Sent 4/26/69

Memorandum

INFORMAL PROPOSAL FOR RESEARCH ON MECHANISMS OF DNA-REPAIR IN RADIATION-RESISTANT STRAINS OF BACTERIA.

General Background.

The study of DNA-repair after damage from ionizing radiation, ultraviolet light, or chemical attack has been extremely fruitful in recent years both from the standpoint of elucidating the primary mechanism of radiation damage and in uncovering several enzymatic steps important in the normal synthesis in DNA and in genetic recombination. The work of Jane Setlow, M. E. Boling, and B. E. D. Mosely (1,2,3) at Oak Ridge on Micrococcus radiodurans has been the specific stimulant to my own interest in correlating genetic and molecular biological studies on radiation resistance. They found, briefly, that this radio-resistant organism has an unusual effective repair-mechanism which facilitates recovery from radiation damage not only of homospecific DNA but also of the DNA of other species. Their discovery that Micrococcus radiodurans could be studied by genetic transformation by purified DNA was instrumental in this work. This organism suffers, however, from a number of serious technical limitations: in particular the great difficulty of extracting DNA and the complexity of the growth media needed to cultivate it. For our own purposes, therefore, we would prefer to have organisms of similar degrees of radiation-resistance but which can be handled by techniques more familiar to us from our own previous studies with E. coli, Samonella, Bacillus subtilis.

In a pilot approach to this idea one of my graduate students, Mr. Howard Eisenstark, examined a number of coil samples from the gamma-ray field at Brookhaven and had no difficulty in isolating a number of other radiation-resistant organisms of a level of resistance quite comparable to that of Micrococcus radiodurans. Some of these seem to be aerobic spore-formers similar to Bacillus subtilis and capable of growth on the same simple media as our conventional B. subtilis strains. These are at the present moment being examined for their amenability for genetic analysis.

Our immediate proposal is to collect soils from similar sites which may be expected to be subject to natural selection for radio-resistant organisms and to survey a wide range of isolates for suitability for further genetic and enzymological studies. Briefly the samples would first be screened by rather obvious microbiological techniques for the isolation of distinctive radio-resistant forms. Those that appear promising from a bacteriological culture standpoint will then be studied more carefully for the kinetics of radiation-killing with respect to x-ray and untraviolet light and also sensitivity to a number of chemical agents believed to have comparable effects on target DNA.

They will also be examined for their utility as sources of enzyme preparations capable of restoring damaged DNA, particularly of B. subtilis which can be tested for biological activity in the transformation system.

These strains will also be scrutinized for the development of systems of genetic analysis, be it sexual recombination, viral transduction, or DNA transfer. To the extent that such genetic analysis appears to be possible it will be exploited for a thorough examination of the genetic basis of radiation resistance by the examination of further mutants which are deficient in one way or another aspect of this function.

Particular attention will be paid to bacterial strains for which we can obtain circumstantial evidence that double strand lesions of DNA can be repaired. There is little evidence for this at the present time in bacterial systems but it seems necessary to invoke some such phenomenon to account for restoration of the molecular weight of DNA of irradiated lymphocytes in higher organisms. Also the growing evidence that favors the occurrence of very long molecules of DNA as the essential structural unit of the chromosomes make it necessary to postulate double strand repair to account for such cyto-genetic phenomena as inversion and translocation. We are pursuing the examination of higher organism cell systems for such a function but would like also to expand our examination of microbial systems for it.

The possibility of double strand repair of DNA has implications much broader even than an understanding of radiation damage and therapy for it. It could also lead to approaches for the introduction of foreign genetic information from an arbitrary source into an appropriate genotype of a bacterium for both investigative and practical applications. To quote a fairly obvious example it could be possible, for example, to insert the genetic information for human growth hormone into a bacterial strain in order to produce this very valuable material by an industrial microbial fermentation process.

Some of these points are elaborated in the attached material which was submitted in an application to the NIH for work co-ordinate with the present proposal. My own research support from NIH has been at a stable level for several years and has been subject to an obligatory cut-back in the last year or two as well. Without additional financial support, it will, therefore, not be possible for me to expand these research activities to a level appropriate either to the facilities and man power potentially available here or to the inherent interest of the problem.

A tentative budget estimate is also attached.

Sincerely yours,

Joshua Lederberg
Professor of Genetics

Enclosure

JL/rr

BUDGET: (Direct costs only)

Research Associate	$14,000
Research Assistant	$ 7,500
	$21,500
Payroll costs @ 12%	$ 2,580
	$24,080
Equipment	
Biochemical lab apparatus	$ 2,000
Maintenance & repairs	$ 2,000
Chemicals, isotopes & glassware	$ 5,000
Travel	$ 500
Reprints, page charges, reproduction, photography, reference material	$ 500
Computer time (strain files; data plots; tracer calculations)	$ 2,000
Total:	$36,080

REFERENCES

1) Boling, M.E., and J. K. Setlow
The resistance of Micrococcus radiodurans to ultraviolet radiation. 3. A repair mechanism.
Biochem. Biophys. Acta 123:26. 1966.

2) Moseley, B. E. B.
The isolation and some properties of radiation-sensitive mutants of Micrococcus radiodurans.
J. Gen. Micro. 49:293. 1967.

3) Moseley, B. E. B. and J. K. Setlow
Transformation in Micrococcus radiodurans and the ultraviolet sensitivity of its transforming DNA.
PNAS 61:176. 1968.