Progress Report

PHYSICS OF CELLULAR SYNTHESIS GROWTH AND DIVISION

NsG-324

Period covered: Oct. 1, 1965 - March 31, 1966

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The Biophysics Department of The Pennsylvania State University is continuing to progress in research and, simultaneously, to expand its facilities and student accommodations. Much of this progress can be attributed to the support of the National Aeronautics and Space Administration. Publications and attendance at professional meetings continues at a high level and specific lists of both types of papers are given at the end of this report. Consistent with the past policy of this laboratory, the following is a brief summary of the progress during the first half of the grant period, and a more complete report will be submitted in the fall.

The investigation of the structure and function of the bacterial cell is now moving along several lines in the principal investigator's laboratory. Probably the most significant data, at the moment, is that resulting from the study of the nature of the effect of irradiated medium. We have previously reported data which indicate this effect is due to hydrogen peroxide generated in the medium by irradiation and that the nature of the action is to temporarily inhibit transcription. Our recent work suggests that hydrogen peroxide interferes with aerobic metabolism by reducing the ATP supply in the cell and depleting the amino acid pool, resulting in a shutdown of translation.

Study of direct irradiation effects continues to be at the core of the program since it is felt that this method, through studies of localized damage, will prove a very useful tool in elucidating the heart of the research problem, cell structure and function. Fast proton bombardment

has revealed a fast and a slow component in DNA degradation. The DNA-agar studies reported in the last status report have been accepted for publication by Radiation Research. The studies of pressure effects on cell structure and DNA synthesis are being emphasized in an attempt to understand the varied effects detailed in the last status report. The preliminary work, also previously reported, indicating loss of synchrony in daughter cells, in colonies originating from a single cell, has now shown that hydrogen peroxide not only increases this loss of synchrony but also increases the non-identity of daughter cells as well. Temperature studies and TTP pool measurements are continuing, also.

In the last report emphasis was given to the discovery in Dr. Person's laboratory of localized mutation resulting from uracil-5-H^3 decay in E. coli WWU. Since that time, Dr. Person has found that nearly all revertants produced by uracil-5-H^3 decay will suppress one or more amber mutants of T4, while the parent WWU will not. Hence, it is inferred that the forward mutational event in the parent WWU was a base change giving rise to a nonsense codon. Reversion, then, must be the result of another DNA base change resulting in the production of a suppressing factor, probably an altered s-RNA, which inserts an amino acid at the site of the nonsense codon giving a functional protein product.

Further investigation has shown a differential response for different mutagens. That is, uracil-5-H^3 decay and ethyl-methane-sulphonate produce revertants, nearly 100% of which can suppress several T4 ambers. Other mutagens, such as 5-bromo-deoxyuridine and 2-amino purine produce no revertants by suppression.

If the assumption is made that suppression occurs by alteration of the DNA information specifying the anticodon of one of several sRNA's such

that the new sRNA can recognize the nonsense codon, one can predict the specific base changes produced by all of the mutagens tested.

Further work is in progress to determine the number of different amino acids which can be inserted by suppressing factors. This number should be identical to the number one would predict from the altered anticodon hypothesis or seven. Also, the specificities of UV and ionizing radiation as mutagens are being studied.

In Dr. Snipes laboratory ESR studies of the effect of hydrogen and other atoms on biological systems is continuing. At present irradiated materials are being studied in the single crystal form since free radicals can be identified with much greater certainty in this state. However, the eventual goal of this work is to extend the ESR studies to DNA.

Physical-chemical studies of DNA are progressing in Dr. Taylor's laboratory. The efficiencies of production of single and double strand breaks are being investigated by boundary and band centrifugation of native and denatured calf thymus DNA. Sedimentation, viscosity and light scattering studies are being conducted using low molecular weight DNA. This material is also being used for studies of irreversible melting transitions. The structure and radiation and enzymatic inactivation of the replicating form (RF) of ØX174 DNA are being studied using similar techniques. This work was reported at the Biophysical Society Annual Meeting in Boston in February of this year.

This DNA is also being used in Dr. Ginoza's laboratory for target analysis studies. Three hypotheses have been postulated for the greater than expected radiation resistance in double strand DNA viruses. The possibility of a lethal double strand scission or base-pair damage has been ruled out as the main cause of inactivation by direct physical analysis of

inactivated RF-DNA of ØX174 and studies are now in progress on the two remaining alternatives: 1) the host cell repairs radiation lesions in double-stranded DNA's and 2) some critical target determines the frequency of inactivation, e.g. early enzyme functions.

Dr. Strother's experimentation with, and continued improvement of, the microspectrophotometer brings closer the possibility of examining irradiated single cells as another approach to an understanding of the mechanism of DNA degradation and radiation inactivation. Recent improvements include the use of a phase-lock amplifier to increase the signal-to-noise ratio. The instrument was described at the February Biophysical Society Annual meeting this year.

Meetings Attended

Members of the department presented ten papers at the Biophysical Society Annual Meeting held in Boston in February, 1966. They are listed below. Several faculty members and students also attended the "Symposium on Molecular Metabolism" in December, sponsored by the New York Heart Association. In addition, the principal investigator has just returned from a meeting of the NASA Bioscience Committee on the Exploration of Mars for Life, in Pasadena, California.

Biophysical Society Abstracts

Mechanism of Action of Ionizing Radiation on Genetic Transcription. T. F. Barone and E. C. Pollard, Biophysicals Society Abstracts, 10th Annual Meeting, 1966, TC12.

Depurination Analysis of Transforming DNA's. J. Bramwell, B. Nichols and W, Ginoza, Ibid., TC1.

Radiation Inactivation of the Replicative Form of ØX174-DNA. W. D. Taylor, R. C. Miller[1] and W. Ginoza, Ibid., FC2.

The Degradation of Bacterial DNA by Proton Bombardment. D. C. Huston and E. C. Pollard, Ibid., TC13.

DNA-Agar Annealing of the Residual DNA in Escherichia Coli 15T⁻L⁻ After Degradation Due to Ionizing Radiation. J. Swez and E. C. Pollard, Ibid., TC14.

Free Radical Formation in Amino Acids Exposed to Thermal Hydrogen Atoms. W. Snipes and J. Schmidt, Ibid., FC9.

Use of a Silicon Photodiode for Microspectrophotometry in the Near Ultraviolet and Visible Spectrum. G. K. Strother, Ibid., TE10.

The Effect of Irradiated Medium on the Thermal Sensitivity of Cells and of Enzymes. A. Yayanos, P. K. Weller and E. C. Pollard, Ibid., FC7.

[1] Graduated (Ph.D.), December, 1965. Present Address: UCLA.

Publications

1. W. Bernhard and W. Snipes. Electron Spin Resonance of a Gamma-Irradiated Single Crystal of Barbituric Acid Dihydrate, J. Chem. Phys., April 15, 1966.

2. S. Person, S. Phillips, F. Funk and M. Osborn. Suppression of Nonsense by Revertant Bacteria, Submitted for publication.

3. E. C. Pollard. The Fine Structure of the Bacterial Cell and the Possibility of Its Artificial Synthesis, Amer. Scientist, 53, 437-463 (1965).

4. E. C. Pollard. The Degradation of RNA by Ionizing Radiation in Dilute Solution, Nature, in press.

5. E. C. Pollard, J. Swez and L. Grady. Physical Characteristics of the Residual DNA in Bacterial Cells After Degradation Due to Ionizing Radiation, Radiation Research, in press.

6. W. Snipes and J. Schmidt. Free Radical Formation in Amino Acids Exposed to Thermal Hydrogen Atoms, Radiation Research, in press.

7. J. Swez and E. C. Pollard. DNA Agar Annealing of Residual DNA After Degradation by Ionizing Radiation, Radiation Research, in press.

8. E. C. Pollard. The Action of Ionizing Radiation on Post-Irradiation Synthesis and Degradation of DNA in E. coli 15 T⁻L⁻, Radiation Research, 27, 419 (1966).

Personnel

E. C. Pollard	Principal Investigator
W. Ginoza	Associate Professors
S. Person	
G. K. Strother	
W. Snipes	Assistant Professors
W. Taylor	
M. Trask	Research Assistants
G. Morris	
H. Newton	
B. Benson[1]	Graduate Assistants
W. Bernhard	
D. Chapman	
J. Elder	
C. Gay	
L. Grady	
D. Kearns	
N. Pascoe	
M. Sclair[1]	
A. Yayanos	
R. Hallet	
L. Harris	
B. Hellewell	
M. Mogus[1]	
J. Swez	

[1]One term only (12 weeks per year)

C. Ross	Secretary

Technical Assistants (Part time)