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RENEWAL PROPOSAL: Year 2

February 1975

REPETITIOUS NATURE OF REPAIRED DNA IN MAMMALIAN CELLS

U.S.E.R.D.A. Contract No. AT(40-1)-4761

Project Period: June 1, 1975 - May 31, 1976

Submitted to:

Energy Research and Development Administration
 Oak Ridge, Tennessee 37830

Submitted by:

Martin L. Meltz, Ph.D.
 Principal Investigator



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SOUTHWEST FOUNDATION
for RESEARCH and EDUCATION

P. O. Box 28147 (8848 West Commerce Street) • San Antonio, Texas 78284
 Bexar County • 21st Congressional District

MAR 3 1975

Renewal Proposal: Year 2

U.S.E.R.D.A. Contract No. AT-(40-1)-4761

1. Title

REPETITIOUS NATURE OF REPAIRED DNA IN MAMMALIAN CELLS

Submitted by:

Martin L. Meltz, Ph.D.

Associate Foundation Scientist

2. Institution

Southwest Foundation for Research and Education

8848 W. Commerce at Loop 410

P. O. Box 28147

San Antonio, Texas 78284

Martin L. Meltz
Principal Investigator
Martin L. Meltz, Ph.D.

FEB 27 1975
Date

Edward F. Feith
Official Authorized to Sign for
Southwest Foundation for Research
and Education
Edward F. Feith, Treasurer

FEB 27 1975
Date

Project Period

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June 1, 1975 - May 31, 1976

3. PROJECT ABSTRACT

Two general objectives are being pursued; the first is to determine the amount of radioactivity incorporated into normal, aged, transformed, diseased, and cancerous mammalian cells during repair replication after and during treatments with agents known to damage DNA. This is being determined as a function of dose and exposure time, allowing for the cellular activation of any chemicals which require metabolic alteration before being capable of interacting with DNA. The second is to determine, where sufficient amounts of repair replication radioactivity are present, the repetitive nature of the DNA undergoing repair replication. Additionally, the DNA being synthesized upon treatment with the damaging agent will be studied for abnormal distribution of incorporated radioactivity. The method of fractionating the DNA is the DNA/DNA hybridization "Cot" technique, which distinguishes between highly repetitive, intermediate repetitive, and unique DNA. The distribution will be observed as a function of cell age, determined by the criteria of either passage number or age of the donor, and as a function of the state of the cell, i.e., normal, transformed, diseased, or cancer. Cell lines being investigated include human lymphocyte, Kaplan's mononucleosis, Burkitt's lymphoma (Raji and EB₃), mouse L-929 fibroblast, mouse L5178Y lymphoma, WI-38 (lung origin), Xeroderma pigmentosum, and normal human skin fibroblasts. An attempt will be made to determine whether repair replication occurs initially in one class of DNA, e.g., in highly repetitive DNA, before it occurs in intermediate or unique DNA; the cell cycle relationship between unscheduled synthesis and repair replication will be investigated if abnormal patterns are detected. Special attention will be paid to relating repair observations to survival and mutation rate in L5178Y cells. The agents being studied are ultraviolet light and chemical carcinogens and mutagens which either do or do not require metabolic activation, and which are or are not water soluble.

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4. SCIENTIFIC BACKGROUND

Repair of Damage to DNA

The phenomenon of repair of DNA has been described in several short notes (1-4) and more extensively in several review articles (5-10). Three of the types of molecular repair discussed are (a) rejoining of single and double strand breaks, (b) unscheduled DNA synthesis, and (c) repair replication. The evidence available indicates that unscheduled synthesis, which is measured using autoradiographic techniques (11), is equivalent to repair replication, which is measured using isolated DNA and density gradient centrifugation procedures (12,13). Repair of DNA has been reported not only after damage by ultraviolet irradiation, but also after treatment of cells with chemical carcinogens (14-44). The measurement of repair after treatment of the cells with precarcinogens does not result in detection of repair synthesis, while the latter is detected when their respective ultimate carcinogens are used for treatment (22). The distribution of repair in repetitive and unique sequences of human DNA has been reported (13,45), and the distribution in mouse satellite versus mainband DNA has also been described (46).

General Objectives

During the first year of this proposal, two major lines of investigation, relevant to the cellular and molecular responses of mammalian cells in culture to biohazardous physical and chemical agents, were initiated. The first was to measure the amount of radioactivity incorporated into the DNA

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of normal, aged, transformed, diseased, and cancerous mammalian cells during repair replication after treatment with agents known to damage DNA and induce repair. This was to be determined as a function of dose and exposure time.

The second line of investigation was to determine, where sufficient amounts of repair replication radioactivity were present, the repetitive nature of the DNA undergoing repair replication. The method of fractionating the DNA is the DNA/DNA hybridization "Cot" technique. This technique allows us to determine whether the repair replication radioactivity is distributed uniformly in highly repetitive, intermediate repetitive, and unique DNA sequences. A discussion of the known and possible biological functions of these different classes of sequences was presented in the original proposal.

Chemical Considerations

Because we are particularly concerned in our laboratory with developing in vitro cellular assay systems for assessing the environmental danger of pollutant chemicals, the choice of chemical carcinogens to be used in these studies is being based on a number of factors: (a) are the chemicals found in the environment as pollutants; (b) are they water soluble or relatively insoluble in aqueous systems; (c) are they readily taken up by the cells; and (d) do they require metabolic activation from a precarcinogenic state to the form of an ultimate carcinogen before they are capable of interacting with or otherwise damaging the DNA. This latter consideration, which has been extensively discussed (47-52), is of importance in deciding upon the length of time of exposure of the cells to the chemical when attempting to measure DNA repair.

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Cellular Systems

Several basic questions were raised in the initial proposal concerning relative repair capabilities of different human cell lines, and the progress and direction of these studies is presented in the accompanying report. In particular, questions were raised about the possibility of differences in repair replication capability of normal versus cancerous human cells. This might depend on differences in available sites in their chromosomal configuration for chemical attack, or be related to the availability of repair enzymes associated with viruses or viral information present in the cells. Additionally, the question was raised of possible quantitative or qualitative differences in the ability of cells from humans of different ages to respond to UV light or chemically induced damage. In the development of our biohazard assessment capability, a major consideration is to select cells for study which are derived from tissue systems which are potential targets for environmentally biohazardous agents. In this regard, we are particularly interested in the "normal" human lymphoblastoid cells, of blood origin, the WI-38 human diploid fibroblast line, of embryonic lung origin, and skin fibroblasts from humans of different ages.

In treating these cell systems with chemical agents, it will be necessary to distinguish in each case between the ability of different cells to repair equivalent damage in their DNA, caused by direct acting agents, and their handling of chemicals requiring metabolic activation. As indicated in the accompanying progress report, a choice of agents which may help accomplish this has already been made for the first year of this study; additional chemicals will be investigated as described below.

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In addition to the above described investigations with human cells, studies have already been initiated during the first year of this project on the extent of repair and its distribution in the DNA of mouse L-929 fibroblasts. During the second year of this project, the studies on mouse cells will be extended to the L5178Y mouse lymphoma cell line. The latter is currently being employed in our laboratory for mutation assay.

As indicated in the initial proposal, it is our ultimate goal to relate our observations on the quantitative and qualitative distribution of repair to the molecular development of cell death, mutagenesis, and carcinogenesis. During the second year of this project, we will pay particular attention to the effect of repair inducing treatment on cell survival, and initiate studies on its correlation with mutagenic action.

5. SCIENTIFIC SCOPE

The scientific scope of this project is essentially as described in the initial proposal; as described in the accompanying progress report, these studies are well underway. In addition to the aims originally listed, however, we will also be investigating two additional parameters.

The first relates to the probability that treatment of cells in culture with known carcinogens or mutagens will disturb the semi-conservative DNA synthesis process, while at the same time inducing repair replication. One approach to investigating the question of whether any disturbance to normal DNA synthesis is non-uniform in nature is to study the DNA/DNA Cot pattern of the DNA incorporating ^3H -TdR immediately after exposure to the

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mutagen or carcinogen. As indicated in the accompanying progress report, such a study is currently underway with DNA of mouse L-929 cells labeled immediately after exposure to UV light, and which appears to be "radio-resistant". This type of labeling protocol is a composite of both normal DNA synthesis and repair replication labeling.

An alternative procedure would be to allow repair replication labeling to occur after or during treatment of the cells with the chemical agents, but without hydroxyurea present, and then to separate the heavy density BrUdR containing semi-conservatively synthesized DNA from the repair replication labeled normal density DNA using the two sequential alkali CsCl-Cs₂SO₄ density gradient procedure now standard in our laboratory. Both fractions, with appropriate controls, would then be subjected to DNA/DNA Cot fractionation. This second procedure, which would require minimum perturbation by the BrUdR on the Cot pattern, remains to be investigated.

The second parameter relates to preliminary studies to be performed with L5178Y cells. We are currently using these cells for mutation assay at the thymidine kinase locus, as described by Clive et al. (53-55). It is our intention to begin preliminary studies on the relationship of extent of repair replication and mutation frequency per survivor during the second year of this project.

Procedures and Experimental Design

The experimental designs to be used for these repair replication studies, as well as procedural modifications to be applied to future studies, have been described in the initial proposal and in the accompanying progress report.

For preliminary studies involving mutation rate, we are currently developing cell clones which will allow us to perform the mutation assay described by Clive and co-workers (53-55) at the thymidine kinase locus. At the present time, we are maintaining in our laboratory an L5178Y clonal line which is resistant to BrUdR at a concentration of 50 $\mu\text{g/ml}$; this cell clone is believed to be lacking in thymidine kinase activity. We have also isolated a potential revertant clone, a presumptive heterozygote, which will be used in testing chemicals for their induction of forward mutation to BrUdR resistance and for their induction of DNA repair under equivalent treatment conditions.

Choice of Chemical Agents

In the initial proposal, the agents listed for cell treatment included methyl methanesulfonate (MMS), 4-nitroquinoline-1-oxide (4NQO) or its derivatives, N-acetoxy-2-acetylaminofluorene, nitrogen mustard, and N-methyl-N'-nitro-N-nitrosoguanidine (MNNG). As indicated in the accompanying progress report, the chemicals being employed in the first year of this study are MMS, benzo(a)pyrene, and 4NQO. In the second year, we will additionally be investigating N-acetoxy-2-AAF, representative of the aromatic amines, and dimethylnitrosamine, representative of the nitrosamines, as well as ethyl methanesulfonate (EMS), a standard mutagen. Investigation of other carcinogens, e.g., the hepatic carcinogens ethionine and urethane, will be considered during the third year of this study.

Significance

The significance of this study, in terms of the environment, biological damage, and repair, was presented in the original proposal. Our very ability to measure repair makes available to us a tool for assessing the harmful potential at the DNA genetic level of biohazardous agents in the environment.

6. SCIENTIFIC PERSONNEL

Martin L. Meltz, Ph.D., Principal Investigator

Associate Foundation Scientist

Social Security No. [REDACTED]

50 percent of time for 12 months

Birthdate: [REDACTED]

Place of Birth: New York City, New York

Education:

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

Honors:

Atomic Energy Commission Postdoctoral Fellow

National Defense Education Act (NDEA) Predoctoral Fellow

Sigma Xi

Research and Professional Experience:

September 1971 to present - Associate Foundation Scientist, Southwest Foundation for Research and Education, San Antonio, Texas.

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September 1973 to present - Adjunct Assistant Professor of Radiology,
University of Texas Health Science Center at San Antonio.

October 1970 to August 1971 - Graduate Research Biophysicist, Laboratory
of Radiobiology, University of California, San Francisco.

Dr. Martin L. Metz is an Associate Foundation Scientist in the
Environmental Science Program of the Southwest Foundation for Research and
Education, where he has been employed for 3 1/2 years. He is responsible
for the cell and molecular biology aspects of the Environmental Sciences
Program.

Dr. Metz received his B.S. degree in Physics in 1963 from the State
University of New York at Stony Brook, Long Island, N. Y., after attending
public school in New York City. He was enrolled in a program in Nuclear
Science and Engineering at the University of Buffalo for one year, before
transferring to the Health Physics program at the Department of Radiation
Biology and Biophysics of the University of Rochester, N. Y. Dr. Metz
completed his degree in January of 1970. His thesis research, under the
direction of Dr. Shigefumi Okada, was concerned with the effects of
X-irradiation on synthesis of RNA in the mouse lymphoma L5178Y cell line
grown in suspension culture. His studies of RNA synthesis as a function
of cell cycle time and after X-irradiation required use of pulse labeling,
sucrose gradient, and RNA/DNA hybridization techniques.

In October 1969, Dr. Metz began an Atomic Energy Commission post-
doctoral Fellowship under the supervision of Dr. Robert Painter at the
Laboratory of Radiobiology, University of California Medical Center in
San Francisco, California. His studies were involved with the repetitious
distribution of the DNA undergoing repair replication after ultraviolet
irradiation, and the cell lines studied were the L-929 mouse fibroblast
line and the human HeLa 229 cell line. In September 1971, Dr. Metz arrived
at Southwest Foundation for Research and Education and was involved for
two years in a research program studying RNA and DNA synthesis in the
ventral prostate of rats as a function of age and hormonal status.

In June of 1974, Dr. Metz was awarded a research contract by the United
States Atomic Energy Commission to study the repetitious nature of repaired
DNA in mammalian cells; this program involves a number of different mammalian
cell lines and is concerned with chemically induced damage, as well as ultra-
violet light-induced damage. Dr. Metz spends 50% of his time on this study
(AEC Contract No. AT-(40-1)-4761).

Societies:

Radiation Research Society
American Society for Cell Biology
Biophysical Society
Sigma Xi
American Association for the Advancement of Science
Environmental Mutagen Society

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Publications:

1. Meltz, M. L., and Okada, S.: Ribonucleic acid synthesis in the cell cycle of L5178Y mouse leukemic cells: Time of replication of the DNA template or rapidly labeled hybridizable RNA. *Exp. Cell Res.* 67:90-96, 1971.
2. Meltz, M. L., and Okada, S.: Characterization of the rapidly labeled hybridizable RNA synthesized in L5178Y mouse leukemic cells: Hybridization lifetime studies. *Biophys. J.* 11:582-595, 1971.
3. Meltz, M. L., and Okada, S.: Increased hybridization of RNA synthesized immediately after irradiation in cultured L5178Y mouse leukemic cells. *Int. J. Radiat. Biol.* 19:287-292, 1971.
4. Meltz, M. L., and Painter, R.: Distribution of repair replication in the HeLa cell genome. *Int. J. Radiat. Biol.* 23:637-640, 1973.
5. Meltz, M. L.: Nucleic acid precursor incorporation patterns in ventral prostate tissue from rats of different ages. In *Symposium of the Normal and Abnormal Growth of the Prostate*, Chapter 20. M. Goland (ed.), Charles C. Thomas, Springfield, 1975 (in press).

Abstracts:

1. Meltz, M., and Okada, S.: RNA synthesis in lethally irradiated cultured mammalian cells. *Radiat. Res.* 35, No. 2, Abstract DE-2, 1968.
2. Meltz, M., and Okada, S.: RNA synthesis in L5178Y mouse leukemic cells: RNA/DNA hybridization studies. *Biophys. Soc.*, Abstract TPM-M6, 1970.
3. Meltz, M., and Painter, R.: Non-uniform distribution of repair replication in mouse L-cell DNA after ultraviolet irradiation. *Biophys. Soc.*, Abstract FPM-B5, 1972.
4. Meltz, M., Gause, E. M., and Rowlands, J. R.: Baboon cell systems for environmental hazard determination. I. Alveolar macrophages. *Biophys. Soc.*, Abstract (February, 1975).
5. Meltz, M. L., and Thornburg, W. H.: Repair replication and its distribution in "normal" and cancerous human lymphoblastoid cell lines after physical and chemical damage. *Radiat. Res. Soc.*, Abstract (for presentation May 1975).

7. OTHER PERSONNEL

One Assistant Research Scientist, already employed, will spend 100% of his time on this project; one laboratory assistant, already employed, will spend 50% of his time on this project.

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8. OTHER FINANCIAL ASSISTANCE

At this time, proposals for other research projects involving Dr. Meltz participation have been submitted. Any money derived from any other project would be used solely for that project, and would not be available for funding any personnel or material for this research program.

9. PREMISES, FACILITIES, EQUIPMENT, AND MATERIALS TO BE FURNISHED BY THE CONTRACTOR

All premises, facilities, and equipment will be provided by the Southwest Foundation for Research and Education. The facilities available for this study include a tissue culture laboratory and an adjacent biochemistry laboratory.

1. Tissue Culture Laboratory

- a) For incubation of cells in culture
 - 1. 37° C air incubator, double door (NAPCO)
 - 2. 37° C double door Hydro-Jac incubator (Forma Scientific)
 - 3. Full-view temperature and controlled gas-flow culture incubator for spinner bottles (Precision Scientific)
 - 4. Temperature controlled warm room, 37° C (Honeywell)
 - i) 9 position roller bottle apparatus (Wheaton)
 - ii) Rocker platform (Bellco Glass, Inc.)
- b) Microscopy
 - 1. Inverted microscope (American Optical Co.)
 - 2. Light microscope (AO)
 - 3. Zeiss Ultraphot II microscope
- c) Three sterile transfer rooms, with UV lights for sterilization: Two rooms with table top hoods, and one biohazard room with Biogard Vertical Laminar Flow hood and Lab Con Co. glove box for weighing and diluting carcinogens.
- d) Miscellaneous
 - 1. Drying oven
 - 2. Sterilizing oven
 - 3. Portable autoclave (Wilmot-Castle)

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e) Ultraviolet irradiation source

An ultraviolet exposure chamber has been built for this work following the specifications of H. Steier and J. E. Cleaver, Laboratory Practice 18:1295 (1969), Exposure chamber for quantitative ultraviolet photobiology.

2. Biochemistry Laboratory

a) Fume hood

b) Equipment for DNA isolation

1. Vortex Genie Mixer
2. Precision variable control shaker water bath
3. Eberbach recipricol shaker
4. International PR-J refrigerated centrifuge

c) Equipment for shearing DNA

1. Virtis "65". homogenizer
2. Aminco DNA cracker

d) Equipment for hydroxylapatite separation procedure

1. Batch method
 - i) Sorvall SS-3 Automatic Centrifuge
2. Column method
 - i) ISCO Model UA-5 Absorbance Monitor and optical unit, and ISCO Golden Retriever Fraction Collector.
 - ii) Water-jacketed column apparatus (as described in progress report).

3. Equipment Available in Southwest Foundation Common Equipment Pool

a) UV and visible spectrophotometry

1. Beckman DU Spectrophotometer with Gilford accessories
2. Cary Model II Recording Spectrophotometer
3. Spectrofluorimeters

b) Equipment for scintillation counting

Southwest Foundation Research and Education has a centralized isotope counting facility. The counting center contains Model 3322 and two Model 3000 Packard Liquid Scintillation Counters which are on line to a G.A. 18/30 Computer, and will also have the unique feature of being under the operational control of the computer as well.

c) Centrifuge Equipment

Beckman Spinco Models L, L2-65, and L3-50 preparative ultracentrifuges are available with a variety of rotors, including two type 50Ti rotors, SW39 and SW25.1 rotors, etc.

<u>BUDGET</u>	<u>2nd Year Salary</u>	<u>2nd Year Fringe Benefits</u>	<u>2nd Year Total</u>
1. Salaries and Wages.	\$ 21,200	\$ 3,285	\$ 24,485
Scientific Discipline Personnel			
Principal Investigator, M. L. Meltz, Ph.D., Associate Foundation Scientist-- 50% of time for 12 months			
Soc. Sec. No. [REDACTED]	9,800	1,519	11,319
N. J. Whittam, Assistant Research Scientist-- 100% of time for 12 months			
Soc. Sec. No. [REDACTED]	8,900	1,379	10,279
Support Personnel			
A. Arteaga, Lab Assistant--50% of time for 12 months			
Soc. Sec. No. [REDACTED]	2,500	387	2,887
2. Equipment			\$ -0-
None			
3. Travel.			\$ 250
Domestic: American Society for Cell Biology Meetings, Puerto Rico, November 1975			
4. Other Direct Costs.			\$ 16,350
Supplies and Materials (see attached sheet)			14,550
Publication			300
Communication			100
Computer time, xerox charges, linen, etc.			1,000
Scintillation Counting			400
5. Indirect charges - 56.16%			23,073
Total Direct and Indirect Costs.			\$ 64,158
Fixed Fee at 6% = \$3,849			3,849
Total Project Costs			<u>\$ 68,007</u>
Percentage and Amount to be Contributed by Southwest Foundation for Research and Education = 5.66%			\$ 3,849
Percentage and Amount Requested of AEC = 94.34%			\$ 64,158

It is the policy of Southwest Foundation for Research and Education that all contracts carry a fixed fee. This is usually an amount equivalent to 6% of the total cost of the contract, which in this case would amount to \$3,849. Because of our desire to encourage the undertaking of this research project, which is a component of our Environmental Research Program, we will, as our method of cost sharing, not apply the fee requirement to this renewal proposal.

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Justification for Supplies:

a.	Radioactive isotopes, including predominantly ^3H -BrUdR, also ^{14}C - and ^3H -thymidine.	\$ 4,500
b.	Counting vials and scintillation solution chemicals.	3,500
c.	Tissue culture media, serum, disposable tissue culture pipets and labware.	1,500
d.	Cesium chloride and cesium sulfate.	1,500
e.	Chemical reagents.	1,500
f.	Glassware.	1,500
g.	Cost of cell lines from ATCC.	200
h.	CO_2 for forma incubator.	<u>350</u>
		\$ 14,550

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FINANCIAL STATEMENT

Contract Number AT-(40-1)-4761

6/1/74 - 5/31/75

	Actual Project Cost to <u>1/31/75</u>	Estimated Total Costs <u>2/1/75-5/31/75</u>	Total Costs	Total Costs Chargeable to ERDA
Salaries and Wages	\$ 13,602	\$ 7,336	\$ 20,938	\$ 17,148
Travel	250	-0-	250	205
Other Direct Costs	<u>8,289</u>	<u>6,377</u>	<u>14,666</u>	<u>12,011</u>
	22,141	13,713	35,854	29,364
Indirect Costs	<u>11,823</u>	<u>7,323</u>	<u>19,146</u>	<u>15,636</u>
	\$ 33,964	\$ 21,036	\$ 55,000	\$ 45,000
Accumulated ERDA Support Ceiling, as stated in Article III of Contract				\$ 45,000
Total Estimated ERDA Funds remaining under Contract which may be used to reduce amount of new funds required from ERDA for proposed renewal period				\$ -0-

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