Meetings

Techniques in Somatic Cell Genetics as They Pertain to Senescence of Diploid Cells

Ever since a finite life-span of human diploid fibroblasts in cell culture was described by Leonard Hayflick (Stanford University) and Paul Moorhead (University of Pennsylvania), these cells have been in use as a model system to elucidate a variety of cellular phenomena, some of them related to biological aging. A workshop was held 8 to 10 March at the Institute for Medical Research, Camden, New Jersey, sponsored by the National Institute of Child Health and Human Development, to discuss methods in somatic cell genetics and related areas as they might be applied to studies of senescence of these diploid cell cultures. The participants included 18 investigators in somatic cell genetics and 7 in theoretical biology.

Leslie E. Orgel (Salk Institute, San Diego) reviewed the current status of his theory that aging and senescence result from an accumulation of cellular misinformation in the form of altered proteins, which then function with decreased efficiency and eventually lead to grossly impaired function and cell death. He stressed that this type of misinformation is only one of several mechanisms that might contribute to the aging process. He reviewed the observations of Robin Holliday (National Institute for Medical Research, Mill Hill, London) and his collaborators demonstrating that specific activities and thermal stabilities of certain cellular enzymes decrease with increasing age of cultured cells, and noted that the degree of thermolability of cellular protein is thought to be predictive of the degree of aging. He noted that protein errors and also possibly gene mutations tend to increase precipitously in cultured cells just before senescence. Also discussed was the question of what changes are produced when a population of cells with a finite in vitro life expectancy are transformed to a "permanent" cell line with an apparently unlimited survival potential. An idea of clonal evolution might be invoked to explain the difference between normal and transformed cell lines, because events occur which alter the selection intensity compared to the rate of accumulation of misinformation. That is, transformation might not necessarily increase the accuracy of the synthetic processes, but might provide more opportunity for cellular selection.

Selective methods to detect spontaneous and induced auxotrophic and drug-resistant mutants were discussed by E. H. Y. Chu (University of Michigan), Morgan Harris (University of California, Berkeley), and Liselotte Mezger-Freed (Institute for Cancer Research, Philadelphia). Methods have recently been developed to study these mutagenic events in cells in culture in ways similar to those used in microbial genetics. Interpretations of the results of this type of experiment have been questioned. There is some doubt as to whether the variants selected are true mutants. Chu pointed out that 5-bromodeoxyuridine (BrdU)—a deoxyriboside analog of thymidine which is used in conjunction with visible light to kill actively dividing cells and thereby select for dormant (nonreplicating) mutant cells—is itself mutagenic to cells at the concentrations used; this fact must be considered in interpreting either spontaneous or induced mutagenic events. The interpretation of data from this type of system was questioned by Harris and Mezger-Freed when experiments with cells of different ploidy did not yield the mutation frequencies predicted for the gene dosage. Harris found that mutation frequencies in tetraploid and octoploid Chinese hamster cells were not those expected on the basis of the mutation frequency in diploid lines. In similar studies, Mezger-Freed used haploid and diploid frog cells; again, the frequency of the variants differed from that expected on the basis of gene dosage. Another kind of evidence suggests that thymidine kinase deficiency in frog cells might not arise from gene mutation; a clonal analysis revealed that a haploid, isogenic population is heterogeneous in its competence to yield the enzyme-deficient variants. These results led Mezger-Freed to ask whether some of the variants described are stable alterations in gene expression, like those that occur in differentiation, rather than genetic mutants. Orgel suggested that this question might be answered by seeing whether variants could be induced by treatment with highly reactive nonmutagenic compounds. Harris also reported data from cell fusion experiments indicating that mutations to vinblastine resistance are dominant, whereas mutation to arabinosylcytosine resistance is recessive in hamster cells.

The role of DNA repair mechanisms in genetic systems of cultured somatic cells and in senescence was described by Michael W. Lieberman (National Cancer Institute, Bethesda, Maryland), Samuel Goldstein (McMaster University, Hamilton, Ontario), and Frederick J. de Serres (National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina). Lieberman discussed the three types of repair known to occur and indicated that excision repair is believed to be the common one in human cells. Repair can be detected in diploid cells by virtue of unscheduled DNA synthesis. When diploid fibroblasts are in confluent culture, approximately 1 of 200 cells is synthesizing DNA. If an electrophilic chemical that reacts with DNA is added to these cultures, unscheduled DNA synthesis is initiated in a high percentage of the cells. There is some evidence that the newly synthesized DNA is true to the original DNA, but there is also evidence that repair is not complete and that 15 to 50 percent of DNA damage produced by the chemical is removed and repaired.

Goldstein reported that fibroblasts from patients with xeroderma pigmentosum repair damage induced by x-irradiation but not that produced by ultraviolet irradiation or electrophilic chemicals. These cells have a consistently low level of excision repair, as measured by mean grain counts in autoradiographic plates after induction of unscheduled synthesis in the presence of [^{3}H]thymidine. In normal human

fibroblasts, repairability is reduced as the age of the culture increases but never falls as low as that in xeroderma cells. Despite the serious and constant defect in excision repair in xeroderma fibroblasts, these cells do not have curtailed life-span in vitro, even when no special precautions are taken to shield them from ambient light during routine manipulations.

De Serres reported on the role of repair in the production of mutations in neurospora. Several strains of neurospora have been isolated with repair deficiencies. Some of these repair-deficient strains are much more sensitive to mutagens, others are much more resistant, and a third group shows no difference from the wild type.

Hayden Coon (National Institute of Child Health and Human Development, Bethesda, Maryland) discussed the genetic heterogeneity found in patients with xeroderma pigmentosum. Different types of defects can be detected by complementarity experiments. In these studies, cell cultures from two patients are fused and the percentage of repair (measured as grain counts after the induction of unscheduled DNA synthesis) is compared in the two parent lines, fusion products, and normal controls. These studies have revealed four complementation groups for xeroderma pigmentosum, each with a different repair capacity. When cells of two complementary groups are fused, the repair capacity returns to 100 percent of normal.

Selma Silagi (New York Hospital–Cornell Medical Center) led a discussion on alterations of genome expression in differentiated cells. Silagi, studying mouse melanoma cells, has demonstrated that low concentrations of BrdU that do not appreciably alter the growth rate of the cells can markedly change genome expression. In the presence of BrdU, these cells stop producing melanin, alter their growth characteristics in culture, and have markedly reduced tumorigenicity when injected into recipient animals. Treated cells with reduced tumorigenicity have been used to immunize mice with up to 90 percent efficiency against untreated highly tumorigenic cells. The enzyme tyrosinase, detectable electrophoretically in the untreated cells, is progressively decreased in amount during growth with low concentrations of BrdU, and one of the two electrophoretic bands becomes smaller earlier than the other. These alterations are not true mutations, since the cells revert when BrdU is withdrawn. They can be manipulated in either direction by sequential additions and withdrawals of BrdU. Also, if the cells do not synthesize DNA and divide, they are not affected by the BrdU addition.

Goldstein, working with D. P. Singal (McMaster University) on clonal studies of human fibroblasts, reported a loss of some HL-A surface antigens with aging. He noted that in similar studies with mass culture techniques, these antigens usually appear to maintain stability to senescence. In comparison, fibroblasts of patients with progeria, a so-called premature aging syndrome, are negative for these antigens.

George Martin (University of Washington) discussed gene expression in diploid cells in culture with regard to a variety of possible mitotic segregation mechanisms. These mechanisms included somatic crossing-over, for which there is considerable evidence, and diploidization, a process in which cells go from two to four and back to two chromosome complements, with concomitant recombination of the original homologous pairs as measured by increase in chromosomal variants. Similar mechanisms involving haploidization resulting from multipolar mitoses, aneuploidy by mitotic nondisjunction, and recombination by translocation were also discussed. Coon noted some current work with hybrids of human and rodent cells in which the rodent chromosomes are preferentially lost. This phenomenon apparently occurs only when fresh primary rodent cultures are hybridized with cell lines of human origin. Such hybrid lines may lose the total rodent chromosome complement in addition to certain human chromosomes, resulting in a cell line monosomic for as many as eight human chromosomes.

Susumu Ohno (City of Hope National Medical Center, Duarte, California) presented a hypothesis that mutations in transfer RNA might explain the senescence phenomenon. He felt that the data on mutation rate and cell degeneration time were most compatible with mutations in this system.

James F. Danielli (Center for Theoretical Biology, Amherst, New York) expressed the view that the senescing cells in culture probably represented a complex dynamic system with a trajectory in time, rather than a continually repetitive system. Cells, he said, have many possible sets of behavior. One of these may be expressed for a time, and may initiate a period of transition to expression of a new set of characteristics, which is followed by a third set, and so forth. In this complex system, he felt, mutagenesis and mutants can be tools for studying the mechanism of aging, but this does not mean that senescence or aging is necessarily due to mutagenesis. Indeed, Danielli considers it unlikely that random mutagenesis is the primary cause of aging in mammals.

The presentations at the workshop will be published as a supplement to *International Review of Cytology*. The workshop was organized by Warren W. Nichols (Institute for Medical Research), Moorhead, Danielli, and Donald G. Murphy (National Institute of Child Health and Human Development.

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Control of Transcription

An international symposium on control of transcription was held in Calcutta, India, from 12 to 15 February 1973. This symposium was organized by the Bose Institute, Calcutta, in collaboration with the Oak Ridge National Laboratory, and was funded by the National Science Foundation and the government of India. Participants presented papers on different aspects of transcription in prokaryotes, eukaryotes, and their viruses to a group of about 100 scientists and 50 graduate students who attended the symposium as observers and took part in discussion.

In the opening meeting, Alexander Hollaender of the Oak Ridge National Laboratory and the University of Tennessee described the genesis and objectives of this symposium. The scientific sessions started with the presentation of electron microscopic pictures of genes active in transcription by Barbara Hamkalo (Oak Ridge National Laboratory). In bacteria, active structural genes could be identified by gradients of attached polyribosomes because transcription and translation are tightly coupled in these cells. Active ribosomal RNA genes were distinguishable from structural genes by the absence of attached ribosomes. Active loci synthesizing