STATUS REPORT

PHYSICS OF CELLULAR SYNTHESIS, GROWTH AND DIVISION

NGR 39-009-008

(Period Covered: July 1, 1968 - December 31, 1968)

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INTRODUCTION

Most of the activity under this grant can be seen by looking at the separate reports. What is not so easily apparent is the degree of unity that is felt among the faculty and students who are working on this project. There are frequent small seminars held by sub-groups, for example one on the structure of the cell, and there is considerable interaction on each of the specific areas as research results arrive. One of the objectives of the original proposal was to develop a group with competence in the general area of the function of a small cell and this we hope to be able to bring usefully to bear on problems when they arise. We are currently doing some thinking about the analysis of soil from the Moon when it is available; we are wondering whether there will be any possibility of obtaining some and specifically working on it. In any event, on demand, our group stands ready to do specific work on this subject.

The productivity of the group has been high during this report period, as evidenced in part by the list of publications, and its contributions to the Summer Colloquium on Theoretical Biology and Biophysics at Traverse City, Michigan, was considerable. We are going to attend the future one in Fort Collins, though not in quite such large numbers.

The ability to attract good students and to develop good Ph.D.s who are serving usefully has already shown itself. Several examples of recent graduates who are serving acceptably are: Brent Benson, Assistant Professor of Physics at Southern Illinois University; William Bernhard, Postdoctoral

Research Associate at Argonne National Laboratory; D. J. Chapman, Medical Biophysics Section, Atomic Energy of Canada, Ltd.; L. J. Grady, Postdoctoral position at Carnegie Institution of Washington; G. J. Kantor, U. Calif. Biomedical Research Group at Los Alamos; C. David Lytle, Physicist with Department of Health ,Education and Welfare at Rockville, Maryland.

We think that the sum of the individual contributions is probably not so great as the whole entity that is present here in the University.

INDIVIDUAL REPORTS

1. Bacteriophage Studies in Filamentous Cells. W. Ginoza

Our radioautographic studies in the replicating T4 DNA in UV-induced, septa-free filaments of E. coli B have shown that this DNA pool remains localized and confined throughout the latent period in a volume (3-6 μ^3) which is small relative to the length (maximum studied, 60 μ) of the filaments. Also they reveal that recombination frequency between two co-infecting T4 amber mutants falls off sharply as the average length of the filamentous host cells increases. These results make topological sense, since genetic recombination must follow physical contact between two interacting genomes, and these contacts are expected to decrease with increasing cylindrical volume of the cell, provided that the replication pool is tethered to a replication site on the bacterial membrane.

The absence of septa inside the filamentous cells that may be a barrier to diffusing macromolecules, such as DNA and enzyme molecules, was confirmed in the following way. Two different T4 mutants, am B22 (polymerase$^-$) and ts L56 (thermal-sensitive polymerase) were made to co-infect filaments at an m.o.i. of 0.5 each and the infectious complexes allowed to develop at 30°C for 50 min. Amber alone does not grow on this su$^-$ host, but the ts mutant which does might be expected to supply the polymerase required for replication

of the amber genome at a distal site provided there is no membrane (septum) barrier. This expectation has been borne out as shown by plating the progenies on selective hosts and at permissive and non-permissive temperatures. The decrease in the frequency of recombination with filament length, mentioned above, is thus not due to septa formation.

From this observation we are led to the conclusion that the replicating DNA pool of T4 is a compact mass. This raises some intriguing questions as to its properties. Is the apparent compactness due to tangling by random incestuous mating known to take place among the replicating pool DNA? How large is the vegetative pool relative to a normal cell volume, and is this question relevant to the observed variation in the number of recombinants among individual cells of a population in mating experiments? Will the efficiency of multiplicity reactivation, a well known phenomenon in radiobiology, be affected by filament length? Can functional rescue alone (i.e., without genetic recombination) explain multiplicity reactivation?

2. Structure of Ribosomes of Chromatoid Bodies. R. S. Morgan

In last June's progress report the results of my first attempt at reconstructing the three-dimensional electron density of a single ribosome, from the Fourier transform of an image of a section through a Chromatoid Body, were given. These results have since been published in Science 162, 670, 1968, and reprints are enclosed.

In order to perform these calculations, I assumed that the phases of the transform could be taken from those calculated from a set of points arranged with the same helical symmetry as these ribosomes. This assumption, though plausible, forced the reconstructed ribosomes to have a two-fold (or dyad) axis of symmetry, perpendicular to the helical axis, a result which, as I then stated, was not necessarily a true feature of these ribosomes.

Since that time I have chiefly engaged in checking and refining these calculations. I have approached the problem of phase determination both optically (with my own optical diffractometer) and computationally, with a combination of scanning microdensitometry of one helix of the electron micrograph and IBM 360 computation of the Fourier transform of these numbers. This latter approach was strongly urged by the originators of the technique of reconstruction, Drs. DeRosier and Klug. It was made possible by the kindness of Technical Operations, Inc., Burlington, Mass., who furnished gratis a trial run of my micrograph on their scanning microdensitometer. (If I am to employ this extremely powerful aid to reconstruction regularly in the future, I will need either to purchase or rent the use of this major item of equipment.)

At this time I have gone far enough to see that the computational approach to phase determination will allow me to make a reconstruction that is both free of the unwanted perpendicular dyad axis of symmetry and, moreover, has no other questionable assumptions built into it. The results of this new reconstruction are nearly but not yet ready, and it should be possible to give a full description of it in my next report.

3. The Functions of Nonsense Codons. S. Person

Of the 64 code words in the genetic code only 61 have been shown to specify amino acids; the other three, called nonsense codons, have been shown either in vivo or in vitro (or both) to cause the termination of polypeptide synthesis. These three code words are UAG, UAA and UGA. When a transfer RNA is present in the cell that can recognize UAA, cell growth is significantly decreased, even in nutrient medium. The transfer RNAs that can recognize UAA are called ochre suppressors. These suppressors are produced by single base substitutions in DNA triplets specifying the anticodons of transfer RNAs that are one base

different from the complement of UAA. After mutation, the transfer RNA anticodon is complementary to UAA.

Our work in the past six months has been to continue to characterize cells containing ochre suppressors so that we can determine the molecular mechanisms by which UAA codons function in protein synthesis. Amber and ochre suppressor tRNAs are named according to the amino acid inserted. For example, the amber suppressor tRNA causing the insertion of serine is called a class 1 amber suppressor. Recently we were able to convert particular amber suppressors to their corresponding ochre suppressors using specific mutagens. In this way cells containing a class 1 ochre suppressor were derived from cells containing a class 1 amber suppressor. We have examined the beta-galactosidase and transcriptase activities in these two types of cells as well as in the original parent cell that contains no suppressors at all. These two enzymes are the proximal and distal enzymes in the genetic message of the lac operon in E. coli. Cells were grown in the same growth media, harvested, and extracts examined for the two enzyme activities. Total protein in the extract was also determined using the method of Lowry. Our finding (manuscript now in preparation) was that the enzyme units/mg of protein were smallest in the extracts from cells containing ochre suppressors. This confirms our previous belief that UAA codons have a normal regulatory function in protein synthesis and that cells containing a tRNA that recognizes UAA (ochre suppressor) interferes with that function.

4. Work in Dr. Pollard's Laboratory.

TEMPERATURE EFFECTS ON PHAGE T4 (W. Solosko). Following the discussions at Traverse City this past summer, it became apparent that the method of melting of phage DNA might be an important factor in the thermal inactivation of viruses. With this in view specially directed experiments were made to see whether in the process of phage inactivation the expansion of DNA and the

disruption of the protein code were factors in inactivation. Experiments show quite clearly that this is the case because DNAase in the presence of the heated phage greatly potentiates the destruction of the virus even though the enzyme cannot penetrate the cell in the ordinary way. We thus have evidence that the heating effect causes some kind of rupture in the protein code. This is an interesting new view of the method of inactivation of viruses by heat and is a significant contribution to that field.

PRESSURE EFFECTS ON RIBOSOMES AND ENZYMES (C. E. Hildebrand). Following another suggestion that developed at the Traverse City Colloquium, the idea that local pressure effects might be influential in systems containing hydrophobic regions, work has been initiated on looking for hydrostatic pressure effects on such systems.

Work has been started on the concept that distortion of ribosomes might permit the reading of the genetic code in such a way as to allow phage to develop in cells where they had been unable to complete their translation. Such systems are the famous amber systems; in non-permissive hosts such viruses do not finish their cycle. We have tried one or two of these systems using hydrostatic pressure to see whether an increased virus yield is observed. The results would be interpreted as misreading due to the distortion of the ribosomes. The suggested effects are seen, but they are complicated by the high rate of revertants (which could also be a secondary explanation for the experiment). Thus although this line of work is encouraging, it has been decided to move the pressure studies into three other areas.

We have already had some success in showing that hydrostatic pressure does influence temperature-sensitive enzymes. Cells which have a temperature-sensitive rate of DNA synthesis show that there is a reduced amount of synthesis when pressure is applied. We are going to follow this up, but we are more interested in examining a cell free system in which we directly

look at the influence of pressure on ribosomes. In addition, we are going to look at the induction of a prophage under pressure.

This work has been set up in a new laboratory and looks as though it should be productive.

5. Memory and Learning. A. Rake

The laboratory is being equipped* to a) study the biochemistry of learning and memory in mice, b) culture neurons in tissue culture, c) study genetic relationships of organisms and the evolution of genetic DNA sequences. Most of the necessary equipment has arrived and is, after suitable modifications, operable. Mouse training and the effects of drugs on mice have been initiated. Plant material for the genetic studies has been collected from the surrounding area or procured from other countries, and some of these have been induced to grow in tissue culture.

6. Effects of High Sugar Concentrations on E. coli. P. Scheie

Subtle variations in the constituents of hypertonic media have been found to be important in determining whether or not E. coli placed in such media will be plasmolyzed. Purity of the water used is one factor. Investigation is underway to establish what these crucial ingredients are, and how cells which are not plasmolyzed achieve their osmotic equilibrium. We have already obtained numerous descriptive data on both the plasmolytic and the non-plasmolytic response, but neither the cause nor the mechanism of the non-plasmolytic response has been established.

Microscopic Study of Cells. P. Scheie and H. Dalen.

Continued use has been made of a scanning electron microscope to study microextensions on cultured Chang's liver cells, in situ, at various stages

* Dr. Rake joined the Biophysics group in September.

of the division cycle. It appears that microvilli become attached to the substrate during interphase and when the cell rounds up for division these microvilli form the distal ends of long retraction fibrils. After division the cytoplasm spreads back into the flattened interphase configuration by following these same retraction fibrils.

7. Nerve-Muscle Relationships in Insects. T. Smyth, Jr.

It is well known that acetylcholine, the excitatory neuromuscular transmitter substance of vertebrates, is released in multimolecular packets. In certain insect muscle fibers which receive inhibitory as well as excitatory innervation, Mrs. Greer has demonstrated that the inhibitory transmitter is similarly packaged. This was done by observing the electrical response of the muscle fibers to transmitter release in an ionic environment which enhanced the response to inhibitory transmitter. Resting fibers were found to show spontaneous miniature hyperpolarizing potentials at random intervals. When the calcium/magnesium ratio was sufficiently low, neurally evoked inhibitory post-junctional potentials became smaller, eventually failing in a stepwise manner. The sizes of the steps were the same as the spontaneous miniature hyperpolarizations. Each miniature hyperpolarization is taken to reflect the escape of a packet of transmitter. An impulse in the inhibitory neuron permits the synchronous release of several packets.

8. Microspectrophotometry. G. K. Strother

Work during this report period has been directed towards solving some of the problems concerned with preparing red blood cells for analysis with the microspectrophotometer. Various media were tried in order to reduce diffraction effects; a 50% glycerol solution, mixed with .6% NaCl to preserve platelet form, was finally chosen. The problem of the cells slowly drifting out of the microscope field of view still has not been completely solved. However,

the best technique found to date is to deposit the blood cells on a 300 mesh (40 micron opening) coated electron microscope grid. With several cells per opening, drifting is minimal or zero.

Some effort has been directed towards analysis of stained epithelial cancer cells, prepared by Dr. S. Stein. The cells are being scanned at fixed wavelengths to determine the dye distribution. Preliminary results indicate that differential staining of membrane vs cytoplasm is being obtained, depending upon the dye being used.

9. Radiobiological Studies of Aqueous DNA Solutions. W. D. Taylor

A. Single-stranded ØX174 DNA. It has been found that single-stranded ØX174 DNA can be prepared in pure doubly distilled water by exhaustive dialysis and that the biological activity of this DNA does not decrease if the solutions are frozen. To restore the full biological activity the salt concentration must be raised to 0.5 Molar and then lowered to .01 Molar for assay.

Using this system and the addition of specific radical scavengers such as potassium iodide, sucrose, broth, etc., the complex dose response of the infectivity of this DNA can be divided into a simple exponential direct effect, an exponential effect due to the reaction of OH radicals, and an effect saturating by 100 kr tentatively assigned to reactions of aqueous electrons and/or hydrogen atoms.

The studies are continuing and will be correlated with strand-break measurements which were performed simultaneously.

B. Double-stranded DNA. Studies of radiation damage to the replicating form, RF, of ØX174 DNA have been made in order to compare the effects of single-strand breaks caused by gamma-rays with those caused by pancreatic DNase on the infectivity of RF in a protoplast assay. ^{32}P-labeled RF frozen at 77°K and irradiated with ^{60}Co gamma-rays was separated by sucrose gradient

sedimentation into two components according to the presence or absence of single-strand breaks, and the ^{32}P activity and biological activity of these components measured. Similar analyses were made on DNAse-treated XP. The data show that single-strand breaks cause less than 10% of the observed killing, 90% or more being due to radiation effects other than strand breaks. The nature of this damage and the mechanism of its production are under investigation.

10. Mammalian-Cell Research. P. Todd.

The projects described in the August, 1967, Status Report* have been under continued pursuit with the following findings:

A. Modification of Radiation Effects

1. Temporal fluctuations in radiation sensitivity occur in cells harvested from confluent monolayer cultures, and the fluctuations are due to the synchronous passage of cells through a division cycle, as determined by radioautography with ^{3}H-thymidine and mitotic monitoring.

2. The carcinostatic drug methotrexate has no immediate effect on the radiation sensitivity and recovery of cultured human liver cells. There are at least two direct effects of this drug on these cells, one related to thymidylate synthesis and one or more related to the scheduled completion of mitosis. The reversibility of these effects by thymidine and by folate coenzymes differs.

3. The extreme sensitivity of mitotic cells to ionizing radiation appears to be related to the frequency of interchromatid bridge formation in cells irradiated in late prophase and early metaphase.

4. Preliminary evidence indicates a temporal correlation of intracellular thiol content and/or distribution with radiation resistance in partially synchronized cell populations.

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* Dr. Todd was on leave of absence from August, 1967 to August, 1968.

B. Properties of Cultured Cell Surfaces.

1. Glycoproteins participate in the attachment of cultured cells to plastic surfaces.

2. Ionic and hydrophobic bonds are both important in adhesion of cells to polystyrene.

3. Increased surface charge density on the substratum enhances mobility of cells in the absence of serum.

4. Cells wan be cultured on millipore filters for preparation for electron microscopy. Electron micrographs show a preponderance of microvilli on the upper surfaces of attached cells but on the lower surfaces only when the substratum is sufficiently porous to allow microvilli to penetrate.

5. There is an increased reactivity of charged groups due to protein-bound arginine on cell surfaces or membranes during mitosis and accompanying other conditions that free cells from the substratum, such as infection by Herpes simplex virus.

6. Scanning electron microscopy, in conjunction with ordinary cytology, reveals that cultured human liver cells maintain many of their surface contacts through mitosis ny means of long microextensions which persist although the cells become spherical during mitosis.

7. Scanning electron microscopy has revealed cell surface structures associated with intercellular chromatin bridges.

C. Ultraviolet Photobiology.

1. A Chinese hamster cell mutant with 21 chromosomes has been found to be sensitive to ultraviolet light. Its rate of reversion to wild type lies somewhere in the range 10^{-1000} to 10^{-5}.

2. There is no obvious relationship between inherited cellular sensitivity to ionizing radiation and sensitivity to ultraviolet light.

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PERSONNEL

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W. Ginoza	Professors
S. Person	
R. S. Morgan	Associate Professors
T. Smyth, Jr.	
G. K. Strother	
W. Taylor	
A. Rake(4 mo.)	Assistant Professors
P. Schele	
P. Todd (4 mo.)	
H. Dalen (1 mo.)	Post-doctoral
R. Rehberg	Research Assistants
K. Newton	
L. Ritchie	
B. Benson	Graduate Assistants
M. Bernhard (3 mo.)	
D. Dickinson (3 mo.)	
D. Griffiths	
M. M. Greer	
R. Hallett	
P. Kellogg (3 mo.)	
G. Strniste (3 mo.)	
Z. Meckevicius (3 mo.)	
S. Reisteter (3 mo.)	
R. Donnelly	Secretary
A. Duke	Technical Service
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