Progress Report

(August 16, 1971 - August 31, 1972)

This report covers part of the fifth and most of the sixth year of contract AT(30-1)3797. The report is divided into two sections: I - Work completed, II - Work still in progress.

I. Work completed

1. Base damage in x-irradiated DNA. In the previous progress report it was stated that O₂-dependent damage (base damage) prevents expression of λ genes which are injected on DNA fragments. The genes studied were connected to their promoter and, therefore, only require transcription for expression. It had been observed that the effect of the presence of O₂ during the irradiation was so great that base damage had to be more prevalent than previously proposed in 1965. At that time we showed that in x-irradiated phages, strand breakage accounted for half of the lethal hits and we assumed that base damage accounted for the remainder. Since irradiated (O₂) injected λ DNA rarely engages in complementation, apparently every molecule sustaining a double-strand break also has significant base damage. To get an estimate of the frequency of base damage, we have studied E. coli phage ϕ X₁₇₄, a single-strand DNA phage. We have found that the rate of production of single-strand breaks (ssb) in ϕ X₁₇₄ phage is the same as that in double-strand DNA phages and is ten times that for double-strand breaks. Nonetheless, there was ca. a 3-fold effect of deoxygenation on radiosensitivity of ϕ X₁₇₄. To show that this O₂ effect was not on ssb, we irradiated phage, titered them, opened them with 0.3 M NaOH, and looked at the DNA by electron microscopy. By measuring the conversion of ϕ X₁₇₄ circles to filaments as a function of x-ray dose, it was shown that: (i) O₂-dependent damage is 2.6 times as frequent as ssb, and 26 times as frequent as dsb. Therefore, in phages, O₂-dependent damage is the major source of lethality.
2. Atis Folkmanis received his Ph.D. with a physical and genetic study of the development of E. coli phage λ . Most of his work is reported in the "in progress" section.

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Ter system is either a long molecule containing two joined ends or a double circle (which naturally contains two joined ends). We have detected an activity in vivo that acts only on two tandem prophages and we tentatively assume that this is the Ter activity. We are screening all λ genes suspected of being involved in the Ter system; i.e., genes A, W, B, C, D, E, and F. We have shown that F is not; and within a month, we will have positive information concerning the others. In an attempt to set up an in vitro assay for Ter, we have tried to prepare the appropriate substrate; i.e., a mixture of linear and circular trimers and circular dimers with p32 in the joined ends. This substrate has been prepared, but the student doing this work has just left school. This work is temporarily being held up.

We have also investigated the role of the Ter system in the formation of recombinant molecules. Grete Kellenberger in Geneva proposed that a major mechanism of recombination in λ is the formation of a double circle from a single circle followed by cleavage by the Ter system to give the recombinants. We have examined this indirectly. Since the Ter system is not expressed during lysogenization because it is a well-repressed late function and since double circles can probably be inserted to make a double lysogen, we have investigated the genotype of lysogens from a mixed infection of two mutant phages which yield wild type phage. If production of a wild type recombinant requires Ter, then no single lysogens should yield recombinant phages - all recombinant yielders should be polylysogens. So far, 40/40 such yielders tested are polylysogens, supporting the hypothesis of Kellenberger.

3. Electron microscopic techniques. We have learned how to do protein monolayer (i.e., Kleinschmidt-type) electron microscopy with samples containing detergent. We have been working on a method to look at non-chromosomal DNA directly by lysis of spheroplasts in a protein film. We have developed several improved processes for denaturation mapping of DNA.
4. Mechanism of lysogenation and insertion of phage λ . To study this, we have used a strain of E. coli deleted for the λ attachment region (att) and containing a sex factor, F'450 which includes att. Hence, an inserted prophage can be detected and the processes of insertion and excision can be followed simply by looking at sex factor circles, which we detect principally by sedimentation through alkaline sucrose gradients. Two basic procedures are used. For excision studies, cells containing prelabelled F'450(λ) are induced and the conversion of F'450(λ) to F'450 and λ is followed. For insertion studies,

the F'450 is prelabelled and one follows its conversion to F'450(λ). Sometimes differentially labelled λ is used and frequently labelling is carried out during induction or infection.

In the previous report twelve results were stated. To these we add:

A. The Xis gene product is a non-specific nuclease in addition to its role in excision. Therefore, its role in excision is probably nucleolytic rather than as a modifier of the Int product.

B. The left prophage att must be transcribed in order for excision to occur.

C. The DNA origin must be transcribed in order to initiate DNA synthesis.

D. The phage att must be transcribed in order for insertion to occur.

E. The mutant, t_{12} has a defect in initiation of DNA synthesis.

F. The Int product is not limiting in the insertion reaction.

G. DNA replication is not necessary for insertion to occur; but when it does occur, it must be turned off prior to insertion. Otherwise insertion does not occur.

H. Transcription does not interfere with the insertion reaction.

I. Excision is a slow reaction and usually comes well after DNA replication has begun.

J. At 42°, an Xis-double lysogen cannot excise a single prophage, all excision is by Ter. At 37°, a single prophage can be excised. Therefore, the crosses att PP¹ and att^R x PP¹ are temperature sensitive of the prophage is Xis-

K. A double lysogen is normally excised as a double circle. If the tandem prophages contain the $\Delta B'$ att between them, it interferes with excision so that Ter becomes the major excision system.

L. The Xis product affects the rate of insertion—Xis-mutants insert more slowly.

III. Publications:

Folkmanis, A. and Freifelder, D. Studies on Lysogeny with E. coli Phage λ . I. Physical Observation of the Insertion Process. J. Mol. Biol. 65, 63 (1972).

Freifelder, D. Mechanism of Formation of λ Polylysogens. submitted.

Freifelder, D., Kirschner, I., Goldstein, R. and Baran, N. Physical Studies of the Curing and Excision of λ Prophage. J. Mol. Biol. in press.

Publications (cont.)

Freifelder, D. and Levine, E. Stimulation of Nuclease Activity by Thymine Starvation. *Biochem. Biophys. Res. Comm.* 46, 1782 (1972).

Obel, J. and Freifelder, D. The Termini of Bacteriophage T7 DNA. *J. Mol. Biol.* 64, 439 (1972).

Freifelder, D. and Levine, E. Studies on Lysogeny with *E. coli* Phage λ . II. A Requirement for Transcription New att λ . Submitted to *J. Mol. Biol.*

Freifelder, D., Donta, S. T., and Goldstein, R. X-Ray Inactivation of Bacteriophages: Role of O₂-dependent Damage in Single- and Double-strand DNA Phages. *Virology*, in press.

Freifelder, D. Zonal Centrifugation. Methods in Enzymology. In press.

Inokuchi, H. Freifelder, D. and Dove, W. F. Physical Studies of RNA Involvement in λ DNA Replication and Prophage Excision. *J. Mol. Biol.* In press.