MASTER

THE RELATIONSHIP OF DNA REPAIR PROCESSES
TO
MUTAGENESIS AND CARCINOGENESIS
IN
MAMMALIAN CELLS

Three Year Progress Report
for Period August 1, 1977 - October 31, 1980

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Three-Year Report

I. Main Research Accomplishments

A. Originally Stated Objectives

The objective of this research is to determine the role of DNA repair in mutagenesis and carcinogenesis in mammalian cells. More specifically, we hope to select mutant strains which are deficient in various DNA repair pathways and to study these strains with regard to (1) the nature of the defect in repair, and (2) the mutability and transformability of the defective cells by various agents as compared to the wild type parental cells.

B. Progress During the Last Three Years

The results of the research that we have carried out in the last three years can be organized into five categories: (1) Determination of the optimum conditions for the growth and maintenance of the cells and for the quantitative measurement of various cellular parameters; (2) Investigation of the effect of holding mutagenized cells for various periods in a density inhibited state (in which DNA synthesis does not occur) on survival and on the frequencies of mutation and transformation; (3) Examination of the repair capabilities of BHK cells, as compared to repair-proficient and repair-deficient human cells and to excision-deficient mouse cells, as measured by the reactivation of Herpes simplex virus (HSV) treated with radiation and ethylmethane sulfonate (EMS); (4) Initiation of the host cell reactivation

viral suicide enrichment and the screening of survivors of the enrichment for sensitivity to ionizing radiation; and (5) Investigation of the toxicity, mutagenicity, and carcinogenicity of various metabolites of 4-nitroquinoline-1-oxide (4-NQO). These five topics are discussed below.

1. Determination of the optimum conditions for the growth and maintenance of the cells and for the quantitative measurement of various cellular parameters. The cell lines which we have used in these experiments include normal and transformed C3H 10T 1/2, 3T3, and BHK cells. With regard to the growth and maintenance of these cells, we have investigated the effects of various media and serum lots, methods of media preparation, and the effect of various media constituents on the rate of growth and plating efficiency. For the purposes of allowing DNA repair in the absence of DNA synthesis, we have determined the effects of low serum concentration and of high cell density on cell multiplication and DNA synthesis (Table 1). The effects of various drugs on the growth of these cells were determined in order to establish acceptable levels of mutagens and selectants. The maximum concentrations of various mutagens which did not result in lethality are shown in Table 1. For selectants, we have determined the minimum level of the drug necessary to reduce the plating efficiency of the cells by a factor of 10^6 (Table 1). Various effects of putative inhibitors of DNA repair on BHK cells are shown in Table 1A, as well as the characteristics of the dose-response of these cells to radiation.

In order to investigate the optimum conditions for the measurement of mutagenesis and neoplastic transformation, we have isolated transformed cells, as well as ouabain-resistant cells, and have conducted

Table 1. Effect of Medium Constituents and Various Drugs on the Growth of C3H 10T 1/2, and BHK Cells

Condition or Substance	Cell Type	
	10T 1/2	BHK
<u>Medium Constituents</u>		
Optimum medium type	BME	DME
Doubling Time, hours	16	10
Optimum pH	7.4	
Fungizone effect 2.5 µg/ml	reduced PE to 85%	
Gentamycin effect at 50 µg/ml	one batch non-toxic, other batches toxic	non-toxic, all batches
Fetal calf serum, (FCS) optimum %	10	10=20
FCS filtered through 0.1 µ millipore filter	Loses growth promotion with shelf-time of serum. Non-filtered retains activity..	
FCS concentration for no cell division or ³ H-thymidine incorporation/cell	0.1%	0.3%
Cell density at which cell divi- sion stops, cells/cm ²	8 x 10 ⁴	3 x 10 ⁵
Cell density at which ³ H-thymidine incorporation per cell decreases, cells/cm ²	1 x 10 ⁴	2 x 10 ⁵
<u>Chemical Mutagens: Maximum non-lethal level</u>		
Ethylmethane sulfonate, 2 hours	5mM	3mM ⁺
N-methyl-N'-nitro-N-nitroso- guanidine, 1 hour		<2µM
4-nitroquinoline-1-oxide (10 min)	0.1µM	
4-Hydroxyaminoquinoline-1-oxide (30 min)	10µM	
<u>Selectants: Minimum level reducing plating efficiency by 10⁶</u>		
Ouabain	1mM ⁺	0.5mM
Azaguanine	10uM	
6-thioguanine		0.1mM
Hydroxyurea	0.1mM	0.1mM

⁺ Similar values were obtained for BALB/3T3 cells.

Table 1A

Condition or Substance	Cell Type
BHK	
<u>Putative Repair Inhibitors</u>	
acriflavin, 22 hours, 1 μ g/ml	PE reduced to 75%
caffeine, 1 mM, continuous	increases lag phase, reduces growth rate slightly
6-(p-butylanilino)uracil, 0.1mM, 24 hours	complete inhibition DNA synthesis and cell division, reversible
<u>Radiation Effect</u>	
UV radiation, D_0 , J/M ²	6
X-radiation	D_0 variable, under investigation

reconstruction experiments. The results of these experiments are discussed in an accompanying manuscript (Appendix A). In general, we have found that a minimum of three days is required for expression when determining the frequency of induced mutation to ouabain-resistance and that the density of cells seeded in the selection plates should not exceed $1 \times 10^3/\text{cm}^2$. For the assay of neoplastic transformation by anchorage-independent growth, we have found that soft agar, agarose, and methyl cellulose give similar plating efficiencies for transformed cells, and that the number of cells plated in 2.5-5.0 ml of the semi-solid medium should not exceed 1×10^5 . The plating efficiencies of various transformed strains of C3H 10T 1/2 which we have isolated varied from 8% to 23.5% in soft agarose.

2. The effect of holding periods on the survival and on the frequencies of mutation and transformation of mutagenized cells. When C3H 10T 1/2 cells were held in a confluent state for various periods following EMS treatment, the survival, as well as the frequencies of mutation and transformation, were altered. If the cells were held in the density-inhibited state for four hours, survival as well as the frequencies of mutation and transformation increased, whereas if the holding period was extended to 24 hours, the frequencies of both mutation and transformation decreased from the values observed after a holding period of four hours. These results are similar to those obtained by Terzaghi and Little (1) and Chan and Little (2) who subjected C3H 10T 1/2 cells to holding periods following treatment with ionizing and UV radiation. It is possible that the increase in mutation and transformation frequencies observed after short holding periods is due to the occurrence of error-prone excision repair (3). Alternatively, the observed increase in mutation and transformation frequencies could be due to a

differential repair of potentially lethal lesions in cells still sustaining potentially mutational and transformational lesions. These results are presented and discussed in an accompanying manuscript (Appendix A).

3. Repair capabilities of BHK cells as measured by the reactivation of HSV treated in various ways. The DNA repair capabilities of BHK cells were investigated in terms of the reactivation of HSV as compared to its reactivation by normal and repair-deficient human diploid fibroblasts and excision-deficient mouse cells. BHK cells were found to have an intermediate ability to reactivate UV-irradiated HSV relative to normal human fibroblasts and xeroderma pigmentosum (XP) group A cells. With mouse L929 cells as the host, the dose response of the UV-irradiated virus was biphasic, with $D_{0.1}$'s of 5 and 24 J/M^2 for the low and high dose components, respectively. In contrast, BHK and normal human cells were equally able to reactivate HSV treated with ionizing radiation, whereas XP cells were slightly less efficient in the repair of virus treated with ionizing radiation. The effect of EMS on HSV was biphasic, a 15 minute treatment with concentrations of 0.3, 1.0, and 3.0 mM EMS yielding 85%, 60%, and 54% survival, respectively. These results are presented and discussed in an accompanying manuscript (Appendix B).

4. Isolation of DNA-repair deficient strains using a host-cell reactivation viral suicide enrichment procedure. We have decided to use BHK cells for these experiments because of their diploid character (rather than the hypertetraploid C3H/10T 1/2 cells), in order to increase the probability of isolating cells carrying recessive mutations. Indeed, BHK cells carrying recessive mutations have been isolated by several groups of investigators (e.g., 4-6). BHK cells can be transformed in vitro by viruses, chemicals, and

radiation (6-9), the supernormal clones have a spontaneous transformation frequency of less than 1×10^{-6} (6), and the induction of neoplastic transformation can be measured by the growth of the transformants in semi-solid medium (6). For the reactivation-suicide enrichment procedure, we have used HSV as the viral agent. We have been encouraged in this work by the report of Shiomi and Sato (10) who have used the technique successfully in the isolation of repair deficient human cells. We have therefore followed their procedure in the enrichments we have carried out to date. Small batches of BHK cells have been mutagenized with EMS and outgrown for 10-13 generations. Controls were treated similarly except for the addition of EMS. Aliquots of the mutagenized and control cells were tested for survival and for the frequency of induced ouabain-resistant mutations. The remaining cells were frozen in liquid nitrogen for future use. For the enrichment procedure, mutagenized and control cells were seeded in 150 mm petri dishes (2000 cells per dish) and incubated until the colony size was approximately 50 cells. HSV, (previously subjected to an irradiation of 1090 gray which reduced its survival to 14% of the initial value), was added to the plates, which were then incubated for 90 minutes with gentle rocking. After removal of non-adsorbed HSV, medium containing human gamma globulin was added to prevent liberated, repaired virus from infecting repair-deficient colonies. This level of gamma globulin (500 μ g/ml) had no effect on the plating efficiency of BHK cells but reduced the plaquing efficiency of HSV to 4% of the initial value. The cells were infected as colonies to reduce the number of virus particles needed for inactivation of repair-proficient cells, one successfully infected cell per colony being sufficient to kill the colony (10). When the surviving colonies from the first infection had grown to a size of approximately 400 cells, the cells were washed free of gamma globulin and were subjected to a second infection with irradiated

virus as described above. Survivors of the second infection were isolated with cloning rings and grown up in normal medium. We have isolated nine surviving colonies from 36,000 cells infected with irradiated virus. Three of these were lost during subculture. When sufficient numbers of the remaining six strains were obtained, samples were frozen, and aliquots were tested for their sensitivity to ionizing radiation. Details of the enrichment procedures carried out to date are shown in Table 2, and the response of the survivors to ionizing radiation is shown in Fig. 1. It appears that at least two of these strains are somewhat sensitive to ionizing radiation, as determined in repeated experiments.

5. Investigation of the toxicity, mutagenicity, and carcinogenicity of various metabolites of 4-NQO. We have found that in C3H/10T 1/2 cells 4-hydroxylaminoquinoline-1-oxide was much less toxic than 4-NQO, that catalase addition did not alleviate the toxic effects of 4-NQO, and that cells held in a confluent state for 6-22 hours following treatment with 4-NQO, partially recovered from the toxic effects of this compound.

II. Plans for Continuation of the Research

We have been encouraged by the high percentage of radiation-sensitive strains among the survivors of the host cell reactivation viral suicide enrichment procedure. We plan to continue the enrichments, using different batches of mutagenized cells and virus inactivated by a variety of methods in order that strains bearing different mutations may be isolated. We are in the process of characterizing the radiation-sensitive strains which we have obtained and will characterize other sensitive strains as they are isolated

TABLE 2

SUMMARY OF TWO HOST CELL REACTIVATION - VIRAL SUICIDE ENRICHMENTS

	EMS CONC. mM	% SUR- VIVAL	OUA ^R /OUA ^S (X 10 ⁶)	CELLS TREATED ^a	HSV ^b added/plate (PFU X 10 ⁻⁸)		SURVIVING COLONIES
					1st infec- tion	2nd infec- tion	
EXP. 1	0	100	1.7	4000	1	0.3	1 ^c
	2.5	119	73	4000	1	0.3	2 ^c
	10	29.4	347	4000	1	0.3	0
	20	2.0	114	4000	1	0.3	1
EXP. 2	0	100	0.83	-	-	-	-
	2.5	228	5.84	8000	1	0.3	0
	2.5			6000	0.5	0.3	2
	2.5			6000	0.3	0.3	3

^a Number of BHK cells treated with irradiated virus. Each plate was seeded with 2000 cells.

^b The PFU of the HSV has not been corrected for inactivation by γ irradiation.

^c Did not survive upon subculture.

Figure 1. Effect of X-Radiation on the Growth of Strains Isolated Using the Viral Enrichment Procedure.

Cells were suspended in complete medium, counted and treated with various amounts of X-radiation. Appropriate dilutions of each sample were then plated in triplicate. After incubation for ten days, colonies were counted. The survival curves for the various isolates are labeled (V₁, etc.). Control cultures are included in each set (x—x).

In a second experiment, the response of V₁ and V₆ to X-radiation was similar relative to the parental cells as that shown above. Retests on the sensitivities of V₂, V₃, V₄ and V₅ are in progress.

with regard to their mutability and transformability upon treatment with one or more alkylating agents. Those strains which display altered frequencies of mutation or transformation as compared to the wild type strain will be characterized as to their DNA-repair capabilities both in vivo and in vitro. Correlation of specific repair defects with alterations in mutability and transformability should yield valuable information as to the nature of the lesion(s) involved and the role of DNA repair in these processes.

III. Graduate student trained: May Sadiq, in second year of study; Full-time research on this project.

Postdoctoral fellows:

Carter Schroy, Ph.D., in third year of post-doctoral fellowship; Part-time research on this project.

Perry Mulleavey, Ph.D., in second year of postdoctoral fellowship; part-time research on this project.

IV. Publications

Evans, H.H., C. Santoro, M.F. Horng, T.E. Evans, K.G. Glazier, and L.M. Wilkins, Transformation and Mutagenesis of the Mouse Cell Line C3H/10T 1/2 by Ethylmethane Sulfonate, Abstracts of the 6th International Congress of Radiation Research, Tokyo, 1979.

Glazier, K.G., M. Weber, and H.H. Evans, Host-Cell Reactivation of Herpes Simplex Virus in BHK Cells, Radiation Research 83, 425, 1980 (Abstract).

Evans, H.H., L.M. Wilkins, M.F. Horng, C. Santoro, T.E. Evans, and K.G. Glazier, Mutagenesis and Transformation of C3H/10T 1/2 Cells Treated with Ethylmethane Sulfonate, Draft of manuscript enclosed (Appendix A.)

Glazier, K.G., M. Weber, and H.H. Evans. Host-Cell Reactivation of Herpes Simplex Virus in BHK Cells, Draft of manuscript enclosed (Appendix B).

V. State of Knowledge in the General Area of this Research and Subjects in Need of Further Investigation

The topics discussed below are (1) of current interest in the investigation of chemical carcinogenesis and (2) in need of further investigation.

1. Somatic Cell Mutation as the Initiating Step in Carcinogenesis

In recent years a large variety of chemicals have been found to be carcinogenic. Common properties of these carcinogens include their electrophilicity (or their ability to be activated to produce electrophilic compounds by cellular metabolic processes) and their ability (following activation) to react with DNA (11). Chemical mutagens, as well as carcinogens, possess these properties. Since there is a close correlation between the mutagenicity and carcinogenicity of chemicals (12,13), it has been suggested that the first step in the carcinogenic process is a somatic cell mutation (14-17). However, this hypothesis has not been proven, nor has it been accepted by all investigators (18-21).

2. Significance of the Differences between the Processes of Mutagenesis and Carcinogenesis in Cell Culture

Differences between mutagenic and carcinogenic processes include (1) the higher frequency of neoplastic transformation as compared to the frequency of specific mutagenic events observed in cell cultures treated with carcinogenic agents (19,21) and (2) the extended time required for the expression of the transformed phenotype (22). In order to explain the differences in frequency, it has been suggested (1) that the target size and/or the target number could be larger for transformational events than for specific mutational events (for instance, a lesion in any one of a number of genes could lead to transformation, whereas a lesion in a specific area of a specific gene may be necessary for the production of a specific mutational event (16,23)), and (2) that the frequency of in vitro transformation (which varies with the assay used (22)), may not always be indicative of tumorigenesis in vivo. The need for a long expression time may be due to (1) the necessity for recombinational events for the expression of recessive mutations involved in transformation (24,25), or (2) the requirement for the dilution of a product necessitating many cell divisions (26). It is evident, however, that these reasons for the expression time requirement could also apply to the expression of specific recessive mutations.

3. The Chemical Nature of the Lesions Causing Cell Death, Mutation, and Transformation

If the first event in the carcinogenic process is a somatic cell mutation, it is possible that the DNA lesions involved in mutation and transformation are similar, the difference between the two processes residing in the genes affected. Carcinogens include all types of mutagens (i.e., those that yield point mutations, frame-shift mutations, or deletions (12,15)), although few mutagens are absolutely specific in the type of lesion and mutation that they produce (27). The integration of viral DNA into the cellular genome can also result in neoplastic transformation, either by the donation of new information to the cell (28), or by inactivation of cellular genes by the insertion of foreign DNA sequences (29,30). Similarly, some carcinogenic agents may exert their effect by enhancing the insertion or transposition of genomic elements (31).

Alternatively, the lesions that result in transformation may differ from those that result in mutation. There is good evidence that lesions which produce cell death differ from those yielding mutation and transformation (32-34), and it even appears that different mutational events may be caused by different types of lesions. Thus, Cole and Arlett (35) have compared the frequency of mutations at various loci following treatment of cells with ethylmethane sulfonate (EMS) and methylmethane sulfonate (MMS). EMS alkylates the oxygen as well as the nitrogen atoms of DNA purines and pyrimidines, while MMS preferentially alkylates the nitrogen atoms of DNA bases (36,37). Cole and Arlett found that EMS produced a higher frequency of thioguanine-resistant mutations (which can be due to a loss of gene function) and of ouabain-resistant

mutations (which result from an altered but active gene product) than did MMS (35). The frequencies of thymidine-resistant mutations (which also can be caused by a loss of gene function) induced by the two alkylating agents were similar (35).

If mitotic recombination or gene conversion plays an important role in the expression of neoplastic transformation, lesions which are most efficient in producing recombinational events might be especially efficient in the induction of transformation. It is known that various agents which damage DNA can enhance mitotic recombination (25,38-41).

4. The Role of DNA Repair in Chemical Carcinogenesis

The role of DNA repair in neoplastic transformation is indicated by the high incidence of cancer in individuals born with hereditary defects in DNA repair processes (42). If cultured cells are treated with a carcinogen and then are subjected to a holding period long enough to allow DNA repair before the normal occurrence of DNA replication, cell survival increases, while the incidence of transformation and mutation decrease, at least in repair proficient cells (1,2,43,44). (In repair deficient xeroderma pigmentosum (XP) cells, the fraction of cells surviving or expressing mutations is not altered by a holding period (44)). These results suggest that transformation and mutation are caused by the persistence of lesions in DNA. If lesion-containing DNA is replicated, mispairing is probable at the sites of the lesions, and mutations would be expected in the surviving cells. In bacteria, replication through templates containing cyclobutane dimers requires the "SOS" system, involving the induced synthesis of the rec A gene product (45). There is evidence that the processes

involved in the toleration of DNA lesions are inducible in mammalian cells as well (46-52). The nature of such an inducible process in eukaryotic cells is unknown.

5. The Higher Frequency of Induced Neoplastic Transformation in Rodent Cells as Compared to Human Cells

It is of interest that rodent cells, which are deficient in excision repair, are not unusually sensitive to the lethal and mutational effects of DNA-damaging agents (53-55). Rodent cells, therefore, appear to have the ability to replicate damaged DNA accurately. However, rodent cells in culture have been found to be more subject to the induction of neoplastic transformation than are repair-proficient human cells (55). This higher susceptibility to transformation could be due to (1) persistence of specific transformational lesions in the rodent DNA; (2) a higher capacity of the rodent cells for mitotic recombination or gene conversion; or (3) a higher level of heterozygosity in a gene or genes involved in growth control. Evidence exists that the persistence of an unrepaired lesion in DNA can result in transformation. Thus, those tissues which are deficient in the repair of DNA O6-alkyl guanine have been found to be especially susceptible to the carcinogenic effects of alkylating agents which produce this lesion (56). It appears that the study of cells which are deficient in various aspects of DNA repair can yield valuable information as to the nature of the lesions and the mechanisms involved in mutagenesis and neoplastic transformation.

6. The Isolation and Characterization of Repair-Deficient Strains of Mammalian Cells.

Many studies of the role of DNA repair in mutagenesis and carcinogenesis have been carried out using either (1) radiation-sensitive mutant cells obtained from patients with hereditary defects in DNA repair or (2) chemical inhibitors which depress various repair processes. These studies suffer from (1) the lack of isogenic wild-type parental cells with which phenotypic comparisons can be made with confidence and (2) the lack of specificity of chemical inhibitors. Several groups of investigators have isolated mutants of mammalian cells which are sensitive to ionizing- or UV-radiation or to chemical carcinogens. In a number of cases, the isolation procedure has involved the irradiation of the entire mutagenized cell population, so that the sensitive cells could be separated from the wild-type cells on the basis of their sensitivity (57-61). The mutants isolated by this type of procedure, having been subjected to at least two treatments with mutagenic agents, have a high probability of carrying more than one mutation, an occurrence which makes correlation of phenotype with genotype difficult. Other investigators have avoided this disadvantage by isolating sensitive mutants using replica plating techniques (62-66). Some of the sensitive strains isolated have shown altered frequencies of induced mutation. Busch et al. (61) and Sato and Hieda (64) have reported the isolation of UV-sensitive strains which exhibited a higher frequency of UV-induced mutations than did the wild-type cells. Another strain isolated by Sato and Heida (65) was found to be sensitive to ionizing radiation: The yield of induced mutations in this strain did not increase with radiation dose in contrast to the wild type cell (67).

In the research described in this application, the isolation and study of mutant strains which are sensitive to various alkylating agents are proposed. For these studies we plan to use lightly mutagenized cells, and to use the host-cell reactivation suicide as an enrichment procedure, rather than to subject the cells to further damage during a selection process. We plan to conduct the studies with BHK cells which undergo both mutation and neoplastic transformation in vitro following treatment with carcinogens. In this research, we hope to correlate specific defects in DNA repair with alterations in mutagenesis and transformation and thus obtain information as to the nature of the lesion(s) involved in these processes and the mechanisms whereby they proceed.

7. Significance of These Studies

Modern epidemiologic studies have indicated that 80-90% of all human cancers are caused by agents present in the environment. Of these agents, chemicals are undoubtedly the predominant carcinogens (68). Since the number of carcinogenic chemicals present in the environment appears to be increasing each year, it is important to elucidate the mechanisms involved in chemical carcinogenesis so that preventive measures may be established.

VI. Federal Support for Overall Research Program.

A. NIH: CA 19283; Radiation Oncology Research Core Grant, E.C. Gregg, P.I.; \$238,632 annual direct costs; 25% salary H. Evans provided; 6/30/76 - 6/30/82

entire project period.

B. DOE: DE-AC02-77EV04472; The Relationship of DNA Repair Processes to Mutagenesis and Carcinogenesis in Mammalian Cells; H. Evans, P.I.; \$59,556 annual direct costs, \$83,000 total annual costs; H. Evans, 20% effort; 2/1/80 -1/31/81 contract period.

C. NIH: CA 23427; Radiation Induced Mutagenesis; H. Evans, P.I.; \$72,538 annual direct costs; H. Evans, 20% effort and salary; 6/1/78-5/31/81 entire project period. Renewal applied for.

D. NIH: T32ES07080; Environmental Biology Training Grant, H.H. Evans, Program Director; \$53,101 annual direct costs; H. Evans, 10% effort, 5% salary; 7/1/78 - 6/30/81 entire project period. - Renewal applied for.

E. Unencumbered funds for remainder of H. Evans salary support.

Pending Application

Department of Energy, Mechanism of Nuclear DNA Replication in Physarum polycephalum, T.E. Evans, P.I.; \$64,793 total direct costs requested for 1 year, H. Evans, 20% effort; T. Evans, 60% effort.

References

1. Terzaghi, M. and J.B. Little, *Nature* 253, 548, 1975.
2. Chan, G.L. and J.B. Little, Abstracts of the 6th International Congress of Radiation Research, Tokyo, p. 179, 1979.
3. Hanawalt, P.C., P.K. Cooper, A.K. Ganesan, and C.A. Smith, *Ann. Rev. Biochem.* 48, 783, 1979.
4. Meiss, H.K. and C. Basilico, *Nature New Biol.* 239, 66, 1972.
5. Caboche, M., *Genetics* 77, 309, 1974.
6. Bouck, N. and G. di Mayorca, *Nature* 264, 722, 1976.
7. Stoker, M. and I. Macpherson, *Nature* 203, 1355, 1964.
8. diMayorca, G., M. Greenblatt, T. Trausathen, A. Soller and R. Giordano, *Proc. Natl. Acad. Sci. USA* 70, 46, 1973.
9. Ishii, Y., J.A. Elliot, N.K. Mishra and M.W. Lieberman, *Cancer Res.* 37, 2023, 1977.
10. Shiomi, T. and K. Sato, *Somatic Cell Genetics* 5, 193, 1979.
11. Miller, J.A., *Cancer Res.* 30, 559, 1970.
12. McCann, J., E. Choi, E. Yamasaki and B.N. Ames, *Proc. Natl. Acad. Sci. USA* 72, 5135, 1975.
13. Huberman, E. and L. Sachs, *Proc. Natl. Acad. Sci. USA* 73, 188, 1976.
14. Ames, B.N., W.E. Durston, E. Yamasaki, and F.D. Lee, *Proc. Natl. Acad. Sci. USA* 70, 2281, 1973.
15. McCann, J. and B.N. Ames, *Proc. Natl. Acad. Sci. USA* 73, 950, 1976.
16. Huberman, E., R. Mager and L. Sachs, *Nature* 264, 360, 1976.
17. Bouck, N. and G. di Mayorca, *Nature* 264, 722, 1976.
18. Rubin, H., *J. Natl. Cancer Inst.* 64, 995, 1980.
19. Parodi, S. and G. Brambilla, *Mutation Res.* 47, 53, 1977.

20. Holliday, R., Br. J. Cancer 404, 513, 1979.
21. Marquardt, H., Carcinogenesis 1, 215, 1970
22. Barrett, J.C. and P.O.P. Tso, Proc. Natl. Acad. Sci. USA 75, 3297, 1978.
23. Chan, G.L. and J.B. Little, Proc. Natl. Acad. Sci. USA 75, 3363, 1978.
24. Kinsella, A.R. and M. Radman, Proc. Natl. Acad. Sci. USA 75, 6149, 1978.
25. Kinsella, A.R. and M. Radman, Proc. Natl. Acad. Sci. USA 77, 3544, 1980.
26. Jacobs, L. and R. Demars, Mutation Res. 53, 29, 1978.
27. Drake, J.W., The Molecular Basis of Mutation, Holden-Day, San Francisco, 1972.
28. Collett, M.S. and R.L. Erikson, Proc. Natl. Acad. Sci. USA 75, 2021, 1978.
29. Howe, M.M. and E.G. Bade, Science 190, 624, 1975.
30. Calos, M.P. and J.H. Miller, Cell 20, 579, 1980.
31. Hrai, K., V. Defendi, and L. Diamond, Cancer Res. 34, 3497, 1974.
32. Baker, R.M., W.C. Van Voorhis, and L.A. Spencer, Proc. Natl. Acad. Sci. USA 76, 5249, 1979.
33. Newbold, R.F., W. Warren, A.S.C. Medcalf and J. Amos, Nature 283, 596, 1980.
34. Slapikoff, S.A., B.M. Andon, and W.G. Thilly, Mutation Res. 70, 365, 1980.
35. Cole, J. and C.F. Arlett, Mutation Res. 50, 111, 1978.
36. Lawley, P.D. Mutation Res. 23, 283, 1974.
37. Singer, B., J. Natl. Cancer Inst. 62, 1329, 1979.
38. Holiday, R., Genet. Res. Camb. 8, 323, 1966.
39. Holiday, R., Nature New Biol. 232, 233, 1971.
40. Manney, T.R. and R.K. Mortimer, Science 143, 581, 1964.
41. Fabre, F. and H. Roman, Proc. Natl. Acad. Sci. USA 74, 1667, 1977.
42. Setlow, R.W., Nature 271, 713, 1978.
43. Ikenaga, M. and T. Kakunaga, Cancer Res. 37, 3672, 1977.
44. Maher, V.M., D.J. Dorney, A.L. Mendrata, B. Konze-Thomas, and J.J. McCormick, Mutation Res. 62, 311, 1979.

45. Witkin, E.M., *Bact. Rev.* 40, 869, 1976.
46. Lytle, C.D., S.G. Benane and L.E. Bockstahler, *Photochem. Photobiol.* 20, 91, 1974.
47. DasGupta, U.B. and W.C. Summers, *Proc. Natl. Acad. Sci. USA* 75, 2378, 1978.
48. Jeeves, W.P. and A.J. Rainbow, *Biochem. Biophys. Res. Commun.* 90, 567, 1979.
49. Sarasin, A. and A. Benoit, *Mutation Res.* 70, 71, 1980.
50. Buckley, J.D., P.J. O'Connor and R.W. Craig, *Nature* 280, 76, 1979.
51. Altimirano-Dimass, M., R. Sklar and B. Strauss, *Mutation Res.* 60, 197, 1979.
52. Montesano, R., H. Bresil and G.P. Margison, *Cancer Res.* 39, 1798, 1979.
53. Takebe, H., S. Nii, M.I. Ishii and H. Utsumi, *Mutation Res.* 25, 383, 1974.
54. Rauth, A.M., M. Tammemagi and G. Hunter, *Biophys. J.* 14, 209, 1974.
55. Spandidos, P.A. and L. Siminovitch, *Cell* 13, 651, 1978.
56. Goth, R. and M.F. Rajewski, *Proc. Natl. Acad. Sci. USA* 71, 639, 1974.
57. Sinclair, W.K. *Radiat. Res.* 21, 584, 1964.
58. Isomura, K., O. Nikaido, M. Horikawa T. Sugahara, *Radiation Res.* 53, 143, 1973.
59. Schultz, R., C. Chang and J.E. Trosko, *Proc. Am. Assoc. Cancer Res.* 19, 28, 1978.
60. Thompson, L.H., J.S. Rubin, J.E. Cleaver, G.F. Whitmore and K. Brookman, *Somatic Cell Genet.* 6, 391, 1980.
61. Busch, D.B., J.E. Cleaver, and D.A. Glazer, *Somatic Cell Genet.* 6, 407, 1980.
62. Stamato, T.D. and C.A. Waldron, *Somatic Cell Genet.* 3, 431, 1977.
63. Kuroki, T. and S.Y. Miyashita, *J. Cell. Physiol.* 90, 79, 1977.
64. Sato, K. and N. Hieda, *Int. J. Radiation Biol.* 35, 83, 1979.
65. Sato, K. and N. Hieda, *Radiation Res.* 78, 167, 1979.
66. Sato, K. and N. Hieda, *Mutation Res.* 71, 233, 1980.
67. Shiomi, T. and K. Sato, *Somatic Cell Genet.* 5, 193, 1979.
68. Heidelberger, C. *Ann. Rev. Biochem.* 44, 79, 1975.