8	Sasaguri, Kyushu Island	+/+
9	Yonakuni Island	+ <sup>m</sup> /+ <sup>m</sup>
10	Okinawa Island	+ <sup>1</sup> /+ <sup>1</sup>

+ : Positive C-band in No. 18. +<sup>1</sup> : Large positive C-band. +<sup>m</sup> : Positive band in the middle of No. 18.

The geographical distribution of those polymorphic chromosomes likely suggests possible immigration processes of *molossinus* mouse into Japan. Probably the first immigrants with a large positive C-band in No. 18 chromosome came to this country through the Korea Peninsula and spread all over Japan. The next was those with positive C-band in the middle of No. 18 and the latest those with positive C-band of No. 18 as similar as that of *M.m. domesticus*. The last ones are distributed still in the restricted area of Northern Kyushu. Geographical distribution of No. 18 polymorphism summarized in Table 1 may reflect the possible processes of repeated immigration of *M.m. molossinus* into the southwestern Japan as proposed above.

**Hybrids among Three Geographical Variants of the Black Rats, 1.**  
**Hybrids between Asian and Ceylonese Type Black Rats,**  
**and Their Karyotypes**

Toshide H. YOSIDA

Delivery of hybrids in the laboratory matings between the Asian (*Rattus rattus tanezumi*) ( $2n=42$ ) and the Oceanian type black rats (*R. rattus rattus*) ( $2n=38$ ) and their karyotypes have already been reported (Yosida *et al.*, 1971, *Chromosoma* 34: 40; Yosida, 1976, *Proc. Jap. Acad.* 52: 304). However, any hybrids between the Asian and Ceylonese types ( $2n=40$ ) and also between Oceanian and Ceylonese types have not yet been reported. The present report deals with the  $F_1$  hybrids between Asian ( $2n=42$ ) and Ceylonese type black rats ( $2n=40$ ) and their karyotypes. The Asian type (*R. rattus flavipectus*) and Ceylonese type (*R. rattus kandianus*) were collected in Hong Kong and Sri Lanka, respectively, and then bred in this laboratory for several years.

Ten  $F_1$  hybrids were obtained by matings between Ceylonese type (♀) and Asian type (♂) black rats. They invariably had 41 chromosomes, among which pair no. 11 was composed of one large metacentrics ( $M_2$ ) and one acrocentrics. The acrocentric no. 12 was not paired. The partner of the no. 12 is homologous to the short arm of the  $M_2$  chromosome. Pairs no. 1 and 9 were acrocentric and subtelocentric heteromorphism. It is obvious that the subtelocentric pairs no. 1, 9 and the large metacentrics ( $M_2$ ) originated from the Ceylonese type, while the acrocentrics in the pairs no. 1, 9, 11 and 12 were derived from the Asian type rat. All chromosome pairs were detected by the conventional and G-band sequential stainings. The karyotype of the  $F_1$  hybrid was then formulated as follows:  $2n=41$  ( $4S+20A+14SM+1M+XY$ ) (S=subtelocentrics, A=acrocentrics and SM=small metacentrics). Although the effort to get the  $F_2$  hybrid from them had been taken place, it has not yet been obtained until this time. The  $F_1$  hybrids seem to be semisterile as seen in the hybrids between Asian and Oceanian type black rats.

**Hybrids among Three Geographical Variants of the Black Rats, II.**  
**F<sub>1</sub> and F<sub>2</sub> Hybrids between Oceanian and Ceylonese Type**  
**Black Rats and Their Karyotypes**

Tosihide H. YOSIDA

By laboratory matings between Oceanian type (*Rattus rattus rattus*,  $2n=38$ ) originally from the United States and Ceylonese type black rats (*R. rattus kandianus*,  $2n=40$ ) originally from Sri Lanka, 15 hybrids (♀ 8: ♂ 7) were obtained. They invariably showed 39 chromosomes. The karyotype was remarkable in having 3 large metacentrics. One of them was the  $M_1$  chromosome resulting from Robertsonian fusion of the pairs no. 4 and 7 and the other two are the  $M_2$  pairs resulting from the fusion of the pairs no. 11 and 12. Pair no. 4 is heteromorphic by the  $M_1$  (4/7) and one acrocentric no. 4. Pair no. 7 was acrocentric and not paired. The partner of the pair no. 7 is translocated to the  $M_1$  chromosome. Pairs no. 1, 9 and 13 were subtelocentrics which were derived from both parents. Seven small metacentric (SM) pairs (no. 14-20) were not different from those of the other black rats. Thus, the karyotype of the  $F_1$  hybrids was formulated as follows;  $2n=39$  (6S+14A+1 $M_1$ +2 $M_2$ +14SM+XY).

A total of the 12 (6♀: 6♂)  $F_2$  offspring were obtained in three litters from the hybrids matings. The average litter size of the  $F_2$  was 4.0 which was an almost normal condition. Karyotypes of 9 (6♀: 3♂) rats among them were analysed. Three karyotypes, namely Oceanian type ( $2n=38$ ),  $F_1$  type ( $2n=39$ ) and Ceylonese type ( $2n=40$ ), were expected theoretically at the ratio of 2.25: 4.5: 2.25. The observation, therefore, resulted in 6  $F_1$  types and 3 Oceanian types. No Ceylonese type was observed at this time.

$F_2$  offspring were not obtained from the hybrids between Asian and Ceylonese types, but the hybrids between Ceylonese and Oceanian types were apparently fertile. Based on these findings, it is suggested that the Oceanian type is more closely related to the Ceylonese type than the Asian type black rat. This result seems to coincide with the karyotype evolution in these three geographical variants already postulated by the present author (Yosida *et al.*, 1974, *Chromosoma* 45: 99; Yosida 1977a, *Chromosoma* 60: 391, Yosida 1977b, *Cytogenet. Cell Genet.*, 18: 149).

**Hybrids among Three Geographical Variants of the Black Rats, III.  
An XO Female Occuring in the F<sub>2</sub> Hybrid between Oceanian  
and Ceylonese Types**

Tosihide H. YOSIDA

Among 6 females in F<sub>2</sub> offspring from the hybrids between Oceanian and Ceylonese type black rats, one female showed 37 chromosomes. The fundamental karyotype of the female was the Oceanian type. All 20 cells from bone marrow and cultured tail tip tissue of the rat had invariably 37 chromosomes. By analysis of G-band staining it was clearly shown that the sex chromosome mechanism of this rat was of the XO type. The rat was apparently a normal female, but its fertility was not confirmed because it was killed for the chromosome observation. The other 5 female and 2 males born of the same parents which produced the XO female showed normal XX (♀) and XY (♂) types. Both parents had also a typical karyotype comprising the regular sex chromosome mechanism with the XX (♀) and XY types (♂).

Presence of an XO female has already been reported in one Oceanian type black rat obtained in Davis, California (Yosida *et al.*, 1974, *Jap. J. Genet.* 49: 49). The monosomic X has been confirmed by G-band staining. The fertility of the animal was also unknown, because the rat died about 6 months after capture without reproducing. Therefore, whether the XO female of the black rat is fertile or not is uncertain at present. Another example of the XO female was found among the black rats collected in New Guinea in 1968. This rat was also basically an Oceanian type (Yosida, unpublished). Based on these findings, it can be said that the present XO female found in the F<sub>2</sub> offspring is not a specific case in the hybridization, but rather a natural and sporadic case as seen in the other black rats.

**Candidates for a New Experimental Animal, I.  
The Soft-Furred Rat, *Millardtia meltada***

Tosihide H. YOSIDA

The soft-furred rat, *Millardtia meltada*, collected in India has been bred successfully in the laboratory by brother and sister matings for about 10 generations by use of our breeding system. The relative size of this

animal is about midway between the mouse and the rat. The mean body weight of 30 males and 30 females in 12 weeks old is  $82.7 \pm 16.0$  g and  $59.9 \pm 8.1$  g, respectively. The number of mammae in the animal is eight (2:0:2). They are easily handled in the laboratory due to its gentle behaviour. Laboratory breeding of the animal is easy by use of the usual rat cage. Average litter size is 4.09 (based on 21 litters observed). The gestation period of this animal is 20 days on the average, and about 12 days after the birth the eyes open. Diploid chromosome number of the animal is 50 among which two autosome pairs (pairs no. 1 and 2) are large metacentrics, 21 autosome pairs (nos. 3 to 23) are acrocentrics, and the one autosome pair (no. 24) is a small metacentric. The X is a large metacentric and Y a median sized acrocentric.

Some interesting characteristics of this animal were as follows: 1) The new born baby within one day has thin hair on its body. 2) Well developed inciser teeth have been found in the new born. 3) The age of the animals seems to be shorter (about one year) than the other rodent species. 4) Individuals with low blood pressure are often found among the animals. Based on the above characteristics, this animal is one of the more promising candidates for a new experimental animal.

### Subtelocentric No. 3 Chromosome in the Japanese Black Rat—a New Inversion

Toshihide H. YOSIDA and Yuriko OCHIAI

The Japanese black rat, *Rattus rattus tanezumi*, was generally characterized as having 42 chromosomes (21 pairs). Among them pairs no. 1, 9 and 13 were characterized by acrocentric and subterocentric polymorphism. Pair no. 3 in about one thousand black rats collected in several countries in the world, however, was always characterized by an acrocentric centromere. One male black rat collected in the field of Gotenba, Shizuoka-ken, Japan, had 42 chromosomes as the other Asian type rats. Pairs no. 1 and 9 of this rat were acrocentrics, but pair no. 13 was an acrocentric and subtelocentric heteromorphism. Out of the heterologous pair no. 13, the pair no. 3 was remarkable because it had an unexpected acrocentric and subtelocentric heterologous pair. As the length of the acrocentrics and subteleocentrics was almost similar, the subtelocentrics would seemed to have developed by pericentric inversion of the originally acrocentric chromosome.

From the G-banding analysis, it was proved that pericentric inversion could have occurred in about one-third of the acrocentric no. 3, and then the subtelocentrics developed.

On the origin of polymorphism in pairs no. 1, 9 and 13 in the black rat, the present author suggested that the original type of these chromosomes were acrocentrics. The subtelocentrics of these members have developed by pericentric inversion. New observation of the subtelocentric no. 3 seems to prove the above suggestion. The rat demonstrating thus is being maintained to observe fertility and no. 3 chromosome behavior.

### **An Experiment on Artificial Insemination between the Norway Rat and the Black Rat**

Toshihide H. YOSIDA and Choji TAYA

To ascertain the relation between the black rat and the Norway rat, artificial insemination between these two species was taken. As the female recipient, inbred strains of the Norway rats (*Rattus norvegicus*) were used. Semen was obtained from the dissection of the cauda epididymis of the black rat (*Rattus rattus flavipectus*). When the female rat was at the proestrus stage of the estrus cycle, the cervical uterus was stimulated by faradic current. Then the semen was injected into the uterine horns through each cervical canal by the hypodermic needle. For the control experiment, the semen from the Norway rat was inseminated artificially.

Among 20 Norway rats which were inseminated artificially with the semen of the black rat, 9 rats were successfully fertilized. In 5 of the rats the impregnation was confirmed by dissection. Between one and 4 embryos (2.2 on average) were confirmed in the impregnated rats. Offspring, however, were never born in the case of interspecific hybridization, and the embryo always died at a maximum age of about two weeks after the insemination. In the control, 25 rats were treated and 23 among them were impregnated successfully. Seven impregnated females were dissected and the number of impregnated embryos were counted. In this case there were between 3 and 11 embryos (6.3 on average). From the other females impregnated young were born normally. Thus, the number of embryos in the case of interspecific hybridization was significantly less than the control.

For the observation of chromosomes, the blastocysts were obtained from the uterus 5 days after insemination, and then chromosomes were observed



by drying technique. The Norway rat and the black rat together have 42 chromosomes and their karyotypes are similar (Yosida 1973, Chromosoma 40: 285). Pairs no. 1, 9 and 13, in the black rats (*R. rattus flavipectus*) were acrocentrics, but those in the Norway rat were subtelocentrics. Five hybrid blastocysts examined showed invariably 42 chromosomes, among which the nos. 1, 9 and 13 chromosomes were remarkable as having heteromorphic pairs. The blastocysts from the control had the regular karyotype of the Norway rat which consisted of the subtelocentric pairs no. 1, 9 and 13. Based on the above investigations, it can be said that although the Asian type black rat has the same chromosome number and a similar karyotype to the Norway rat, evolutionary difference between these two species is much larger than those among the geographical variants in the black rats, even though each of them is characterized by having a different chromosome number and karyotype.

## VI. MUTATION AND MUTAGENESIS IN ANIMALS

**Genetic Analysis of Spontaneously Derived Mosaics from  
Abnormal Fertilization in the Silkworm**

Akio MURAKAMI and Hisashi FUJIOKA

In the course of experiments on the spontaneously-induced egg-colour specific locus (*pe: re*) mutations of the silkworm females, the mosaic mutant having yellow (*pe*) and black (*pe*<sup>+</sup>) serosa cells at the ratio of 1:1 has repeatedly been detected at the high frequencies ( $103-645 \times 10^{-5}$ ) in wild type (C108 or *Aojuku* strain) female pupae treated with (or without) 0.85% NaCl solution. These treated females were mated to marker strain (*pe: re*) males. However, the frequency of the other type mosaics having red (*re*) and black (*re*<sup>+</sup>) serosa cells ( $0.0-2.1 \times 10^{-5}$ ) was not significantly differed from usual ones. In addition, such the phenomenon was not observed when the marker females were mated to the wild type males.

Hatchability of these mosaic mutant eggs derived from the former mating cross was approximately 5% and this was corresponded to about one sixteenth of non-mutated F<sub>1</sub> eggs. Of nineteen mosaic eggs hatched so far, six were gynandromorphs and two females and eleven males. Both females and males were again mated to a marker strain homozygous for the *pe* gene and they were produced without exception yellow and black eggs with a 1:1 ratio, indicating that no germ-cell of the yellow part (*pe: re/pe: re* or *pe/pe*) might be produced or survived probably due to lethal events caused by androgenesis. It should be emphasized that in this mutation test system the egg-colour of the non-mutated F<sub>1</sub> (+: +/*pe: pe*) individual is black and the *pe* gene is epistatic to the *re* gene, so that the eggs homozygous for both genes (*pe: re/pe: re*) are yellow. Thus, it is likely that the black part of the mosaics (+: +/*pe: re*) may be formed by normal fusion of egg (+: +) and sperm (*pe: re*) nuclei and would be expressed the maleness and femaleness with an equal probability, while the yellow part may be formed via either fusion of two sperm nuclei (*pe: re/pe: re*) or development of one sperm (*pe: re*) nucleus showing only the maleness. When cleavage begins, the derivatives of the two resulting cleavage nuclei with different gene constitutions (either *pe*<sup>+</sup>/*pe* or *pe/pe*) populate the opposite (right and left, in general) sides of the ovum (or egg). If the derivatives of the two cleavage

nuclei having different sex-chromosome constitutions (XX for male, XY for female) were populate the opposite sides resulting in a sexual mosaic or gynandromorph. Lack of  $BF_1$  progeny produced only yellow eggs may be supported the view that germ-cells of the yellow part (probably maleness) is formed as the result of androgenesis and that would be showed an extremely high lethality by recessive lethal mutations on the X chromosome. As to the origin of the black part, it can be rule out a possibility of autogramic fusion of two haploid derivatives in products of female meiotic divisions, since both yellow and black eggs were observed in  $BF_1$  progeny, regardless of sex of the mosaics analyzed.

To sum up, the origin of the half-and-half type mosaics can be interpreted on the assumption that one part of an ovum is formed via normal fertilization between oocytes ( $pe^+ : re^+$ ) and sperm ( $pe : re$ ) and the other part is formed by either homozygous diploid or monospermic androgenesis, suggesting that the origin of these mosaics is essentially differed from that of a hereditary mosaics (*mo*), due to fertilization of an egg-nucleus and one of three polar-bodies (Goldschmidt and Katsuki, 1931). Unfortunately, the mechanism(s) of spontaneous appearance of the mosaics are not known. It is more likely, however, that the phenomenon may depend on a functional disorder of cytoplasm in oocytes/eggs being due to genetic or infectious factors.

### Chemically-Induced Transmitted Translocations in the Silkworm

Akio MURAKAMI and Tetsuro MUROTA<sup>1)</sup>

Transmitted translocation, that is translocations induced in the post-meiotic germ-cells and subsequently transmitted to the progeny leading to a reduction in fertility owing to abnormal meiotic divisions, can be able to detect by either genetic or cytogenetic method. In lepidopterous insects, this type genetic incidence is simply detected by an increase of embryonic lethality (or sterility) of the  $F_1$  progeny so that the test method described in this communication would be recommended for the detection of chemicals inducing such the translocations. A practical procedure for the detection of this type genetic damage is to measure the reduction of hatchability in fertilized eggs of the first generation offspring of treated parents or the  $P_1$  progeny. This report describes the result of transmitted translocation tests

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in the sperm after treatment of the mid-stage male pupae with mitomycin C (MC).

In the present test, wild type (C108 strain) male pupae were injected with a 10  $\mu\text{g}$ /pupa of MC, mated to a different strain (*pe: re*) females to minimize a noise being resulted from recessive lethal mutations and survived  $F_1$  individuals were further mated to the marker (*pe: re*) strain to determine the embryonic lethality as the reduction of egg-hatchability of the  $F_1$  progeny as being due to the incidence of heritable translocations.

The results of the experiments summarized in Figure 1 showing a typical case of the transmitted lethality in which hatchabilities of the  $F_1$  progeny derived from the treated male parents were lower than those of the  $P_1$  progeny. However, the hatchability of the  $BF_1$  progeny was higher than those of both  $P_1$  and  $F_1$  progenies. This recovered hatchability in the  $BF_1$  progeny may be interpreted by the selection of the cells having translocations lead to lethality during the course of gametogenesis in the back cross  $F_1$  generation (or  $F_2$ ).

$F_1$	$BF_1$	$BF_2$
$pe: re \text{♀} \times ++ \text{♂}$ (85.7%)	$F_1 \text{♀} \times \text{nontreated } pe: re \text{♂}$ (73.3%)	$BF_1 \text{♀} \times \text{nontreated } pe: re \text{♂}$ (92.2%)
MC $\swarrow$	$\text{nontreated } pe: re \text{♀} \times F_1 \text{♂}$ (64.9%)	$\text{nontreated } pe: re \text{♀} \times BF_1 \text{♂}$ (87.0%)
		$BF_1 \text{♀} \times \text{nontreated } pe: re \text{♂}$ (106.8%)
		$\text{nontreated } pe: re \text{♀} \times BF_1 \text{♂}$ (88.2%)

Fig. 1. Changes in hatchabilities of eggs for three generations after treatment of the mid-stage male pupae (or sperm) with 10  $\mu\text{g}$ /pupa of mitomycin C. The numbers in parentheses indicate the hatchability in percent of controls.

As communicated in the previous report (this Report No. 26: 42-44 (1976)) that the sperm in the mid-stage pupae was the least sensitive to MC for the induction of dominant lethal mutations during the post-meiotic stage of this insect male. The present and other findings tend to suggest that the low sensitivity of the sperm at this pupal stage may be at least in part due to effective reparability for broken DNA molecules or chromosomes. From this context, it can be possible to said that the inherited lethality caused by translocations may be formed via incorrect repair processes for broken DNA.

### Drug Metabolic Activation in the Silkworm

Kyoko SAKAMOTO<sup>1)</sup>, Tetsuro MUROTA<sup>1)</sup> and Akio MURAKAMI

For the past few years, we have been studied on the production of the egg-colour specific locus mutation in pupal oocytes and sperm with a number of known indirect mutagens (such as benzo(a)pyrene, BP; dimethylnitrosamine, DMNA; 4-nitroquinoline-N-oxide, 4NQO; 2-acetylaminofluorene, AAF and so on) which require metabolic activation to exert their mutagenic activity. The first three mutagens so far tested have not yet been shown to be a positive result in these germ-cells, while AAF has been shown to be a very weak mutagenicity in pupal oocytes. This may be interpreted as the deficiency of these drug metabolic activation systems in pupal silkworm.

This communication reports the activity of AAF metabolic activation in pupae as well as other developmental stages of the insect. The metabolic activity for AAF was measured by a slight modification of the *Salmonella*/microsome test (cf., Ames *et al.*, 1973, Proc. Natl. Acad. Sci. U.S. 70: 2281). Each biological sample was homogenized in three volumes of ice-cooled 1.15% KCl solution with a Teflon homogenizer. The homogenate was centrifuged at 5°C and 9000 g for 30 min. The resulting supernatant with the cofactor system was used for the measurement as an S-9 reaction mixture (S-9 mix). The S-9 mix, *Salmonella* tester strains (TA 98 and TA 100) and AAF is incubated at 37°C for 20 min with shaking before pouring on a minimal glucose agar plate with aid of molten top agar. The cofactor system is contained NADPH, NADP, G-6-P, MgCl<sub>2</sub> and is further added G-6-P- dehydrogenase. The plates are incubated at 37°C for 48 hrs and the revertant colonies to His<sup>+</sup> prototrophy are counted.

The result of experiments clearly indicated that the activity of the AAF metabolic activation by homogenates derived from various developmental stage silkworms was markedly changed with the stage. The activity was increased with the larval growth or the amount of mulberry leaves ingested. The marked activity was observed in the homogenate from the last (5th) instar larvae, not in embryos and pupae. It is of worthwhile to note that the activity of AAF metabolic activation by the last instar larvae was almost the same or somewhat higher than that by the rat liver in which rats had been pretreated with polychlorinated biphenyl (KC-500). Homogenates of

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mid-gut and blood in the last instar larvae were shown to be the same activity as the whole insect.

No significant mutagenicity of the induction of the egg-colour specific locus mutations in spermatocytes/spermatids after treatment of even the last instar larvae with AAF was observed, therefore, suggesting that the lack of mutagenicity of AAF in such the test system may be related to the nature of mutants detected by the specific locus method. Namely, much higher doses of active metabolites which interact with DNA would be required to induce the specific locus mutations including deletion and chromosome breakage in contrast to the molecular incidence detected by the *Salmonella*/microsome test system. By the way, one of possible mechanisms responsible for the lower mutagenicity of AAF to pupal oocytes is that this parent compound itself may interact with DNA presumably through intercalation leading to frameshift type mutations.

In any case, the assay method for AAF metabolic activations may be useful to other indirect mutagens in this insect.

### **Comparison of Some Nitrosonitroguanidines in Regards to Their Efficiency in Inducing Recessive Visible Mutations in Silkworm Germ-Cells**

Akio MURAKAMI and Masanori NAKADATE<sup>1)</sup>

It is a well known fact that N-methyl-N'-nitro-N-nitrosoguanidine or Methylnitrosonitroguanidine (MNNG or MeNNG) is a highly mutagenic and carcinogenic chemical in various biological organisms including *Salmonella*, cultured cells, *Drosophila* and so on. In *Drosophila*, it has been reported that MeNNG induces a broad mutational spectrum in all germ-cell stages with a higher frequency of gene or point mutations than that of other class ones (Browning, 1969). According to Nakao and his associates (1966), however, they have reported that in silkworm MeNNG is a low mutagenic chemical for inducing egg-colour specific locus mutations in male silkworms. The present experiment was undertaken to obtain more information for the mutagenicity of MeNNG as well as four other nitrosonitroguanidines (Ethylnitrosonitroguanidine, ENNG; Propylnitrosonitroguanidine, PNNG; Butylnitrosonitroguanidine, BNNG; isobutylnitrosonitroguanidine, iBNNG) to germ-cells of male and female silkworms. The

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mutagenicity was measured by the egg-colour specific locus method. The late stage pupae (or the last few days before emergence) were injected with the nitrosonitroguanidines which had been dissolved in 0.85% NaCl solution.

The incidence of fractional-body or mosaic mutations in pupae treated with nitrosonitroguanidines was more frequently induced than that of whole-body ones regardless of the compounds tested and germ-cell types. The incidence of the latter type mutations in both oocytes and sperm after treatment of pupae with ENNG was slightly higher than that of controls, but not significantly increased with MeNNG, BNNG and iBNNG. On an equimolar basis at the time of injection, a comparison of the frequency for the fractional-body mutations induced with these five nitrosonitroguanidines in pupal oocytes and sperm was made: the mutagenicity was follows, ENNG, PNNG, MeNNG, BNNG and iBNNG in this order of effectiveness. Thus, it can be said from this experiment that MeNNG is not always highly mutagenic for production of the egg-colour specific locus mutations in both oocytes and sperm of the silkworm. This experimental result is in good accord with that after Nakao *et al.* (1966). In any case, it should be said that mutagenicity of ENNG is equivalent to the known potent mutagens such as ethyl methane sulphonate (EMS) and mitomycin C (MC) in this insect. It is of interest to note that MeNNG and methyl methane sulphonate (MMS) are more highly toxic, but lower mutagenic to silkworm pupae than ENNG and EMS. This difference may be caused by lack of an excision repair system in germ-cells of the silkworm as in an excision deficient stock of *Salmonella* in which ENNG is more mutagenic than MeNNG (Matsushima *et al.* 1977).

### **Enhancing Effect of Caffeine on X-Ray-Induced Dominant Lethal Mutations in Silkworm Sperm**

Akio MURAKAMI and Hiyoriko NISHIJIMA

Silkworm females at the stage of mid-pupae or prophase I oocytes were treated with a purine analogue, caffeine (1,3,7-trimethylxanthine) then mated to male moths or mature sperm that had been irradiated with X-rays. The mutation frequency of X-ray-induced dominant lethals in the sperm was measured. The frequency of this class lethal mutation was determined by dividing the number of dead embryos or non-hatched eggs by the total

number of fertilized eggs. For caffeine treatments, one of various doses of caffeine which had been dissolved in 0.85% NaCl solution or physiological saline was injected into the abdominal cavity of the pupae. Controls were injected with 0.85% NaCl solution without caffeine. In the irradiation, male moths were exposed to various doses of 180 kVp X-rays (25 mA, 1.0 mm Al filter and a dose-rate at 300 R/min) ranging from 2,500 to 15,000 R.

When female pupae at the mid-stage were treated with caffeine at the dose over 200  $\mu\text{g}/\text{pupa}$ , she showed extremely high sterility such as inability to mate and infecundity. However, no dominant lethality was induced in sperm after treatment of the mid-stage male pupae with caffeine of doses varying from 0.1 to 300  $\mu\text{g}/\text{pupa}$ . When various doses of X-rays were exposed to male moths and then mated to female moths which had been treated with caffeine (25 to 250  $\mu\text{g}/\text{pupa}$ ), frequencies of X-ray-induced dominant lethal mutations in the sperm were clearly increased with the dose of caffeine. The enhancing effect of caffeine on the radiation-induced dominant lethals in sperm was an approximately 1.3–1.5 times at a caffeine dose of 200  $\mu\text{g}/\text{pupa}$  as compared with the control regardless of X-ray doses (10,000 and 15,000 R). This observation may be interpreted as due to the inhibition of a maternal cytoplasmic and/or probably dark repair system that is responsible for repair of X-ray-induced chromosome (DNA) breaks in sperm. If so, it can be said that most of breaks induced in sperm would stay open prior to syngamy or DNA synthesis at the early cleavage division stages. It is of interest to note here that no significant enhancing effect was observed when a radiosensitive stock, *Kojiki*, female pupae were treated with caffeine. This finding suggests that this stock may be lacking in the cytoplasmic repair system in oocytes.

### **Appraisal of Silkworm Oocyte System for Mutagenicity Testing of Environmental Chemicals**

Yataro TAZIMA and Akio MURAKAMI

For the purpose of mutagenicity testing of chemical compounds, we developed in silkworm a sensitive test system named "oocyte system," which is essentially a specific loci method using egg color genes as markers (Ann. Rept. Nat. Inst. Genet. (25): 40–41). Using this system, we have performed, as a part of cooperative research project under a grant from Ministry of Welfare, mutagenicity testing of various compounds including known muta-



gens, carcinogens and their allied compounds. The results so far obtained indicated that the system could be adequately applicable to the testing of direct mutagens and/or carcinogens, but not necessarily to those compounds

Table 1. Mutagenicity testing of chemical compounds with silkworm

Compounds	Muta- genicity	Compounds	Muta- genicity
1) Aziridine and derivatives		75-12 $\epsilon$ -Caprolactone	-
Ethylene imine	+	76-21 Epichlorohydrin	+
TEM	+	6) Alkylsulfates, Alkane sulfonic esters	
Mitomycin C*	+	Diethyl sulfates (DES)	+
2) Mustards		Methylmethanesulfonate	
Nitrogen mustard	+	(MMS)	+
Nitromine	+	EMS	+
ICR-170	+	75-28 Propane sultone	+
3) Nitrosamines and Nitrosamides		7) Nitrofuranes	
73-20 Dimethylnitrosamine	-	3-Amino-6-2-(5-nitro-	
74-10 Di-n-butyl-nitrosamine	$\pm$	2-furyl) vinyl-1,2,4-	+
73-22 Butylbutanolnitrosamine	-	triazine, (NFT)	
74-14 N-methyl-N-nitroso-urea	+	AF 2	+
73-29 N-n-butyl-N-nitroso-urea	+	Nitrofurazone	+
74-12 N-n-butyl-N-nitroso-urethane	+	Furazolidone	-
Mitomycin C*	+	8) Heterocyclic compounds	
MNNG	+	75-25 Pyridine	-
ENNG	+	75-26 Quinoline	-
75-06 Urethane	-	74-27 4 NQO	-
75-16 Methylazoxymethanol (MAM)	$\pm$	74-28 4 NH <sub>2</sub> QO	-
4) Amines and Amides		9) Acridines and Acridineum salts	
74-09 Di-n-butylamine	-	Acridine Orange	+
73-21 Butylbutanolamine	-	Acridine Yellow	+
74-13 Methylurea	$\pm$	Proflavine	+
73-23 Butylurea	-	10) Polycyclic hydrocarbons	
74-11 N-n-butylurethane	-	73-11 AAF	+
76-08 Thiourea	$\pm$	74-02 Benz( $\alpha$ )pyrene	-
76-07 Thioacetamide	-	73-05 Dimethylbenzanthracene	-
Hydroxylamine	-	Phenanthrene	-
5) Exoxide, Aldehyde, Lactones		11) Aflatoxines	+
Formaldehyde	-	12) Base analogues	
75-13 $\beta$ -Propiolactone	+	BUdR	+*
		13) Others	
		73-18 Nitrous acid	-

\* Appearing in two places.

acting indirectly.

In the Table listed are the results of mutagenicity testing of chemical compounds, the majority of which were obtained by applying oocyte system. Those are prefixed with experimental number to their chemical name. In order to make possible the detection of indirect mutagens several measures are now being investigated in the silkworm.

### **Mutagenic Effectiveness of an Internal $\beta$ Emitter, Tritium. (3) Effectiveness of $\beta$ -irradiation at Low Dose-Rate Range**

Yataro TAZIMA, Kimiharu ONIMARU and Yosoji FUKASE

Mutagenic effectiveness of very low dose-rate exposure to mutagens has been investigated using tritiated thymidine. The assay system applied was a silkworm oocyte system which was developed especially for chemical mutagenesis study in this insect. The injected compound was taken into egg plasm and then into embryo and excreted mostly soon after feeding of hatched larva. The duration of egg stage was controlled artificially.

$^3\text{H-TdR}$  in aqueous saline was injected into the body cavity of female pupae of a wild type strain (20 and 100  $\mu\text{Ci}$  per head). Emerged females were mated to males of another wild type strain to allow deposition. Average radioactivity transmitted to the deposited egg were 2.28 and 12.73  $\mu\text{Ci/g}$ , which corresponded to ca. 0.66 and 3.7 rad/day, respectively. Half of those egg layings were subjected to acid treatment for artificial hatching, whereas the rest half was allowed to hibernate. The durations of egg stage were 29 and 167 days respectively.

Combination of two dose-rates and two durations gave four dose groups; for 29 days, a) 19.3 rad and b) 107.3 rad, and for 167 days, c) 109.0 rad and d) 612.6 rad. Both sexes of those treated and non-treated groups were crossed to *pe re* partners and mutation frequencies were assayed. The induced mutation frequencies were: almost control level in a) and markedly enhanced in d), while those in c) were very low being about half of those obtained in b), although the total dose was almost same. The results indicated the existence of dose-rate effect even at a very low dose-rate range as 0.66 rad/day (J. Rad. Res. 18: 32).

## Nature of Chemically Induced Mutations Obtained by Oocyte System in the Silkworm (continued)

Yataro TAZIMA, Kimiharu ONIMARU and Akio OHNUMA

In continuation of the work reported previously (this Annual Rep. (26), 40-41) analyses of the nature of chemically induced mutations have been carried out in the silkworm.

### 1) Lethality test in homozygote

Test for lethality of chemically induced mutations in the homozygote has been continued using the same procedure as reported previously. The whole data including the previous one is given in the Table 1, in which the number of tested mutants is doubled. It is noted that proportion of viable mutants are more than half of total tested.

Table 1. Results of lethality test for chemically induced mutations in homozygotes

Mutagen	Homozygote				Total
	Viable	Lethal	Semi-L	Transl.	
EMS, DES	35	25	1	2	63

### 2) Complementation test

Complementation test was performed among similar type of induced mutants in two groups; i.e. lethal red and viable red. Samples were extracted one per each mutant strain. In the lethal red group 11 mutant lines were tested, among which 10 were complete lethal and one was semi-lethal by hatching time. Those strains were crossed each other in all combinations. Mutant red phenotypes were segregated in all crosses, which died during embryonic stage excepting crosses with a semi-lethal strain. The finding indicated that no complementing relationship exists among those 10 lethal red strains. This may be accounted for by assuming that all those mutant strains have a deficiency in common in addition to red mutation.

Similar test performed on viable red mutants brought about different results. Nine mutant lines were crossed each other. In all those crosses red eggs were segregated as expected. They were viable. But in some cross combinations frequency of the incidence of batches in which pink eggs are segregated were unusually high, suggesting that those were trisomics in regard to their chromosome constitution. Among tested 5 mutant lines

were assumed to be trisomic and the rest 5 mutant lines were disomic. Taking into consideration the genotypic classification of the recovered mutants and the results of complementation tests, the proportion of trisomics among viable red mutants was roughly estimated to be 13/35, which was unexpectedly high.

In summary, most lethal mutations and some viable mutations were assumed to be disomic but there were a fairly large proportion of trisomics among visible mutations. In this regard, a mere comparison of the viable with the lethal is mere superficial.

### High Incidence of Translocation between W and the Fifth Chromosomes Among Mutants Detected by Specific Loci Method in the Silkworm

Yataro TAZIMA, Kimiharu ONIMARU and Akio OHNUMA

Experimental induction of a translocation involving W chromosome and an autosome has been reported by several workers in the silkworm. Although such translocations had been successfully induced, the frequency of the incidence was fairly low in those experiments excepting a case reported by Hasimoto (1948). We have observed in this connection cases of surprisingly high incidence of such translocation among mutants recovered as a visible recessive mutation at the marker gene locus.

Wild type females of the silkworm were treated by either  $\gamma$ -rays or a chemical mutagen, EMS, and mated to males of a marker strain with *pe re*. In their  $F_1$ , mutants were obtained either as a whole (*pe* or *re*) or as a mosaic (*pe*, + or +, *re*) with regard to the expression of marker genes. These were subjected to lethality test in homozygous condition. For this purpose a mutation bearing chromosome was led to homozygous condition via appropriate genetic procedures. For instance, in a case of *pe* mutation, the mutant females were mated to +*re* males and in  $F_1$  only individuals of wild phenotype were crossed *inter se* to produce  $F_2$ , and homozygotes for mutation bearing chromosome were obtained as one of the segregants in  $F_2$ : 1 *pe*+: 2++: 1+*re*. In some cases of  $F_1$  batches of whole mutants or  $BF_1$  batches of mosaic mutants, all wild type individuals gave rise to females. Further analysis revealed that those females were carriers of translocation chromosome between W and the fifth chromosomes.

The incidence of such translocation was observed in five different experimental series and the culculated frequency of such incidence was

Table 1. Translocations involving W and the fifth chromosome discovered among mutations at loci marked with +<sup>pe</sup> and/or +<sup>re</sup> genes

Name of transl.	Original strain	Mutagenic agent	Phenotype of mutants captured	Frequency of incidence	Year
OT	C108	Spont.	<i>pe</i>	1/12	1961
M28	C108	Spont.	<i>pe</i>	1/5	1975
ON	C108	$\gamma$ -ray	<i>re</i>	1/24	1972
EM21	Aojuku	EMS	<i>pe</i> mos	1/17	1975
EM30	Aojuku	EMS	<i>re</i> mos	1/8	1975

found surprisingly high (Table 1).

The over-all frequency of the translocation was 5/66, or 1/13 per detected mutants at specific locus. This is surprisingly high. The expected frequency is calculated to be 1/27, even when all recovered mutants were assumed to be due to translocation. The discrepancy is obvious and seems to suggest that there might exist some kind of special prejudice working between the W chromosome and the fifth chromosome in the silkworm.

## VII. RADIATION GENETICS AND CHEMICAL MUTAGENESIS IN MICRO-ORGANISMS AND PLANTS

### Effect of Gamma-Irradiation on Growth of Cultured *Haplopappus* Cells

Estuo AMANO

Soft and friable callus of *Haplopappus gracilis* ( $2n=4$ ) can grow in liquid medium. Suspension culture of cell line HSe was used to obtain gamma-ray dose response curve for cell growth. Liquid culture medium used was 1/2 strength of modified Murashige and Skoog's medium without agar. This medium contains 1 ppm of 2.4 D, 0.15% of yeast extract, besides vitamins and inorganic salts. Under rotation culture (4 rpm) at 25°C and initial cell concentration of more than 2 mg/ml by fresh weight, cells grow at the rate of about 1.4 fold increase a day in fresh weight, up to 9th day and reach to plateau on about 20th day. 1 ml of cell suspension cultured for 14 days was inoculated into 20 ml of liquid medium in 100 ml Ehrenmyer flask and irradiated by  $^{137}\text{Cs}$  gamma-rays up to 25 kR. Fresh weight was measured on the 14th day of rotation culture at 25°C. Fresh weight in mg/ml decreased as the gamma-ray dose increased. 50% reduction dose for fresh weight was estimated to be about 6 kR. 10 kR irradiation reduced the fresh weight to about 15% of 0 kR control. Cultured cells seemed to be resistant compared to normal fresh tissues. This might be due to the number of active cells in the population or tissues responsible for the growth.

### Measurement of Waxy Starch Phenotype in Cereal Crops

Etsuo AMANO

In the studies of waxy starch mutation in maize and rice, it became necessary to compare the phenotypic expression of waxy starch mutation quantitatively, among mutants as well as kernels. For this purpose, blue value method was modified to fit for analysis of a single kernel and for large number of samples. Normal endosperm starch contains about 25% amylose which stains blue black by iodine, and 75% amylopectine which stains reddish purple to brown. As the color of iodinated amylose is very

dark, normal starch shows blue black color by iodine stain. Waxy starch mutants delete, or contain reduced amount of amylose, express waxy or opaque appearance in endosperm, and stain purple to brown color by iodine.

In the colorimetric analysis used here, two wave lengths were adopted. Blue light (430 nm) which is absorbed by major component of starch, amylopectine, after iodine stain, is used to adjust concentration of starch solution. Transmission (T value) of red light (660 nm) of the iodine stained starch solution adjusted to the concentration of 50% transmission at 430 nm, was used as index of amylose content, or waxy phenotype. The apparatus developed for this measurement include microtube pumps and a small mixing motor mounted to suit for dilution in the measuring cell. Changes of the T values for the two wave lengths by dilution of the sample solution were recorded on a X-Y recorder. The index of waxy phenotype of each sample was determined from this dilution curve.

Starch solution was prepared in a small test tube from a single kernel. Each kernel was crushed and 5 ml of water per tube was added. Test tubes were then heated in an autoclave for 15 min. at 1 atm. After cooling to room temperature, 1 ml of supernatant was taken into measuring cell and mixed with 0.1 ml of 0.2% I-KI solution. Non-waxy starch showed very dark blue color and waxy mutant starch showed reddish purple. For the preparation of the starch solution, alkali dispersion was tested but it required neutralization before iodine staining, and moreover, difference in the waxy indices was reduced.

T value of normal non-waxy kernels distributed from 10% to 20% and waxy starch around 60% for large kernels of maize as well as small grains of Italian millet (*Setaria italica*). Among six EMS induced waxy mutants in rice so far examined, two showed reduced waxy indices around 40%, indicating they were intermediate or leaky waxy mutants. These were also intermediate in their appearance of homozygote endosperm. Besides these homozygotic intermediate phenotype, often the waxy indices of normal non-waxy kernels in segregating F<sub>2</sub> population distributed from 10% to 40%. This might be due to gene dosage effect in triploid endosperm. In fact, in some mutants, kernels of intermediate or semi-waxy appearance segregated waxy kernels in the progeny indicating that they were heterozygotes. Measurement of mutant and heterozygous kernels will be continued to characterize the mutant phenotype and to confirm the gene dosage effect.

**Isolation of a New Tester Strain of *Bacillus subtilis* for the  
Screening of Environmental Mutagens and Carcinogens  
by Bacterial Cell Fusion**

Koichi HIRANO, Taiji ASANO, Yoshito SADAIE and Tsuneo KADA

*Salmonella typhimurium* strains of Ames system are widely used as tester strains for detecting environmental mutagens and carcinogens (B. N. Ames *et al.* 1973 Proc. Natl. Acad. Sci., 70: 782, 1975 Proc. Natl. Acad. Sci., 72: 979), and *Bacillus subtilis* strains have also been utilized in many laboratories to examine the hazardous nature of chemicals by means of re-assay, prophage induction assay, forward mutation assay using spores, and so on.

Recently, a new tester strain of *B. subtilis* was isolated by Sadaie *et al.* (1976 J. Bacteriol., 126: 1037). It was constructed by introduction of *dna-8132* (Hara, H., Nature New Biol., 244: 200) into the *polA59* strain (Gass, K. B., 1971 J. Bacteriol., 108: 364). The constructed strain, NIG 1124 (*his met dna-8132 polA59*), showed an enhanced mutability for base change type mutagen such as AF2.

In this case, nonsense mutation of *his* gene might be reverted by the error-prone repair function of the *dna* gene product(s) in the absence of the *polA*<sup>+</sup> gene function(s).

To obtain a more sensitive strain, we constructed a new strain by means of the bacterial cell fusion technique developed by Schaeffer *et al.* (1976 Proc. Natl. Acad. Sci., 73: 2151). The fusion was performed between *B. subtilis* strains NIG 1122 (*his met dna-8132*) and UVSS-19-8 (*met thy trp uvr asp*) isolated by Munakata *et al.* (1969 Mutation Res., 7: 133).

The newly isolated strain BKH 5002 (*trp dna-8132 uvr*) showed a markedly increased response to ICR-170, a typical frameshift type mutagen, in producing an increased number of Trp<sup>+</sup> revertant colonies. This strain was also reverted by a base change type mutagen such as AF2.

Furthermore, when the strain was lysogenized with temperate phage SPO2, it was found that dimethyl nitrosamine, which is one of the presumed human carcinogens showing a weakly positive effect in the *Salmonella* system, could be detected at a markedly lower doses by pre-incubation with the S9 mixture.



### Highly Sensitive Rec-assay Using Spores of *Bacillus subtilis*

Koichi HIRANO, Hisako MATSUMOTO and Tsuneco KADA

The rec-assay procedure is widely used for mutagen screening. This method uses a pair of *Bacillus subtilis* strains M45 (*arg trp rec-45*) and H17 (*arg trp*). When a drug produces damage in DNA, it may show increased lethality upon Rec<sup>-</sup> strain over Rec<sup>+</sup> strain (T. Kada *et al.* 1970 Ann. Rept. Natl. Inst. Genet. 21: 72). Indeed, a wide variety of environmental mutagens were detected by this method, including AF2, Dexon, phloxin and 2,4-dinitrophenyl thiocyanate, etc. Moreover, a wide drug spectrum containing frameshift type or base-change type mutagens could be detected by this method. Recently, using this method, many pesticides were screened by Shirasu *et al.* (1976 Mutation Res., 40: 19). Their results showed the existence of close correlation between the rec-assay positiveness and mutation inducibility.

*B. subtilis* is a unique bacterium, compared with enteric bacteria such as *Salmonella typhimurium* or *E. coli* on account of permeability and spore formability. Since spores are very stable at 4°C in water suspension, it may be possible to do quantitative analysis.

Our improvements on this assay were as follows;

#### 1) Sensitivity

Using spore plates which contained about  $1 \times 10^6$  spores in 10 ml of broth agar in a 90 mm plastic dish for M45 and  $1 \times 10^5$  for H17, 10 or more fold high sensitivity was obtained compared with the current method using vegetative cells.

#### 2) Reproducibility and quantitativity

Because of constant viability of spores, it is possible to obtain reproducible results about the differences between diameters of the inhibition zone for the M45 plate and those of the H17 plate. Moreover it is also possible to obtain quantitative values for the minimal inhibition concentration (MIC), determined by means of extrapolation of the dose response curve to the drug concentration producing no inhibition zone.

### DNA Repair Enzymes in Ataxia Telangiectasia and Bloom's Syndrome Fibroblasts

Tadashi INOUE, Koichi HIRANO, Akiko YOKOYAMA, Hatao KATO  
and Tsuneo KADA

Ataxia telangiectasia (AT) and Bloom's syndrome (BS) are autosomal recessive diseases of man characterized by telangiectasia, immunodeficiency, and an increased frequency of malignancy. The ionizing radiation sensitivity of AT patients has been indicated by *in vivo* and *in vitro* studies of skin fibroblasts. On the other hand, fibroblasts from BS patients have been reported to be ultraviolet light sensitive. An enhanced level of spontaneous chromosome instability was also observed in both AT and BS cells. These features suggest that some kinds of impairment in DNA repair are the molecular bases of the diseases. From this point of view, we have compared the activities of "primer activating" enzyme as well as the apurinic site specific endonuclease in extracts from AT strain and BS cells with those from normal cells. The primer activating enzyme was assayed by a new system in which the capacity of cellular extract to enhance the priming activity of gamma-irradiated colicin E1 DNA for purified type I DNA polymerase was measured (Inoue, T. and T. Kada 1977 *Biochim. Biophys. Acta* 478: 234-243). The experiments yielded the results that the primer activating enzyme activity of AT cells was substantially lower than normal cells, and that BS cells had a slightly lower level of the activity. These results indicate that some kinds of enzymes which function in the excision-type repair prior to repair replication by DNA polymerase are defective in the AT and BS cells. However, cellular extracts from these three strains had similar, if not identical, activities of the apurinic site specific endonuclease, suggesting that the decreased activity of the primer activating enzyme in AT and BS cells was not due to the defect in the endonuclease (1977 *Biochim. Biophys. Acta* 479: 497-500).

### Purification and Properties of *Bacillus subtilis* Endonuclease Specific for Apurinic Sites in DNA

Tadashi INOUE and Tsuneo KADA

Considering the multiplicity of lesions created in gamma-irradiated DNA, it is reasonable to expect that a wide variety of enzymes have evolved, each capable of recognizing a different class of lesions and catalyzing the important

step in an excision-type repair pathway. Indeed, we have identified several kinds of enzymes which may function in the repair of gamma-irradiated DNA in extracts of *Bacillus subtilis* cells using a new assay system in which a capacity of cellular extracts to enhance the priming activity of gamma-irradiated DNA for purified type I DNA polymerase was measured. Preliminary experiments revealed that one of them was an endonuclease specific for apurinic sites (Noguti, T. and T. Kada 1975 *Biochim. Biophys. Acta* 395, 294-305) and the other was 'cleaning' exonuclease (Inoue, T. and T. Kada 1977 *Biochim. Biophys. Acta* 478: 234-243). The former enzyme was purified to near homogeneity and had properties described below.

The enzyme had a molecular weight of about 53,000 as determined by gel filtration and SDS-polyacrylamide gel electrophoresis. It had a pH optimum of 8.0 and has no  $Mg^{2+}$  requirement, and was fully active in the presence of EDTA. The depurinated DNA was an especially good substrate, however, alkylated DNA was also attacked to some extent. Associated activities of exonuclease and ribonuclease were not detected. The enzyme appeared to produce 3'-OH near apurinic sites in DNA, since the priming activity of DNA for DNA polymerase was enhanced by the treatment of the enzyme.

### Utilization of a Mutator Strain for the Isolation of Mutants in *Bacillus subtilis*

Yoshito SADAIE, Yoshiko OHTA and Tsuneo KADA

Utilization of strong mutator strains may be useful to isolate mutants in *Bacillus subtilis*. Mutations produced can be easily transferred, purified and stabilized by transformation of a second strain by DNA from original mutants because transforming DNA integrated into host genome usually comprises a small segment of whole chromosome coding for about one cistron. Revertant analysis on the constructed mutants is necessary to see whether or not the mutants carry a single mutation. A strong mutator strain NIG1125 (*met his mut-1*) of *B. subtilis* was constructed by transformation. Two mutations, *met* and *his*, appeared to be suppressible by a nonsense suppressor mutation (Okubo and Yanagida, *J. Bacteriol.* 95: 1187-1188; Shub, *J. Bacteriol.* 122: 788-790) and the mutation *mut-1* reduces DNA polymerase III activity and greatly enhances spontaneous mutation frequency (Bazill and Gross, *Nature New Biol.* 243: 241-243). We used this mutator strain for the isolation of mutants in *B. subtilis* and the isolated mutations

were transferred by transformation into the second strain.

(1) Twenty four  $Rif^rGsp^{ts}$  mutations were isolated at a frequency of about  $1.8 \times 10^{-7}$  per cell. Nine mutants among them grew well at the restricted temperature. All the mutants carried two mutations *rif* and *gsp*, most of which was linked to *cysA14*.

(2) Temperature sensitive nonsense suppressor mutations were isolated from colonies grown on minimal agar at 42°C (or 30°C) at a frequency of about  $1.6 \times 10^{-5}$  (or  $3.2 \times 10^{-6}$ ) per cell. DNA was isolated from the mutants and used to transform NIG1121 (*met his*) to prototroph at 42°C (or 30°C) in order to fix the suppressor mutation.

### The Induction of Monoecious Mutants in Cucumber

Taro FUJII

A female strain (MSU) of cucumber was exposed to 50 kR of gamma-rays, and among 28  $M_1$  plants observed, three were found to develop male flowers, which were designated as MSU-50A, 50B and 50C. The  $M_2$  plants raised from their selfed seeds segregated into monoecious and gynoecious types giving 19: 15, 32: 27 and 23: 11 ratios, respectively. The monoecious segregants showed a wide variation in the number of nodes with male flowers; a plant had only one such node at the basal part, while another had male flowers on 36 of 50 nodes observed. The monoecism: gynoecism ratios in the  $M_3$  generation derived from MSU-50A were 8: 15 in a line and 16: 1 in another. The  $M_4$  plants from the latter  $M_3$  line were exclusively monoecious (35: 0).

The data suggest that monoecism was promoted by selfing, though the number of plants observed was too small to consider segregation ratios. The tendency of selfing to promote monoecism was also observed in a backcrossing experiment carried out previously.

A backcrossing experiment was made again in which 50A, 50B and 50C were crossed with the control plants of MSU. The  $B_1$  generation showed 8: 9, 17: 17 and 11: 16 ratios of monoecious and gynoecious plants, respectively. The control MSU plants showed no monoecism in the same culture (0: 34). Although the incidence of monoecious plants in the  $M_1$  generation, 3/28, was too high to consider it to be a mutation, the inheritance of monoecism in the progeny suggests that some heritable change in the mechanism of sex determination has resulted from irradiation.

## VIII. POPULATION GENETICS (THEORETICAL)

### Population Genetics and Molecular Evolution

Motoo KIMURA

Recent studies of evolution and polymorphism at the molecular level (i.e., at the level of internal structure of the gene) have brought many puzzling as well as enlightening results. It appears that, in addition to natural selection, chance (in the form of random gene frequency drift) is playing a much larger part than previously considered. Furthermore, natural selection is likely to be based more directly on the secondary and the higher order structure of informational macromolecules rather than ecological conditions, usually in the form of negative selection. In this respect, there is growing evidence suggesting that very slightly deleterious mutations whose selective disadvantages are not excessively large as compared with the mutation rates are playing a significant role in variation and evolution at the molecular level. For details, see Johns Hopkins Medical Journal 138 (6), 253-261.

### Role of Very Slightly Deleterious Mutations in Molecular Evolution and Polymorphism

Tomoko OHTA

Several models of multiple slightly deleterious alleles are reviewed and theoretical consequences of slightly negative selection are discussed in conjunction with evolution and variation at the molecular level. Facts are organized which may be satisfactorily explained by the hypothesis of very slightly deleterious mutations. They are: (1) There appears to be an upper limit in heterozygosity for protein loci as measured by electrophoresis. (2) The excess of rare alleles is more pronounced in *Drosophila* than in man. (3) Correlation of heterozygosities at a locus among sibling species of the *Drosophila willistoni* group is too high compared to what is expected by the strict neutral theory, while it is not so among human races and between man and chimpanzee. (4) The rate of protein divergence is exceptionally high in Hawaiian *Drosophila*. For details, see Theoretical Population Biology 10, 254-275.

## IX. POPULATION GENETICS (EXPERIMENTAL)

### Elimination of the Polymorphic Inversions from the Laboratory Populations of *Drosophila melanogaster*

Yutaka INOUE and Takao K. WATANABE

When natural populations of *Drosophila* have been transferred to a laboratory environment, the relative frequencies of the founder chromosomal variants may change. Three natural populations (Akayu, Katsunuma, Ishigaki) of *D. melanogaster* were transferred to 14 laboratory cages. The initial frequency of the polymorphic inversions per autosome arm was 12.0% for Akayu, 16.6% for Katsunuma and 53.0% for Ishigaki populations. For the Akayu and Katsunuma populations, only 40 generations of cage life were quite enough for the reduction of the frequency to less than 1%. For the Ishigaki population, the average frequency of the polymorphic inversions reduced to be 0.4% at the 70th generation.

Thus, we can conclude that the polymorphic inversions found highly polymorphic in natural populations in Japan turn deleterious when they are transferred to laboratory cages. The magnitude of the selection coefficient is about 5 percent.

On the other hand, the frequency of the rare inversions was found to be 0.26% for the major autosome arm both in natural and cage populations. This suggests that newly arisen chromosomal mutations are very deleterious in natural and cage environments enough to be selected against soon after the mutation occurred.

### Equilibrium Frequency of Lethal Genes in Cage Populations of *Drosophila melanogaster*

WON HO LEE and Takao K. WATANABE

Lethal and sterility mutations were accumulated in a cage population which was initiated with lethal- and sterility-free second chromosomes of *D. melanogaster*. It took about 2,000 days for the frequencies of these genes to reach equilibrium levels, *i.e.* the frequencies of 18% lethal and 9% male-sterile chromosomes. Two other cage populations which were initiated with random chromosomes sampled from natural populations and kept for

more than 11 years in the laboratory showed 19–20% lethal content.

In the course of accumulation of mutations, the frequency of lethals was always higher than that of male-steriles, and the frequency of male-steriles was always higher than that of female-steriles.

The elimination rates of lethal chromosomes by homozygosis (0.08–0.19%) in these populations were smaller than the mutation rate (0.73%). Therefore, a large part of selection against lethal genes must occur in heterozygous condition. By using *Nei's* formulae, the deleterious effect of a lethal gene in the heterozygote (*h*) was estimated to be 0.035.

The effective population number in the cage population was estimated to be 1,000–2,900, while the actual population number was 3,500–7,800. Thus, about 30% of the censused adult population contributes to the next generation.

### Enzyme and Chromosome Polymorphisms in Natural Populations of *Drosophila melanogaster*

Takao K. WATANABE and Taishu WATANABE

Collections of *D. melanogaster* from three natural populations (Katsunuma, Akayu, Ishigaki) and three cage populations were analyzed for enzyme and chromosomal polymorphisms. Allelic frequencies at the *Adh* (2–50.1) and  $\alpha$ -*Gpd* (2–17.8) loci were compared with polymorphic inversion, *In(2L)B* (22D–34A) and *In(2R)C* (52A–56F), frequencies. There was a significant positive correlation between the frequencies of *Adh*<sup>S</sup> and *In(2L)B*, and  $\alpha$ -*Gpd*<sup>F</sup> and *In(2L)B*, caused by linkage.

Two fitness components of these enzyme and chromosomal variants were measured in two different environments; neither of the two loci showed heterozygote superiority in viability or productivity, while the inversion heterozygotes showed a superior productivity compared to the corresponding homozygotes. Therefore, allelic frequencies at the *Adh* and  $\alpha$ -*Gpd* loci seem to be determined by the frequency of *In(2L)B* in natural populations where the inversion appears to be maintained by selection, but not by the selection on the enzyme itself.

On the other hand, inversion-free cage populations maintained in the laboratory for a long time showed considerably larger variation in the frequencies of these enzyme alleles, which seem very likely to be a consequence of random drift.

Although the history of the polymorphic inversion remains unknown, we can speculate that this adaptive chromosomal rearrangement, *In(2L)B*, has occurred in one standard sequence chromosome which had *Adh<sup>S</sup>* and  $\alpha$ -*Gpd<sup>F</sup>* alleles and spread throughout the world. For details, see *Genetics* 85: 319-329 (1977).



## X. EVOLUTIONARY GENETICS

### Geographical Distribution of *Drosophila simulans* in Japan

Takao K. WATANABE and Masaoki KAWANISHI

A census of domestic or semi-domestic species of *Drosophila* has been taken throughout Japan in the fall of 1976, attempting to know the present distribution of *D. simulans* which is a recently immigrant species. A total of 85 collection sites throughout the country was chosen in parks, temple or shrine backyards and similar places which were nearby human habitation.

*D. simulans* was already distributed in the two wide areas; Kanto-Tokai district and Kyushu district. The intervening area, Western mainland, is almost lacking *simulans* except for the west end (Shimonoseki). Shikoku is an interesting area in the mosaic occurrence of this species. Other places are at present free from *simulans* with a few temporary introduced individuals.

The two years survey (1975–1976) of the domestic species at Yamanashi and eastern Shizuoka areas suggested that the invasion of *simulans* from the Pacific coast to the inland area was not so fast.

The colonization of *simulans* remarkably affected on other domestic species, especially on *melanogaster*. In the *simulans* lacking sites, *melanogaster* was the most abundant species (33.6%), followed by *immigrans* (28.6%) and *lutescens* (17.0%). While in the *simulans* abundant sites, *simulans* was the most abundant species (36.7%), followed by *immigrans* (27.0%), *lutescens* (12.5%) and *melanogaster* (11.8%).

### How Genes Evolve; A Population Geneticist's View

MOTOO KIMURA

This paper is based on the author's lecture delivered at the College de France on May 14th 1976. After a brief discussion of historical development of evolutionary theories, a history of population genetics itself is reviewed. It is then pointed out that, during the last decade, with the advent of molecular genetics, studies of evolution has been revolutionized. This led to the theoretical framework called "Molecular population genetics" in which the mechanisms of evolution and variation at the molecular level are

treated using the diffusion equation methods in population genetics ("diffusion models"). The neutral mutation-random drift hypothesis ("the neutral theory") and controversies following its proposal in 1968 are reviewed. Also, evidence supporting the neutral theory is detailed particularly in relation to structure and functional constraints of the evolving molecules.

The present paper consists of the following sections;

(1) From Lamarck to the three savants, (2) Development of theoretical population genetics, (3) Dynamics of mutant substitution and the rate of evolution, (4) Progress and degeneration in evolution, (5) Our past and future. For details, see the original paper [Kimura, M. (1976) *Ann. Génét.* 19(3): 153-168.]

### **Archeology, Population Genetics and Studies of Human Racial Ancestry**

Takeo MARUYAMA

Using traditional classification, many people have tried to determine the place and time of origin of the major human races. Two basically conflicting views have prevailed, one asserting that races developed as local variations in a species evolving phyletically over much of the Old World for up to one million years, and the other view holding that present races are local variations on populations recently expanding into most areas and replacing the previous hominid inhabitants, this occurring since the time of the Neanderthals. Both views are based on the same archeological data. Gene frequency data and time-calibrated genetic distance measures have recently been applied to this problem. Here we show that the probable demographic nature of Pleistocene populations has obscured genetic distances to such an extent that they cannot be used to discriminate between the two viewpoints of racial origins. The racial classifications themselves are probably not useful in this context and obscure the question, so that we presently do not have a scientifically valid understanding of racial origins. Published in *American Journal of Physical Anthropology* 44: 31-50.

### **Simple Model for Treating Evolution of Multigene Families**

Tomoko OHTA

The evolution of repeated genes forming a multigene family was studied by assuming unequal crossing-over. As a first step, the single chromosomal line is considered. It was shown that the contraction-expansion of mutant genes due to unequal crossing-over within a chromosome may be treated by the diffusion model developed by Kimura for analysing the behavior of mutant genes in finite populations, if the number of gene units in the multigene family stays constant ( $n$ ). Two consecutive crossovers (involving duplication and deletion) is called one cycle of the process. Then, one cycle is shown to be equivalent to  $4N_e/n^2$  generations of random gene frequency drift, in which  $N_e$  is the effective population size. For details, see Nature 263, 74-76.

### **Simulation Studies on the Evolution of Amino Acid Sequences**

Tomoko OHTA

A model of molecular evolution in which the parameter (intrinsic rate of amino acid substitution) fluctuates from time to time was investigated by simulating the process. It was found that the usual method of estimation such as Poisson fitting underestimates this variation of the parameter when remote comparisons are made. At the same time, four distance measures (minimum base difference, Poisson fitting, random nucleotide substitutions and negative binomial fitting) were tested for their accuracy. When the substitution rate is not uniform among the amino acid sites, the negative binomial fitting gives most satisfactory results, however, one needs to know the parameter beforehand in order to use this method. It was pointed out that the fluctuation of the evolutionary rate is expected if the nearly neutral but very slightly deleterious mutations play an important role on molecular evolution. For details, see J. Mol. Evol. 8, 1-12.

### Reproductive Barriers Distributed in *Melilotus* Species and Their Genetic Bases

Yoshio SANO and Fumiji KITA

In order to look into species relationships, crosses were made in all possible combinations of 9 species belonging to the subgenus *Eumelilotus*; 504 crosses were made among 53 strains in total. The reproductive barriers found among the species were a crossing barrier (death of young  $F_1$  zygotes),  $F_1$  inviability and weakness due to chlorosis,  $F_2$  breakdown due to the occurrence of chlorotic segregants (classified into lethal and weak types), partial  $F_1$  sterility, and a segmental interchange of chromosomes. All the hybrids within species were normal. In crosses between *M. alba* and *M. suaveolens*, the hybrids of annual strains showed more pronounced  $F_1$  and  $F_2$  chlorosis than those of biennial strains. The hybrids of self-fertile species or forms tended to have a low reproductive potential. The  $F_1$  chlorosis between the above two species was considered to be controlled by a set of complementary dominant lethals,  $Ch_1$  and  $Ch_2$ . The  $F_2$  chlorosis in the same species hybrid was controlled by the sets of duplicate recessive genes,  $chl_1$  and  $chl_2$  for lethal type and  $chw_1$  and  $chw_2$  for the weak type. The  $chl_2$  and  $chw_2$  loci were linked with a recombination value of about 13 percent. (Submitted to Can. J. Genet. Cytol.; this experiment was carried out at the Hokkaido University.)

### Analysis of Genes Responsible for the $F_1$ Sterility between *Oryza sativa* and *O. glaberrima*

Yoshio SANO and H. I. OKA

This experiment has been continued since 1966 ( $B_1$  generation) to observe various crosses between isogenic  $F_1$ -sterile lines with the genetic background of either *sativa* or *glaberrima* parent (Ann. Rep. 24: 67 & 25: 85). The genic system responsible for the  $F_1$  sterility, so far obtained was as follows: The *sativa* parent has  $A_1/A_1$  and its isogenic  $F_1$ -sterile lines have  $A_1^s/A_1^s$  introduced from the *glaberrima* parent. When an  $A_1^s$  gene is present in the sporophytic tissue ( $A_1/A_1^s$ ), the gametes (both pollen and embryosac) carrying  $A_1$  deteriorate during the course of development. The same hypothesis also applied to the isogenic lines with the genetic background of *glaberrima* parent, for which genes  $A_2: A_2^s$  were assumed.

A new evidence supporting this hypothesis was that in a  $A_1/A_1^s$  hybrid, a gene controlling apiculus coloration which was introduced from the *glaberrima* parent and tightly linked with  $A_1^s$  showed no  $F_2$  segregation (all  $F_2$  plants were colored) since all  $A_1$  genes were eliminated by gametic selection. However, the presence of other genic systems conditioning sterility was also suggested; for instance, a true-breeding semi-sterile line with *sativa* background was obtained, which seemed to be homozygous for complementary genes controlling sporophytic sterility. To look more closely into the genic systems, a backcrossing experiment was initiated using a *sativa* isogenic line with  $A_1^s/A_1^s$  and a *glaberrima* isogenic line with  $A_2^s/A_2^s$ ; their intercross produced highly sterile  $F_1$  plants.

## XI. HUMAN GENETICS

### **Hereditary Retinoblastoma: Penetrance, Expressivity and Age of Onset**

Ei MATSUNAGA

It is now well recognized that there are two etiologically distinct forms of retinoblastoma, one due to an autosomal dominant gene with incomplete penetrance, and the other being non-hereditary and perhaps due to somatic mutation. As to the hereditary form, the degree of penetrance has been generally supposed to be about 80%. However, analysis of family data published in Japan concerning 29 kindreds with two or more cases of retinoblastoma revealed that, in the children who received the gene from a carrier parent, both penetrance and expressivity increase with increasing degree of expressivity in the parent. This suggests that suppressor genes at other loci play a significant role in manifestation of the major dominant gene for retinoblastoma. The estimate for the average degree of penetrance varies from 0.7 to 0.9, depending upon the method employed. It is certain that the value will be increased with increasing number of survivors of hereditary retinoblastoma. Furthermore, there was some evidence that in the hereditary form of retinoblastoma onset tends to be later in unilateral than in bilateral affection, though in the non-hereditary form onset will be further delayed. For details, see *Humangenetik* 33: 1-15, 1976.

### **A Survey on Maternal Age and Karyotype in Down's Syndrome in Japan, 1947-75**

Ei MATSUNAGA and Hiroko FUJITA<sup>1)</sup>

In the absence of any nation-wide monitoring system in Japan, we analyzed data concerning karyotype and maternal age of 1,954 cases of Down's syndrome to see if the rate of chromosome mutations leading to this abnormality has been enhanced in recent years. Comparison of data for patients born in 1947-60 with those born in 1961-75 revealed little change with time in the proportions of cases due to different karyotypes, the overwhelming majority being of 21 trisomy type in both periods. How-

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ever, there has been a remarkable decline in the mean maternal age from 33.1 years to 29.7 years as well as in the variance from 50.5 to 29.4. While the rate of decline in the variance was almost the same as that for all births occurring in the same periods, the decline in the mean maternal age was much greater for the patients than for all births, suggesting that the rate of nondisjunction might have increased in younger rather than in older mothers. However, when the risk of bearing a child with Down's syndrome for mothers aged 40-44 is taken as unity, no evidence was found for an increase with time in the relative risk for younger mothers. Moreover, results of surveys made in 1960 and thereafter in different parts of Japan indicate that the crude incidence rate of Down's syndrome at birth has been around 0.10%, giving no indication for an upward trend. These findings seem to be rather remarkable when we consider the serious environmental pollution, including possible genetic hazards, with which Japan has been faced in the 1960s. For details see *Humangenetik* 37: 221-230, 1977.

#### **LBA Technique in the Detection of Chromosome Variants in Man**

Yasuo NAKAGOME, Shigehiro OKA, Ei MATSUNAGA and Masataka ARIMA

Both Q- and C-band techniques have widely been used in the detection of chromosome variants (polymorphism) in man. However, many problems remain still open as only very limited number of variants are detectable by them. In the present report, a technique is described which uses DNA-replication pattern for the purpose of identifying new variants which may not be detected by the two conventional techniques.

BUdR (30  $\mu\text{g}/\text{ml}$ ) was added to leukocyte cultures (standard 3-day culture) 24h prior to the harvest. Fourteen hours later, cells were rinsed and fed with preconditioned medium supplemented with thymidine (3  $\mu\text{g}/\text{ml}$ ). The preparations were stained with quinacrine mustard, photographed under a fluorescence microscope, destained with 1:1 mixture of ethanol and acetic acid, stained with acridine orange (100  $\mu\text{g}/\text{ml}$  in Sørensen's buffer solution at pH 6.5 for 5 min) and then photographed again. Both photographs from each metaphase were compared each other.

Segments of chromosomes which replicated at the last part of the S-phase showed intense fluorescence in acridine stained preparations. These segments will be referred as LBA bands and the procedure as LBA technique (late-replicating bands detected by the BUdR-acridine technique) based on

the suggested system of nomenclature in the Paris Conference (suppl. 1975). Most of the LBA bands corresponded to Q-positive bands. Exceptions included qh regions of nos. 1, 9 and 16 pairs, centromeres of most pairs of chromosomes and several other polymorphic regions. All of them fluoresced intensely.

A total of 41 variants were detected in 40 acrocentrics from 4 unrelated individuals. Twenty-eight of them were detected only by the LBA-technique, 11 of them being in short arms and 17 of them in centromeres. Seven variants, including 4 of those in satellites, were detected only by the Q-technique. Six short arm variants were detected by both methods. As to centromeric variants of nos. 3 and 4, both of them worked equally well. In the evaluation of qh variants in pairs 1, 9 and 16, the present (LBA) technique proved itself to be a very effective implement. Because of intense fluorescence, it was very easy to recognize and evaluate them. The technique was practically free of technical variables coherent with the C-band technique and therefore was possible to use the size of a C-negative euchromatic segment of a chromosome as a reference standard. LBA variants were observed in centromeres of about 50% of the members of the remaining 12 pairs of autosomes, i.e., nos. 2, 5, 6, 7, 8, 10, 11, 12, 17, 18, 19 and 20.

It appears that the LBA technique is especially useful in the detection of variants of short arms and centromeres of acrocentrics. It is also useful in the evaluation of qh and centromeric variants of non acrocentrics. On the other hand, the Q technique is more efficient in the detection of variants of satellites. It has been known that C treatment sometimes fails to work after the Q staining. On the other hand, the LBA bands are not inpaired at all by the preceding Q treatment. Thus, it appears that a sequential Q-LBA technique is one of the most efficient procedure available for the purpose of detecting chromosome variants in man (for further details, see Nakagome *et al.* and Oka *et al.*: Human Genetics 38; 307 and 39; 31, 1977).

### **Holoprosencephaly in Human Embryos: An Epidemiologic Study**

Kohei SHIOTA and Ei MATSUNAGA

Among the large collection of human embryos in the Human Embryo Center for Teratological Studies of Kyoto University, 150 cases of holoprosencephaly (cyclopia-arhinencephaly series) were found during the period between January 1962 and December 1974. The incidence of the defect in



the embryos was 4.1 per 1000 against 0.06 per 1000 newborns, which implies that almost all of the affected embryos are destined to be eliminated prenatally. The occurrence was largely at random through time, and no 'epidemic' was noted in particular years or months, but there appeared more cases derived from conceptions in winter than in summer months. The mean maternal age did not differ significantly from that of the general embryonic population, indicating that, although none of our cases were karyotyped, chromosome aberrations such as trisomies 13 and 18 that are closely associated with maternal age may not constitute a major part as causes of holoprosencephaly in human embryos. Maternal age did not differ by the presence or absence of associated external anomalies. No association was found with paternal age, parental consanguinity nor with maternal medical history, including irregularity of menstrual cycles, and smoking and drinking habits. There was an indication that the mothers were prone to have repeated miscarriages, supporting the view that some kind of maternal predisposition is responsible for the causation of holoprosencephaly. Apart from various chromosome aberrations well documented as causes of this malformation, polygenic mechanism probably accounts for the majority of the cases with normal karyotype. Full data are published in *Teratology* 16: 261-272, 1977.

## XII. BEHAVIORAL GENETICS

### Genetic Variation of Walking Ability in *Drosophila melanogaster*

Sumio M. AKAI and Takao K. WATANABE

Genetic variation of walking ability in *D. melanogaster* was measured by a Benzer countercurrent apparatus. A total of 74 homozygous lines for the second chromosomes were obtained from Ishigaki population. The mean walking ability of each line was measured in the dark (DW), in the light (LW) and to the light (toL) conditions. Variances due to lines, sex and line  $\times$  sex were highly significant for each environment. Males walked much more than females but correlation coefficient between sexes was about 0.8.

Flies walked active in the light (LW) and it was more accelerated toward the light source (toL). However, the walking abilities in the three light conditions positively correlated. The correlation coefficient was 0.58 for DW-LW, 0.43 for DW-toL and 0.85 for LW-toL.

Heterozygous effect of the walking genes was examined by 80 offspring-midparent combinations. Correlation coefficient was 0.74 for DW, 0.81 for LW and 0.68 for toL which were all highly significant.

These results suggested that the walking ability of *Drosophila* was controlled by the additive polygenes located in the second chromosomes.

### Adult Emergence Rhythm of Homozygous Strains of *Drosophila melanogaster*

Won Ho LEE and Chozo OSHIMA

Fifty strains, homozygous for each different second chromosome, were isolated by Cy/Pm method from a natural population of Katsunuma in autumn of 1975. Five crosses were prepared from a strain and each cross was composed with 7 females and 5 males. They were permitted to lay eggs on cultural medium of 5 vials for 12 hours under constant light condition and thereafter transferred into new vials and permitted to lay eggs on cultural medium for successive 12 hours. The same procedure was repeated more two times simultaneously with 5 replications prepared from each strain. The first A group of 5 vials, including total about 250 eggs on cultural medium, was introduced into the Insectron at the beginning of light period

of LD 12:12, 25°C constant condition. The next B group of 5 vials, including similar number of eggs on cultural medium, was introduced into the Insectron at the beginning of dark period of LD 12:12 condition. The following C and D groups of vials were introduced into the Insectron at the same light condition as A and B groups respectively. When about 7.5 days passed, adult flies began to emerge. After that, two times per day, that is, flies emerged for dark 12 hours and for light 12 hours were counted respectively. Such counts of flies were repeated for at least 4 days. Mean rates of flies emerged for light and dark periods were calculated by pooled results of four groups (A-D) and mean rates of 5 replications were also obtained. It has been investigated as a normal function of bioclock in brain that most flies emerged synchronously for several hours after dawn period. Then, the difference between mean rates of adult emergence for light and dark periods would indicate the synchronization of bioclock of the homozygous strain. Values of most strains, showing adult emergence rhythms, distributed normally among 0.956-0.500 and four strains showed low values below 0.50. The mean value of 50 homozygous strains was 0.731. From the result, the function of bioclock of *Drosophila* fly would be polygenic character and a few strains would have any defective genes.

**Circadian Locomotor Activity of *Drosophila melanogaster*  
under Noise Environment and Walking Behavior of a Group  
under Shake Environment**

Chozo OSHIMA and Won Ho LEE

Many homozygous strains for the second chromosome were extracted by Cy/Pm method from a natural population of Katsunuma in 1975, and some of them were used in this experiment. Locomotor activity of a male fly in a small glass cell (5×10×50 mm) during several days was recorded continuously by the electronic actograph. The locomotor activity was found to be circadian rhythmic and two high peaks of locomotion appeared at dusk and dawn periods of the program controlled LD 14:10 and NQ 12:12 environments. The locomotion of a male fly under the constant dark environment was inhibited at low level and continued for about twelve hours. On the other hand, the locomotor activity of a male fly under NQ 6:6 (white noise 100 phon) and constant dark environments

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This research was supported by a grant from Ministry of Environment Agency.

increased and continued for about twelve hours at high level. The excitement of a fly, stimulated simultaneously by circadian rhythmic change of light and noise environments, was pacified after several hours and such states were repeated for several days, but the excitement, stimulated by acircadian rhythmic noise, continued without pacification for a week.

Thirty flies (N) of either male or female of each strain were introduced into a terminal tube (dia. 12 mm, leng. 150 mm) and after 15 minutes, number of flies (p) distributed in five connected tubes (a: 1-5) was counted. Walking index ( $\sum ap/N$ ) was estimated. Walking activities of 61 homozygous strains were estimated under light (L) and dark (D) environments and shake (S) (5 cm cross ways 70 times/minute) environment. Total mean walking index of 61 strains was obtained under each SL, QL, QD and SD environments as follows; 1.79, 1.69, 1.44 and 1.34. The correlations between all walking indexes of these strains under two environments except for SL and SD, were highly significant. The effect of shake accelerated walking activity of a group of flies under light environment, but inhibited it under dark one.

Locomotor activities for six days of a male fly of strain 108, showing fast walking index 3.63 under SL environment, and of a male fly of strain 40, were recorded by a new actograph. The activity of the former was lower than the latter, and then these characters, locomotion and walking behaviors, would be regulated by different nerve centers. The former's bioclock lost its function as a nerve center under the constant dark environment.

### **Selection for the Discriminated Avoidance Learning Ability of Mice**

Tohru FUJISHIMA

The avoidance, discriminatory and discriminated avoidance performances of mice were measured in percentage with an automated Y-type maze apparatus, using buzzer and lamp as conditioned stimuli and electric shock as an unconditioned stimulus (cf. Ann. Rep. 25: 82).

Selection was made bi-directionally for discriminated avoidance performance on the within-litter basis. The present report deals with the first generation of selection. A significant selection effect was observed in the first generation. The selection differential, i.e. the difference between the high and low lines selected, was 29.9, and the selection response was

6.1. Accordingly, the realized heritability with standard error was  $0.204 \pm 0.0493$ . The correlated response for avoidance ability was positive and statistically significant, but that for discriminatory ability was significantly negative.

### XIII. ECOLOGICAL GENETICS

#### Submergence Tolerance of Wild and Cultivated Rice Strains

Hiroko MORISHIMA

Seventy eight rice strains collected from different tropical countries (wild—44 strains of *Oryza perennis* and 7 of *O. breviligulata*; cultivated—16 of *O. sativa* and 11 of *O. glaberrima*) were tested for submergence tolerance. Their seedlings at an age of 30–35 days, raised in a seed-bed box, were cut at a height of 15 cm, and were submerged for 14 days in a water tank (water temperature being 21°C). Seven days after they were taken out of the tank, their survival was recorded. This experiment was carried out in May–June of 1975 and 1976 with two replications. The wild species showed a wide range among strains. Among Asian strains of *O. perennis*, the annual type tended to have higher submergence tolerance than the perennial type. The American strains of *O. perennis* has a low tolerance even though they grow in deep water in their natural habitats. Strains of *O. sativa* also showed a wide range but no particular difference between the Indica and Japonica types. *O. glaberrima* seemed to have a low tolerance. It was pointed out that submergence tolerance and floating ability were independent characters.

#### Variations in Copper Tolerance of *Alopecurus aequalis* Strains

Hiroko MORISHIMA

The seeds were collected from copper-polluted and normal rice fields at Ohta, Gunma-ken and Tsuru, Yamanashi-ken. A total of 28 strains were used for this experiment, each representing more than 20 plants standing at the same site. They were tested in pots (20 cm in diameter) at three levels of copper concentration in the soil, *i.e.*, 0, 200, and 400 ppm (in the form of  $\text{CuSO}_4$ ), with two replications. About 500 seeds per pot were sown in early November. In December–January, the seedlings were killed by frost needle at different rates; copper treatment affected the restoring ability of frost-damaged roots and reduced survival. Percent survival was recorded in March for each pot, and the survival rate of a strain in copper-treated pots in percent of that in control (no copper) pots was considered as showing

its copper tolerance. The ratio of survival rates ranged from 26 to 77 percent among the 28 strains tested, and showed a significant correlation ( $r=0.50$ ) to the copper content in surface soil (4–160 ppm) of the fields from which the strains were collected.

The number of plants of this species arising from the buried seed populations in soil samples, in percent of the total plant number including other species, was exclusively high (above 90 percent) in the soil samples from polluted fields (above 50 ppm copper), though it ranged from 20 to 97 percent in the soil samples from normal fields. This indicates the dominance of *Alopecurus aequalis* in copper-polluted fields. The partial correlation coefficient between dominance and copper tolerance was significant ( $r=0.61$ ) when copper content in the soil was used as a fixed variable. It may be suggested that the evolution of copper-tolerant ecotypes plays a role in the dominance of this species in copper-polluted fields.

### **The Impact of Copper Pollution on Barnyard Grass Populations**

Hiroko MORISHIMA and H. I. OKA

To look into the microevolution of weed populations in copper-polluted rice fields, we have been engaged in observations of weed communities. This report deals with the results so far obtained in the barnyard grass (*Echinochloa oryzicola*, 4X, and *E. crus-galli*, 6X). A total of 210 barnyard grass strains (progenies of single plants) collected from copper-polluted (25–310 ppm) and control (1–18 ppm) fields were tested for copper tolerance and other characters. Copper tolerance was evaluated by the ratio of performance in copper-treated plot to that in control (no copper) plot, mainly in gravel culture and partly in water culture. The 4X species tended to be more tolerant than the 6X species. It was also found that 6X strains resembling the 4X species morphologically had higher tolerance than typical 6X strains. In both species, the populations in polluted fields showed a higher frequency of tolerant plants or a higher mean tolerance than those in control fields. But the populations were polymorphic not only morphologically but also in copper tolerance. There were tolerant plants in normal fields and sensitive plants in polluted fields. The tolerant plants were generally small in the size of various organs and inferior in performance when tested in normal conditions. They were also inferior in competitive ability. The tolerant strains showed a lower copper content in the leaves

and stems than normal strains in the copper-treated plot of gravel culture. The data were largely comparable with those reported by British workers in perennial grass species growing on strongly polluted sites, though the level of copper pollution in Japanese rice fields was much lower.

### **The Pollination Systems of *Melilotus* Species**

Yoshio SANO

To look into the pollination systems of *Melilotus* species, variations in self-fertility, floral characters and in the attractiveness of flowers for bees were observed using 87 strains belonging to 19 species. Self-fertility ranged from 99 to 2 percent among the strains. In four species in which both annual and biennial forms were present, the annual forms had higher self-fertility than the biennial forms. Other species which were annual showed high self-fertilities. Nine species of subgenus *Eumelilotus* each represented by a biennial strain significantly differed in the number of bees visiting a plant per minute. The number of bees and self-fertility were negatively correlated. The attractiveness for bees seemed to depend on the number of flowers per raceme and petal size, which also were negatively correlated with self-fertility. These characters might have been selected so as to maintain a balance among them in accordance with the pollination strategy adopted. (Submitted to *OEcol. Plantarum*; this experiment was carried out at the Hokkaido University.)

### **Resource Allocation Patterns in *Oryza perennis* Strains**

Yoshio SANO and Hiroko MORISHIMA

The photosynthetic product in the leaf of a plant species is distributed to various organs according to a certain scheme to achieve its inherent pattern of growth and development. The allocation of resources for reproductive organs, called "reproductive effort", is considered to be an important factor in determining adaptive strategy. The present experiment was carried out with the view to looking into the variations in allocation rates among 38 strains of *Oryza perennis*, which show a continuum between the annual and perennial types. The plants were grown in two concrete-beds with automatic shortday controls, and the size, number and dry weight of various organs were recorded. The proportion of seed weight to total



dry weight ranged from 1 to 50 percent, being high in Asian annual strains as well as in cultivated (*sativa*) strains tested for comparison.

Correlations were computed among 45 attributes recorded, 35 from the present experiment and the other 10 obtained from other experiments on the breeding systems, competition, etc. Those 45 attributes were divisible into two groups showing positive correlations within each, between which the correlations were exclusively negative. One group included indices for reproductive effort, seed productivity, seed dormancy, seed dispersal, drought tolerance, submergence tolerance, etc. The other group included vegetative performance, culm length, regenerating ability, outcrossing rate, anther weight in percent of flower weight, competitive ability, etc. It was suggested that the pattern of resource allocation was related to other ecological characters like tolerances and competitive ability, and could be an agent conditioning the differentiation of *O. perennis* strains in adaptive strategy.

### Variations in Competitive Ability among *Oryza perennis* Strains

Yoshio SANO and Hiroshi YAMAGISHI

On the competitive abilities of wild rice strains, no published papers are found except for a report that a strain of *Oryza perennis* was inferior to a cultivated strain (*O. sativa*) when tested in Ceylon (Narise and Sakai 1960, Ann. Rep. 10: 66). We have tested 18 Asian, 9 American and 2 African strains of *O. perennis*, 2 *sativa* strains and a *glaberrima* strain in a mix-planting experiment with a test-strain belonging to *O. sativa* (Taichung 65). The plants were harvested at the end of maximum tillering stage to measure dry weight, and the competitive ability of a strain (1) against the test-strain (2) was evaluated by the differences in dry weight between mix-planting (12; 21) and pure stand (11; 22), as  $(X_{12}-X_{11})-(X_{21}-X_{22})$ .

The variance of strains was highly significant indicating their differences in competitive ability. Among Asian strains of *O. perennis*, the perennial type tended to have higher competitive ability than the annual type. The intermediate annual-perennial strains of Asia as well as American strains showed a wide range of variation, their means being intermediate between those for the annual and perennial types of Asia. One of the two *sativa* strains tested showed a stronger competitive ability than all *perennis* strains.

Based on covariations between competitive ability and other measurements

of vegetative growth, path coefficients were computed to estimate the effect of growth rate on competition. The result showed that 63 percent of the variance of competitive ability could be accounted for by those measurements of growth in pure stand, and that the numbers of tillers and leaves at 60 to 70 days after germination and their increasing rate played a major role in determining competitive relations.

### **An Experiment on the Interaction between Cultivated Rice and Barnyard Grass at Different Planting Densities**

Hiroshi YAMAGISHI, Hiroko MORISHIMA and H. I. OKA

To examine the relation between density effect and species interaction, a rice and a barnyard grass strain were tested in pure and mix (1:1) plantings at 5 different densities. In pure stands, the data for dry weight per plant and other characters gave a good fitness to the logarithmic regression equation on plant density (derived from  $WN^a=K$ ). As for mix-planting, a parameter showing neighbor effect was added to the equation, and its values were estimated. Rice and barnyard grass had different signs for this parameter. The trend of competition effect to increase with density was not obvious. Both species tended to reduce reproductive effort at high densities. Rice showed logarithmically linear density responses in both size and number of stems, while the barnyard grass showed it only in stem number. (Submitted to Agro-ecosystems)

#### XIV. APPLIED GENETICS

##### Population Size Maintained for the Bulk Method of Breeding in Autogamous Plants

Shinya IYAMA

Bulk method of breeding is widely used for selection of quantitative traits in autogamous crop plants, i.e. after crossing between two parental genotypes, progenies are propagated by selfing without selection for several generations during which recombination between genes takes place and high homozygosity is attained. For the success of selection, one has to have desirable recombinant genotypes in a final population.

Probability that the population includes at least one desirable recombinant was calculated for various propagation scheme mentioned as follows: the population is to be propagated by taking  $n_i$  progeny from each parental plant at  $F_{i-1}$  generation. Then the population size at  $F_i$  generation is  $n_2 n_3 \dots n_i$ . The results of calculation showed that (1) at a given criterion of risk, the size of final population maintained by single seed decent method (SSD for which  $n_2 = \dots = n_i = 1$ ) is the smallest, (2) expanding a population at later generation is not effective to reduce the risk, and (3) under proper scheme of propagation one can reduce the size of  $F_3$  population to about 20% of SSD population so as to keep the final size not considerably larger than that of SSD.

##### Difference in Noise Sensitivity Affecting Egg Laying Performance between Wild and Domestic Japanese Quails\*

Takatada KAWAHARA

A wild strain of Japanese quail propagated for 10 generations in cages after being captured in the field showed changes in various productive and behavioral characters toward the domestic type (Ann. Rept. 24: 72; Kawahara, T. 1976, Experimental Animals 25: 351). The wild strain and a domestic strain were investigated with regard to their sensitivity to noise. The birds to be tested, 92 in total, were reared under a noise treatment with

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\* This research was supported by a grant from the Ministry of Environment Agency.

a buzzer of 95 phons working one hour 12 times a day for 20 days. The photoperiod applied was 18-hour light and 6-hour darkness throughout the experiment.

The egg laying performance decreased in both strains under the noise treatment, but the wild strain showed more remarkable depression than the domestic strain. The egg production rate of the wild strain decreased by 23.8 percent (82.9% in the noiseless period before treatment), while that of the domestic strain decreased by 7.1 percent (90.0% in the noiseless period before treatment). But, such a depressing effect of noise did not last long. In the domestic strain, the egg production rate was recovered in three days even though the noise treatment was continued for 20 days. In the wild strain, the recovery took 9 days. The incidence of shell-less and soft-shelled eggs slightly increased under the noise treatment in both wild and domestic strains. There was no perceptible effect of noise on the "time-of-lay" in both strains.

The wild strain has been domesticated by means of "unconscious selection" under domestic condition. Therefore, those birds may be regarded as semi-domestic, but they have maintained some noise sensitivity of the wild birds.

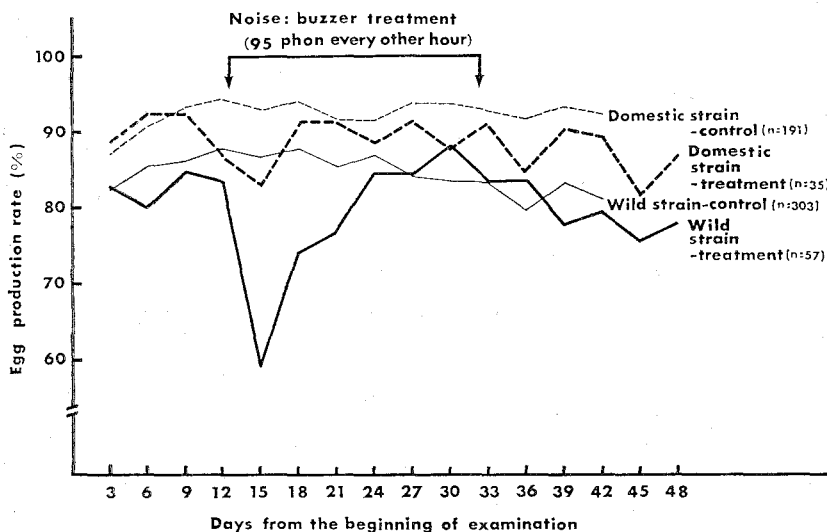


Fig. 1. Egg production curves in noise treated and noiseless control groups of wild and domestic strains.

### A Breeding Experiment in *Oryza glaberrima*

H. I. OKA

*Oryza glaberrima*, cultivated by the native people in West Africa from ancient times, has never been subjected to varietal improvement by the modern methods. Most of the varieties have red pericarp and are strongly sensitive to photoperiod. Looking for plants with colorless pericarp or white grain, three were found out of about 400 strains examined, which were from Gambia. Search for photoperiod non-sensitive plants gave 8 out of 84, which were from Guinea. From hybridization of these two exceptional types (17 crosses in total, in 1970), 37  $F_2$  plants were selected which were insensitive to photoperiod and had white grain. In a part of the crosses, insensitivity was monogenic recessive, while it was digenic (15:1) in the others. Colorless pericarp was monogenic recessive in all crosses. However, the selected plants badly lodged at maturity when heavily fertilized.

With the hope of selecting non-lodging plants, the  $F_3$  lines were intercrossed in 35 random combinations, and their progeny lines were tested for two seasons. Thirty non-lodging  $F_3$ - $F_2$  plants were selected from about 1,000, but their progeny lines showed that the selection was unsuccessful (1974). Their grain yield was 301 g/m<sup>2</sup> on the average (10-6-6 NPK, g/m<sup>2</sup>), while an improved *sativa* variety (Taichung 65) in an adjacent plot gave 447 g/m<sup>2</sup>.

Then, the bulked seeds of the selected plants were irradiated with 20 kR gamma-rays, and about 10,000  $M_2$  plants (by one-panicle-one-kernel method, from about 1,000  $M_2$  plants) were observed, and 21 non-lodging plants were selected. But their  $M_3$  lines lodged again (1976); we had to give up our trial to select non-lodging *glaberrima* strains. Possibly, the lodging of the *glaberrima* rice is related to its annual habit; not only the leaves but also the straw shows senescence with seed-ripenning. It seems difficult to obtain a non-lodging mutant unless the annual habit is modified.

### Plasma Albumin Polymorphism in Japanese Quails

Takeshi SHIBATA,<sup>1)</sup> Seiki WATANABE<sup>1)</sup> and Takatada KAWAHARA

The blood plasma from six Japanese quail strains (4 domestic, 1 wild, and 1 hybrid; 936 birds in total) were examined for albumin polymorphism.

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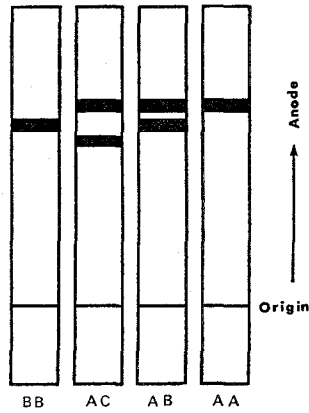


Fig. 1. Diagrammatic patterns of plasma albumin in Japanese quail.

Four band forms, named AA, BB, AB and AC were detected (Fig. 1). The result of mating experiments indicated that these bands were controlled by three codominant autosomal alleles,  $Alb^A$  for the fast band (A),  $Alb^B$  for the medium band (B), and  $Alb^C$  for the slow band (C). The albumin polymorphism was first reported by Haley (1965, *Genetics* 51:983) who found two codominant autosomal alleles and their resultant three band forms. The present experiment proved the presence of the third allele,  $Alb^C$  though its frequency was very low and its homozygote was not obtained. The gene frequencies in the present material were 0.980 for  $Alb^A$ , 0.016 for  $Alb^B$ , and 0.004 for  $Alb^C$ . All 256 wild birds were homozygous for  $Alb^A$ ;  $Alb^B$  was found in domestic strains preserved in the Tokyo University of Agriculture, and  $Alb^C$  was found in a domestic strain of this Institute.

### The Growth Pattern and Competitive Ability of Six Rice Varieties

Hiroshi YAMAGISHI, Hiroko MORISHIMA and H. I. OKA

The "Rice Adaptation Experiment" was conducted in 1968 to 1972 under the International Biological Programme (JIBP/UM), testing a number of rice varieties for their yielding potential and yield stability at different stations distributed in the world. From a part of the data, we have pointed out the variation between the "early vigor" and "late vigor" types and

discussed their bearings on yielding potential (Morishima and Oka 1974, Jap. J. Breed. 24: 226). In the present experiment, this variation was re-examined with six selected varieties which had been used in the Rice Adaptation Experiment and their competitive abilities against a test-strain (Taichung 65) were also observed. The experiment was laid out according to the randomized blocks design with two replications.

The data for growth pattern were generally in good agreement with those from the Rice Adaptation Experiment ( $r=0.89$  in multiple correlation for various indices showing growth). Competitive ability was evaluated by the increment or decrement in performance of two mix-planted strains (1, 2) as compared with their values in pure stand ( $X_{12}-X_{11}-X_{21}+X_{22}$ ). It markedly differed among the varieties tested, and showed strong correlations with the growth rate at 70 to 90 days after germination in high-fertilizer plot ( $r=0.96$ ), or at 90 to 100 days in low-fertilizer plot ( $r=0.89$ ). Competitive ability was also strongly correlated with percent cover of soil surface by the leaves at an age of 75 days ( $r=0.99$ ) and with its increasing rate per day ( $r=0.99$ ). This indicates that in rice competition takes place for light by means of growth rate, and that the "early vigor" type has higher competitive ability than the "late vigor" type.

### Nitrogen-Fixing Ability in Rice Strains

Taro FUJII, Yoshio SANO and Shin-ya IYAMA

The capacity to assimilate aerial nitrogen is limited to certain species of bacteria and algae, and to legumes and some other plants with which nitrogen-fixing bacteria are symbiotic. If the nitrogen-fixing ability was introduced into a non-leguminous crop plant by genetic procedures, a great advantage would be expected in crop production. With the hope of exploring such possibilities, we have initiated experiments on the nitrogen-fixing ability in rice strains. The acetylene reduction assay by gaschromatography (Shimadzu GC-3BF) was adopted for evaluating the ability for nitrogen fixation by the roots with which some bacteria might coexist. Ethylene yield was assessed by conversion from gas-chromatographic profile to a figure in n mol/g fresh root/hour, using the value obtained with soybean roots (T 202) as the standard. In soybean roots, acetylene reduction started immediately after incubation in an aerobic gas phase (10%  $C_2H_2$ +90% air), and an average of 1,400 n mol/g/h of ethylene yield was obtained.

This year, 50 wild (*Oryza perennis*) and 150 cultivated (*O. sativa*) strains of rice were selected at random from our genetic stocks collected from different tropical countries, and used for the experiment. The seeds were sown in seed-bed flats, and the seedlings raised in a green-house were transplanted into an irrigated paddy field in May. Fertilizers applied were limited to the fused phosphate fertilizer (25 g/m<sup>2</sup>) and potassium sulfate (14.5 g/m<sup>2</sup>), as basic dressing. When the plants started heading, the roots were sampled for assay. The whole plants were dug up, and the roots were excised and immediately washed with tap water. The roots were cut into pieces 4-5 cm long and 2 g in fresh weight were put into a 30 ml bottle with a rubber-needle-puncture stopper. The rice roots showed no acetylene reduction under aerobic conditions.

Then the air in the bottle was evacuated and filled with a mixture of 90% helium and 10% acetylene. The bottles were then incubated at 30°C for assay. One ml of incubated gas was taken out from the bottle by using a glass syringe and was injected directly into the gas-chromatograph column. As a control experiment, when acetylene was not added, no ethylene was produced. The assay of field soil gave no ethylene either. These demonstrate the role of roots in acetylene reduction.

The production of ethylene was observed after a lag time of 4 to 6 hours, and increased with incubation time. Most of the strains tested showed about 5 to 15 n mol/g/h. Three strains showed significantly high ethylene yields of 45 to 65 n mol/g/h (Table 1). They were C5444 and C8005 from India and C7588 from Kenya. The variation observed suggests that the nitrogen-fixing ability of the root in the soil is genetically controlled. The assay of the whole root system with the basal part of stems and soils is under way.

Table 1. Variation of acetylene reduction ability in rice strains

	Total No. of strains tested	Acetylene reduction activity (n mol/g/h)						
		5	15	25	35	45	55	65
Cultivated strains	81	45	17	13	4	1		1
Wild strains	46	12	15	16	2		1	



Selection of Induced Mutants in *Oryza glaberrima*

Yoshio SANO

An examination of more than 400 strains of *Oryza glaberrima* collected from West African countries gave quite a limited number of striking morphological differences useful for genic analysis. In order to obtain gene markers and to compare their functions with those of similar genes of *O. sativa*, the seed of a photoperiod insensitive strain with colorless pericarp (GMS, selected by Oka, presented in this Annual Report) was treated with EMS (0.5% solution for 5 hours at 27°C after 2-hour presoaking). The M<sub>2</sub>

Table 1. Mutants selected from an M<sub>2</sub> population (7079 plants, EMS treated) of a *glaberrima* strain insensitive to photoperiod and with colorless pericarp

Type compared with <i>sativa</i> mutants	No. of plants	Frequency ×10 <sup>-4</sup>
Dwarf, "Dwaikoku"	10	14.1
— , tillering	8	11.3
— , others	18	21.4
Chlorosis	13	18.4
Narrow leaf	4	5.6
Broad leaf	1	1.4
Brown discoloration	9	12.7
Rolled leaf	1	1.4
Branching	1	1.4
Brittle culm	2	2.8
Liguleless	12	17.0
Lax panicle	1	1.4
Long empty glume	1	1.4
Double glume	1	1.4
Hairy glume	1	1.4
Awned	1	1.4
Apiculus coloration	1	1.4
Minute grain	2	2.8
Large grain	2	2.8
Round kernel	1	1.4
Long kernel	1	1.4
Glutinous endosperm	1	2.6*
Early heading	3	4.2
Early lodging	1	1.4
Semi-sterile	2	2.8

\* Frequency of heterozygotes; homozygote frequency is its 1/4.

seeds were collected by "one-panicle-one-kernel" method from about 1,000  $M_1$  plants, and about 7,000  $M_2$  plants were raised in an upland field.

The  $M_2$  plants displayed a spectrum of morphological changes. A total of 96 plants were selected; their mutant characters were classified into 24 types, among which were found different types of dwarfisms, plants with narrow or broad leaves, long empty glumes, and necrotic brown spots on the leaves, ligulelessness, early heading, etc. (Table 1).

Further, a glutinous (waxy) mutant was found from iodine test of the  $M_2$  seeds on  $M_1$  panicles ( $1/3760=0.026\%$ ), and glutinous  $M_2$  plants were obtained from the seeds. In heterozygous panicles, the non-glutinous:glutinous ratio of  $M_2$  seeds was 83:25 indicating that the glutinous character was a monogenic recessive. When crossed with a glutinous strain of *O. sativa*, the  $F_1$  seeds had glutinous endosperm suggesting that the induced glutinous gene had a similar function as that of *O. sativa*.

As *O. glaberrima* is annual, all the plants die after seed maturity. To search for mutations affecting the annual habit, the  $M_2$  plants at maturity were cut and ratooning stubbles were looked for. Though it was in late October, 20 ratooning plants were found; they were transplanted in pots and were cultured in a greenhouse. However, all of them died in one month, indicating that the search for perennial mutations was unsuccessful.

### **Breeding of Isogenic Lines of Rice Carrying Marker Genes in Interchanged Segments**

Yoshio SANO and H. I. OKA

The breeding of isogenic marker lines and isogenic translocation lines of Taichung 65 was reported previously (Ann. Rep. 24: 66 & 25: 84). From intercrossing of the marker lines, isogenic lines carrying two independent markers were obtained, that were T65-*ne-bc* (neck leaf and brittle culm), T65-*g-lg* (long empty glumes and liguleless), and T65-*la-gl* (lazy habit and glabrousness). They were each crossed with respective translocation lines, 30 in total number, to identify the chromosomes involved in the reciprocal translocations. Linkage relations between a marker and the point of interchange were detected in 15 out of 55  $F_2$  populations so far observed. From each  $F_2$  population showing such a linkage, one or two plants homozygous for a marker and heterozygous for an interchanged segment were selected, and from their progenies plants homozygous also for translocation were

selected by test-crossing the  $F_3$  plants with the original strain of Taichung 65. Thus, three translocation lines carrying different markers in the interchanged segment were obtained. They will be used for genic analysis. This work is still under way to increase the number of translocation lines carrying gene markers.

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## ABSTRACTS OF DIARY FOR 1976

January	16	231st Meeting of Misima Geneticists' Club
February	9	232nd Meeting of Misima Geneticists' Club
March	4	233rd Meeting of Misima Geneticists' Club
	5	234th Meeting of Misima Geneticists' Club
	8	123rd Biological Symposium
	17	235th Meeting of Misima Geneticists' Club
April	10	124th Biological Symposium
	15	236th Meeting of Misima Geneticists' Club
May	28	237th Meeting of Misima Geneticists' Club
July	28	238th Meeting of Misima Geneticists' Club
September	16	239th Meeting of Misima Geneticists' Club
October	15	240th Meeting of Misima Geneticists' Club

## FOREIGN VISITORS IN 1976

February	24	JACQUARD, P., Centre d'Etudes Phytosociologiques et Ecologiques, Montpellier, France.
	25	PLITMAN, U., Department of Botany, The Hebrew University, Israel.
March	31	PANAYOTOR, I., Institute for Wheat and Sunflower, Bulgaria.
April	9-11	COMSTOCK, R. E., Dept. of Genetics and Cell Biology, University of Minnesota, U.S.A.
	13	RAYCHAUDHURI, S. P., Haryana Agricultural University, India.
	14	NYGREN, A., Department of Genetics and Plant Breeding, Agricultural College of Sweden, Uppsala, Sweden.
	14	SAMMARRAI, S. M., College of Agriculture, University of Rizadh, Saudi Arabia.
July	9	CHUNG, Y. J., Ewha Women's University, Korea.
July	30-31	MACKAY, A., Dept. of Crystallography, Birkbeck College, University of London, England.
August	17	RO, B. J., Ewha Women's University, Korea.
	27	ORAM, M. N., C.S.I.R.O. Division of Plant Industry, Canberra City, Australia.
	27	LAWN, R. J., C.S.I.R.O. Division of Tropical Crops and Pastures, St. Lucia, Queensland, Australia.
September	6	BLUNDELL, T. L., Dept. of Crystallography, Birkbeck College, University of London, England.
	7	RUFFIÉ, J., Centre d'Hémostyologie, CNRS, France.
	7	KOWNACKI, M., Polish Academy of Sciences, Institute of Genetics and Animal Breeding, Poland.
October	1-5	KING, J. L., Dept. of Biological Sciences, University of California, U.S.A.
	1-5	AYALA, F., Dept. of Genetics, University of California, U.S.A.
	1-5	HOOD, L., Division of Biology, California Institute of Technology, U.S.A.

- 1-5 DICKERSON, R. E., Division of Chemistry and Chemical Engineering, California Institute of Technology, U.S.A.
- 1-5 MCLACHLAN, A. D., MRC Laboratory of Molecular Biology, University Postgraduate Medical School, Cambridge, England.
- 9 GANI, J., Division of Mathematics and Statistics, Commonwealth Scientific & Research Organization, Australia.
- November 10-11 VERGNES, H., Centre d'Hématologie de Toulouse, France.
- 18 CHU, E. H., Department of Human Genetics, University of Michigan Medical School, Ann Arbor, Michigan, U.S.A.

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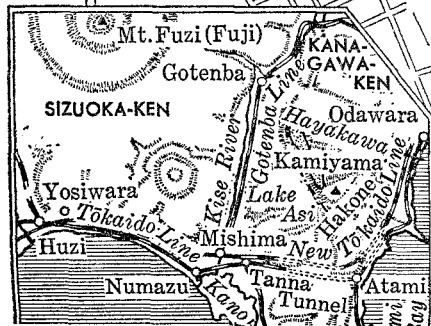
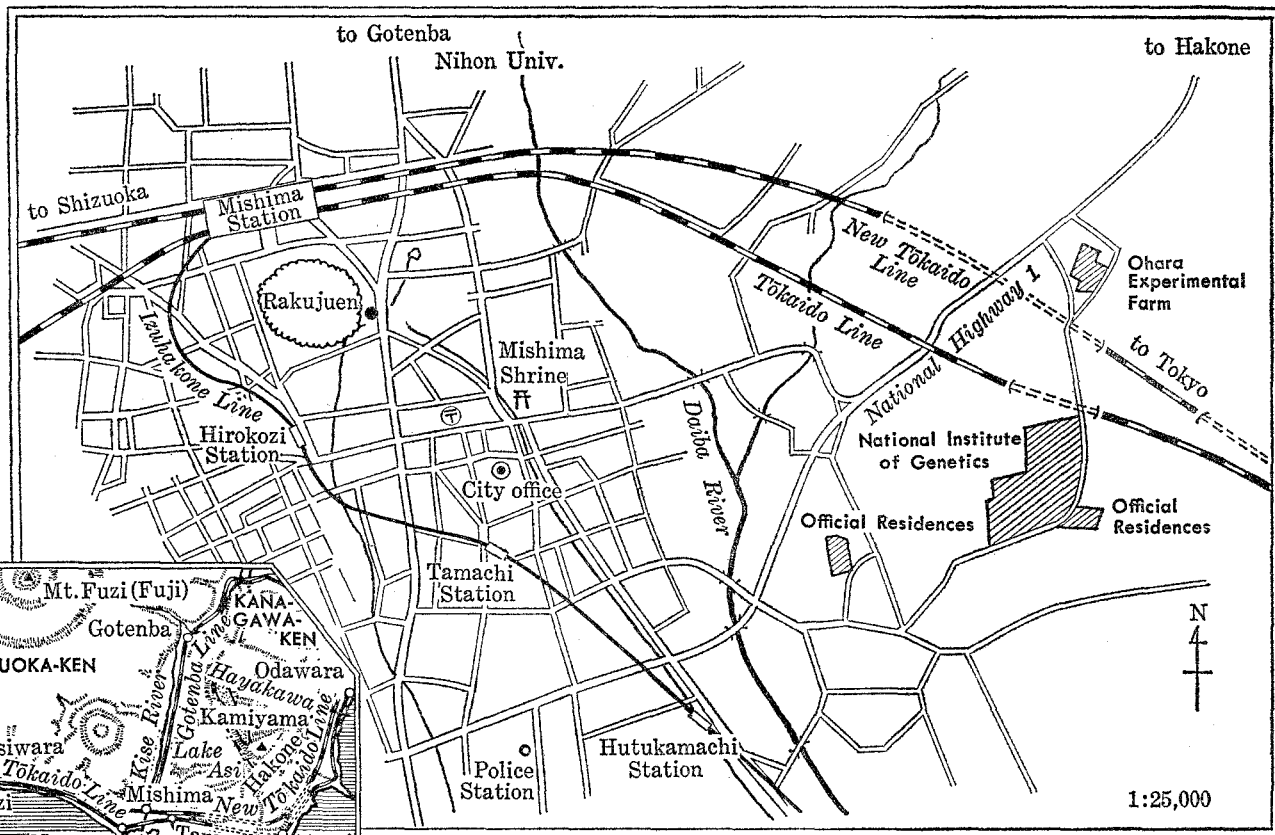
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