Final Progress Report (June 12, 1977)

ERDA contract E-(11-1)-3233 awarded to David Freifelder, Department of Biochemistry, Brandeis University, Waltham, Mass.

Over a period of ten years a great deal of work was carried out under this contract. The results of this work could easily be divided into major and minor findings. In the interest of brevity this report will summarize the major findings only.

I. Radiobiology of phages

In 1966 little was known about the mechanism of inactivation of bacteriophages by ionizing radiation. We made an extensive study of this problem and came to the following conclusions.

1. Phages are inactivated principally by damage to DNA. Only the so-called after-effects observed when phages are irradiated in simple buffers do not involve DNA. This is caused by damage to the phage tail rendering it unable to adsorb to the host bacterium.

2. DNA damage is of two types - base damage and double-strand breakage. Single-strand breaks are not lethal. Base damage is oxygen-dependent.

Double-strand breakage may be lethal because of interruption within a gene. However in phage systems the damage is more fundamental in that only a single DNA fragment is injected into the host.

Base damage probably causes a replicative block.

Base damage is probably the major cause of lethality.

3. E. coli phage T4 is relatively resistant to inactivation by X-rays. This is because it possesses a repair system capable of eliminating base damage.

4. The rate of production of strand breaks and base damage is nearly the same in bacteriophage and bacteria. From the survival curves one can calculate that a major cause of killing of bacteria is also double-strand breakage and base damage.

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II. Studies of E. coli sex factors and plasmids

When this project was begun, plasmid DNA in broken form had been isolated from a few organisms. We spent several years developing methods for the isolation and characterization of plasmid DNA. Many of these methods are still in use in recombinant DNA research. One of our earliest findings was that sex factor DNA is circular. Using a variety of methods the molecular weights of several plasmid DNA's were determined. This enabled us to use molecular weight as a means of identifying different plasmids.

From an extensive study of methods to assay for circular plasmid DNA we developed a means to measure strand breakage in bacteria. Using this procedure we reported the rate of production of single-strand breaks following X-irradiation, ultraviolet irradiation, and thymine starvation. The value obtained for X-irradiation has become a standard for the analysis of experiments involving ionizing radiation and bacteria.

III. Determination of molecular weights of phage DNA

Because of the great difficulty in determining the absolute molecular weight of DNA and the ease in determining relative molecular weight, many labs tried a variety of tricks to obtain an accurate value of the molecular weight of a single phage DNA. The aim was to have a standard against which all other DNA's could be compared. Since all of this work failed, it was decided in my laboratory to use brute force methods to produce such a standard. This was successful and using two different methods, we reported two values for the molecular weight of E. coli phage T7 DNA which together provide a mean value with 2% accuracy. Since then T7 DNA has become the standard.

We used the same methods to get values also for T4 and T5 DNA's and measured very accurately the sedimentation coefficients of T4, T5, and T7 DNA. In so doing, we obtained the most accurate curve to date relating the sedimentation coefficient of DNA to molecular weight. This curve has become the standard curve also.

IV. Studies of lysogenization with E. coli phage λ.

Using an E. coli sex factor which has the prophage attachment site for phage λ and which is a circular DNA, we developed a reliable assay for prophage insertion and excision. Insertion was measured by observing the increase in size of the sex factor circle

when the λ DNA is inserted; excision was detected by the converse process. This method provided the basis for about 75 years' work in which we uncovered many of the details of the lysogenization process. We have determined the kinetics of insertion and excision, identified most of the genes which are involved in both processes and gained a great deal of information about the regulation of insertion. Most of this work has been reported. This work is an ongoing process, though.

For details about items I-IV and minor programs, see earlier Reports and reprints

David Zinder