Meetings

Yeast Genetics

Eighty-three yeast geneticists from ten countries met in Osaka, Japan, 1–5 September 1968. This was the fourth international conference of yeast genetics, including the meeting on nomenclature that was held at Carbondale in 1961. J. Ashida (Kyoto University) welcomed the group and spoke of the accomplishments of Y. Hashitani and Yukio Yamamoto who pioneered studies on yeast biology in the Osaka area.

Nine general topics were discussed —mutation, suppressors, sex control mechanisms, extranuclear inheritance, nucleic acids, gene-enzyme relations and regulation, recombination and gene conversion, techniques, and nomenclature.

The session on mutagenesis was chaired by G. Magni (Parma, now Milan) and S. Nakai (Chiba). Magni opened the discussion with a description of the mutagenic action of 2*M* NaCl; it is mutant-specific rather than strain-specific and can confound hydroxylamine mutagenesis. To reduce the killing action of hydroxylamine, 2*M* NaCl is usually added. One molar pyrophosphate has been suggested as a substitute coadjuvant (Freese, personal communication). Magni reported that nitrosoguanidine and hydroxylamine are highly specific for inducing base substitutions; hydroxylamine does not revert nonsense mutations (also observed by Hawthorne, University of Washington, Seattle), therefore it appears specifically to induce GC → AT transitions. It has been claimed that ICR-170 is specific for addition-deletion mutations in *Salmonella, Neurospora,* and *Drosophila.* Magni stated that at 10 μg/ml in yeast 90 percent of the mutations are base substitutions; at lower concentrations (where it is alleged to be specific) the induced frequency is too close to the spontaneous mutation level to be studied efficiently.

Sherman (Rochester) reported on the collaborative efforts of Stewart (Rochester), Parker (Baltimore), Putterman (North Chicago), and Margoliash (North Chicago) concerning the mutational alteration of iso-1-cytochrome c. They also have not found frameshift mutations with ICR-170. Further, Sherman presented data showing the nonspecificity of mutagens and the change of specificity with different sites in the gene. For example, with ultraviolet radiation, the nonsense codon UAA reverts to a glutamine codon (AT → GC) when it is at amino acid position 2 (strain *cy 1-9*), but it reverts to tyrosine and leucine codons (AT → TA and CG) when it is at position 20 (strain *cy 1-2*). Additional amino acid replacements were found after treating *cy 1-2* and *cy 1-9* with nitrous acid, EMS, and nitrosoimidazolidone. The evidence was reviewed and it was generally agreed that most spontaneous mutations in vegetative cells of *Saccharomyces* (Roman, Seattle) and *Schizosaccharomyces pombe* (Loprieno, Pisa) are base substitutions. Magni presented more evidence that most spontaneous auxotrophs obtained during meiosis in turn have an increased reversion rate during meiosis; von Borstel (Oak Ridge) described an x-radiation–sensitive mutant of yeast, isolated by Resnick (Berkeley), that has a high spontaneous mutation rate for addition-deletion mutations in vegetative cells. Nakai also has been studying a mutant with similar characteristics.

Selective killing of prototrophic cells for enrichment of auxotrophic mutants was discussed. The most widely used method is the nystatin techniques in which cells deprived of nitrogen are vigorously grown until cell number has increased by 20 percent; then the nystatin is added (Snow, Davis). Protoplast formation by prototrophic cells in log phase by snail enzyme in minimum media, where auxotrophs are not growing and are not protoplasted as readily, is strain-specific (Pittman, AEC, Washington, D.C.) and is a promising enrichment technique, although strain-limited. The killing of cells requiring inositol in a medium deficient in inositol (whereas auxotrophs live) was described by Megnet (Berlin) for *Schizosaccharomyces pombe.* This technique has not worked efficiently in *Saccharomyces* in the strain requiring inositol with which it was tried (Snow). Gutz (Dallas) reported that the method worked very well in *Schizosaccharomyces.*

T. Ito (University of Tokyo) and K. Hieda (Rikkyo University, Tokyo) have shown that no liquid-holding recovery occurs from photodynamic killing or mutation of *Saccharomyces* cells after uptake of acridine orange; neither is there an intensity effect from the light. Different wild strains sensitive to ultraviolet radiation show a variety of sensitivities to photodynamic light.

N. Yanagishima (Osaka) induced variants of high DNA content as well as ρ^- mutants with three different auxins. Takahashi (Suita) showed genetically that trisomics and, possibly, triploids were included among the variants induced by auxins.

Pittman and Magni discussed experiences in inducing mutants sensitive to temperature. Pittman used 30°C as the permissive temperature and 36° and 40°C as the restrictive temperatures. Magni used 23°C as the permissive and 33°C as the restrictive temperature. Puglisi (Parma) isolated a mutant which probably affects DNA polymerase; Falaschi (Pavia) is studying its characteristics.

T. Sugimura (National Cancer Center, Tokyo) described the characteristics of 4-nitroquinoline-1-oxide, a powerful carcinogen whose mutation characteristics are not unlike ultraviolet radiation.

Von Borstel and H. Saito (University of Tokyo) were chairmen of the sessions on radiation mutagenesis and suppressors. Takahashi described an increase in intergenic recombination occurring many cell generations after low exposures to ultraviolet radiation of either one or both of the parental strains; an ultraviolet effect on intragenic recombination was also described.

A. Nasim (Chalk River) studied mosaic colony production in *Schizosaccharomyces* following irradiation; by interpreting the action of different mutants sensitive to ultraviolet radiation in producing variable frequencies of mosaics he favors a "repair hypothesis" for production of complete mutants. S. Nakai described the characteristics of several strains of *Saccharomyces* sensitive to x-radiation; they fall into five classes—recombination deficient, reduced catalase activity, abnormal cell division, petite, and unclassified. Mortimer (Berkeley) reported that Resnick (Berkeley) had isolated a mutant that cannot undergo photoreactivation following irradiation with ultraviolet light.

Supersuppressors in *Saccharomyces* have been isolated in a number of laboratories. Mortimer described a new classification and nomenclature scheme that he and Hawthorne (University of Washington, Seattle) developed. Sixteen supersuppressors have been mapped; at least 26 have been shown to be nonallelic. Hawthorne has induced isoalleles of supersuppressors that uniquely suppress UAA or UAG codons. Gilmore, Sherman, and Stewart (Rochester) have shown that eight separate suppressors of class I, set I, are responsible for replacing tyrosine in iso-1-cytochrome c. The most commonly induced suppressible codon appears to be UAA or UAG; since a reversion containing tryptophan has not yet been recovered among separate reversions of a suppressible mutant, they believe it to be UAA.

The sessions on sex-controlling mechanisms and on cytoplasmic inheritance were chaired by Sherman and Yanagishima. Y. Oshima and I. Takano (Osaka) reported on an α-mating–type specific mutator for homothallism, $HO\alpha$, which, with a probability of nearly 100 percent, leads to spontaneous diploidization of cells during the first two divisions. Yanagishima (Osaka) isolated *a* and α hormones and showed that expansion of cells before mating is a response to hormones produced by opposite mating type. Astonishingly enough, cells of the α mating type respond to testosterone; cells of the *a* mating type respond to estradiol. C. Shimoda (Osaka City University) showed that β-1,3-glucanase acts on cell walls of yeast to cause expansion, but only upon diploid cells of the variant responsive to auxins, and of those containing the gene controlling homothallism; normal heterothallic cells do not respond. N. Takao (Kobe Women's College of Pharmacology) has purified the *a* and α hormones and has shown them to be identical with respect to color tests and ultraviolet absorption spectra; he suspects by thin-layer chromatography that they may be stereoisomers of steroids. Gutz (Dallas) has been studying lethal mutations in the mating type region of *Schizosaccharomyces*; these lethal mutations have no effect on the region with respect to meiosis. In diploid strains of the mating-type constitution *h90/h*— a frequent mitotic crossing over (one in 80 divisions) occurs in the mating-type region giving rise to *h90/90* and *h*—/*h*— cells. Mortimer reported that Hartwell (Irvine, now University of Washington, Seattle) found a haploid disomic for the mating-type chromosome which undergoes meiosis yielding incompletely developed spores. Magni reported on the work of Sora (Parma) on tetraploid formation from crosses between a/α diploids; mitotic recombination to yield a/a or α/α cells in at least one parent makes matings possible. Roman (University of Washington, Seattle) reported that rare matings occur between α cells to yield an α/α diploid cell. Matings have not been observed between *a* cells. Diploids of a/a must therefore arise from endomitosis.

K. Wakabayashi (University of Tokyo) presented evidence that adenosine triphosphatase in mitochondria is encoded by a nuclear gene. I. Mifuchi and T. Morita (Shizuoka College of Pharmacology) reported that 4-nitroquinoline-1-oxide induces a variety of respiration-deficient mutants resembling those induced by ultraviolet radiation rather than those induced by acriflavine. T. Sugimura, M. Nagao, S. Miyake (National Cancer Center Research Institute), N. Gunge (Dai-Nippon Sugar Manufacturing Co.), and M. Osumi (Japan Women's University) are mounting an integrated attack on a nuclear mutant sensitive to temperature which lacks all cytochromes. H. Kasahara (Japanese Association of Sake Brewers, Tokyo) found that two respiration-deficient nuclear genes are segregating in the natural population of *S. cerevisiae* used for brewing sake. Y. Arakatsu (Konan University, Kobe) is studying three major components of the structural porteins of mitochondria. With a ρ— mutant that was induced by acriflavine, significant differences from wild type of the amino acid content of all three components were found. Heslot and Goffeau (Paris) have been unable to induce ρ— mutants in *Schizosaccharomyces pombe* with proflavine or ethidium bromide; two mutants resistant to $CoSO_4$ have been shown to lack cytochrome oxidase but they were not ρ— mutants. Nevertheless, researchers have been able to obtain mutants resistant to decamethylene diguanidine which exhibit a cytoplasmic mode of inheritance.

The session on nucleic acids was chaired by J. G. Kaplan (Ottawa) and A. Tsugita (Osaka University). Tsugita and K. Asano have demonstrated that mitochondrial RNA has a quite different base ratio from yeast ribosomal and transfer RNA. Actinomycin does not block RNA synthesis in mitochondria in intact cells, but trypaflavine does. During the transition from anaerobiosis to aerobiosis two types of mRNA are induced in mitochondria. K. Ouchi, H. Saito, and Y. Ikeda (University of Tokyo) measured the homology index among species and genera of yeast by DNA-DNA hybridization. T. Yamamoto (Osaka City Institute of Hygiene) has studied the structure of ribosomal RNA extracted from salt-resistant yeast ($SrCl_2$, 0.75*M*) and found it had minor deviations in base sequence from ribosomal RNA synthesized in salt-free medium. F. Lacroute (Strasbourg) and J. G. Kaplan (Ottawa) have found that UTP inhibits synthesis and activity of both aspartate transcarbamylase (ATCase) and carbamyl phosphate synthetase (CPSase); both proteins are encoded by *ur 2*; Lue (Ottawa) has shown that both activities are present in a single enzyme complex. Kaplan advanced the hypothesis that *ur 2* codes for a single polypeptide chain with ATCase activity alone; CPSase and feedback sites appear after association of subunits. However, Duphil (Ottawa) has found that mutants of *ur 2* which have lost both activities cluster within the locus at a considerable distance from those which have lost ATCase alone. Nagai (Nara) has found a ρ— mutant induced by actidione in *Saccharomyces logos* which is unstable. The mutant generates normal cells, stable petites, and more unstable petites.

The session on regulation and on recombination was chaired by R. K. Mortimer (Berkeley) and T. Takahashi (Suita). Heslot and Nagy (Paris) have found that the *adenine 4* mutant of *Schizosaccharomyces pombe* lacks PRPP amino transferase activity and is also the site for feedback inhibition

of the adenine-guanine pathway; the enzyme has been partially isolated from both the wild strains and a feedback-inhibitionless mutant. Moreover, the enzyme from the mutant is less inhibited by identical concentrations of the end products. Oshima (Osaka) discussed how the enzyme α-glucosidase (maltase), which is polymeric, can become a functional enzyme even when made of isomers encoded by different genes. H. Tamaki (Kyoto) showed that at least two polymeric genes control starch fermentation in *S. diastaticus* and these genes are regulated by inhibitor genes brought in from an industrial alcohol yeast (Hakken No. 1, *S. cerevisiae*).

Roman and Friis (Copenhagen) in studying mitotic gene conversion in a/a, α/α, and a/α diploids have found that after nitrosoguandine treatment, x-radiation, or ultraviolet radiation, the a/α cells convert at a frequency 15 times that of a/a or α/α cells. Mortimer stated that mitotic gene conversion occurs at the same frequency in haploid cells disomic for chromosome VIII as for the a/α diploid. Roman has evidence that induced gene conversion during mitosis is not the result of the formation of two heteroduplexes, as current hypotheses suggest. Fogel (Brooklyn) reported that J. Wildenberg (Brooklyn), by irradiating and obtaining pedigrees of synchronized populations, has proved that the conversion events occur during both G_1 and G_2 in mitotic diploid cells. Fogel and Mortimer proved that gene conversion during meiosis is a process of complete fidelity to the nucleotide level; also, mutants of *arg 4* at distances of 130 nucleotides from one another usually convert together. Occasionally, mutants convert together when they are at distances 1000 nucleotides apart. Hurst (Brooklyn) and Fogel confirmed these findings for *his 1* and *thr 3*, and also find that conversion may occur prereplicatively during meiosis with hybrid DNA extending over two loci. Gutz (Dallas) has also shown that simultaneous conversion is more common between alleles close to one another in the *adenine 6* locus of *Schizosaccharomyces pombe*. Further, Gutz found an *adenine 6* mutant which gives a peculiar pattern of gene conversion; the behavior of this mutation cannot be easily explained by either the Holliday or Whitehouse model of genetic recombination, but fits well into a model suggested by Herbert Taylor.

Roman and Takahashi chaired the last session which included fine-structure mapping and cytology. Snow (Davis) finds that methyl methanesulfonate is as useful as x-rays in fine-structure mapping. Like x-rays, methyl methanesulfonate linearly induces prototrophs at exposures well below levels fatal to cells. Mortimer discussed a selection system that Rodarte (Berkeley) is using to find recombinationless mutants of yeast; at present 18 have been found. Four mutants are sensitive to x-radiation but not to ultraviolet radiation; 14 are not sensitive at all to irradiation of either kind. Roman described a technique for obtaining pure suspensions of spores, virtually free of vegetative cells. Nagai described the use of the flower frog, kenzan, for making identical replica plates.

H. Tamaki (Kyoto) showed pictures of meiotic chromosomes; he uses strong Flemming fixative on young asci before applying snail enzyme followed by Giemsa stain. Gutz described the work of Treichler (Dallas) on Robinow bodies in the cells of yeast nuclei; usually, one can be seen in a haploid cell and two in a diploid.

A committee composed of Mortimer, Sherman, and von Borstel was appointed to undertake changes in nomenclature of mutants; Nakai and Magni are consulting members. Mortimer proposed the use of three-letter symbols following the bacterial system and the elimination of subscripts and superscripts. Fogel is making the arrangements for a yeast genetics stock center at Brooklyn College. The next yeast genetics conference probably will take place in 1970 in Italy or Canada.

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This conference was arranged by a committee composed of J. Ashida (Kyoto University), Y. Ikeda (University of Tokyo), A. Yuasa (Japan Women's University, Tokyo), S. Nagai (Nara National Women's University), T. Takahashi (Brewing Science Research Institute, Suita), Y. Oshima (Suntory Ltd., Osaka), N. Yanagishima (Osaka City University), H. Tamaki (Doshisha Women's College, Kyoto), and S. Nakai (National Institute of Radiological Science, Chiba).

Foreign visitors at the conference were impressed by the courtesy, hospitality, and friendliness of their Japanese hosts. The meeting was made memorable not only because of its scientific interest but also because of the numerous festivities that were so generously provided.

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Behavioral Sciences and the Medical School

It is apparent to most physicians that basic medical and clinical sciences cannot totally explain the phenomena of the sick patient and especially how he is living with his illness. Such explanations come only through work with the behavioral sciences in a clinical setting.

These patient-care focused problems can be approached with psychologists and sociologists. Areas of focus such as the developmental history and psychological theory which could explain patient behavior, or an analysis of the kinds of activities that physicians and other health care personnel are providing in relation to patient care, are pertinent areas for behavioral science investigations. Such investigations were discussed at a conference held by the National Institute of General Medical Sciences, in Bethesda, Maryland, 25 September 1968.

Experience dictates that as this becomes a useful activity, interns and residents and some of the medical students will become interested. Over the years such clinical discussions of patients can be expanded to an exploration of specific research in basic behavioral science and clinical projects. Questions asked in the clinic then become amenable to possible analysis in a much more structured and highly controlled laboratory setting.

For example, investigations on physiologic responses to a set of environmental stimuli can be conducted in parallel to the clinical patient-care process, programmed in part by the sociologists and psychologists to measure both the physiologic and the metabolic, and the psychological and social parameters.

Such a basic-clinical relationship can