Some Aspects of the Mechanism of Bacteriophage Function

PROGRESS REPORT

David Freifelder
Professor of Biochemistry

MASTER

Brandeis University,
Waltham, Massachusetts 02154

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ABSTRACT

We have been concerned with replication, recombination, and maturation of E. coli phage λ . Red-mediated dimers, earlier thought to be recombination intermediates, have been shown to involve extension of a single DNA rather than an interaction between two DNA's. Dimers have also been found in superinfected lysogens and may be an early stage of recombination. A recombination intermediate resembling a Holliday structure has been identified by electron microscopy. The λ Red system has been shown to make non-reciprocal exchanges. The λ Red genes can substitute for the E. coli recBC gene in repair and bacterial recombination. Membrane-bound λ DNA has been shown by electron microscopy to consist of replicating theta-type molecules. UV induction of prophages requires DNA synthesis, presumably to generate gaps which can bind repressor.

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PROGRESS REPORT

Since our work has covered a variety of problems, this report is divided into several sections

I. Problems proposed in previous contract applications

1. Study of genetic systems responsible for production of single-strand breaks in phage DNA. E. coli phage λ DNA, as well as that from many phages, contain single-strand breaks. The genetic system responsible for these breaks is unknown. The potential importance of identifying this system is that the breaks may represent an early step in recombination or an event stimulating recombination. A study of this has been carried out off and on for the past few years - with numerous delays due to changes in technical personnel in my laboratory and to lack of interest in the problem by graduate students and postdoctoral fellows. Now I have a competent and enthusiastic undergraduate doing this work. He has prepared batches of phages from λ lysogens of various E. coli mutants. In an earlier progress report we showed that the E. coli genes *recA*, *recB*, *recC*, *recD*, and *recE* play no role in producing these breaks. We have now added to this list *uvrA*, *uvrB*, *uvrC*, *lig*, and *pol I*, and have prepared purified phages grown on *uvrD*, *uvrE*, *recF*, *recL*. Some bacterial mutants, e.g., *sof*- and *pol X*- have given small yields of phages and it will be necessary to work with larger volumes.

2. Isolation of intracellular DNA by the Worcel-Burgi procedure. Worcel and Burgi (J. Mol. Biol., 1973) showed that E. coli cells can be lysed in such a way that chromosomal DNA is retained as a single supercoiled structure which can be sedimented free of the lysate in a sucrose gradient. We hoped that λ phage DNA would remain in the supernatant and thereby easily isolated. Andrew Braun and Jon Tumin spent about 5 months in an attempt to do this encouraged by a few early successes. The method, however, has been unsuccessful when sensitive cells are infected, although when a lysogen is superinfected about half of the infecting supercoiled DNA is recoverable in this way. We feel that the principal trouble is physical entrainment, since late in infection but before lysis, even completed λ phage particles sediment with the chromosome. We suspect that reports in the literature of membrane association assayed in this way represent an artifact. M. Stodolsky (Loyola University, Chicago) claims to have had success with phage P1 using a variant of the Worcel procedure. In our hands his protocol has failed with λ . This method has been abandoned.

3. In vitro λ Red-mediated recombination using a DNA with a genetic duplication. In this series of experiments recombination was to be assayed by conversion of a duplication DNA to one without the duplication. To my knowledge we have had as little success as have

workers in other laboratories. The reason is probably that the reaction is too inefficient to be detected by standard spheroplast assays. From work in other labs the relatively efficient conversion in vivo is due to inter- and not intramolecular interactions. From work on λ integrase-promoted recombination at NIH it was shown that using present methods only intramolecular events occur with detectable frequency in vitro. Hence this project has been put aside.

4. Study of λ Red-mediated DNA dimers in infection of sensitive cells. Several years ago we observed that in *E. coli* infected with phage λ , there were structures which sediment in alkali like dimeric circles. Of special interest to us were those made by the λ Red recombination system. Two important questions were unanswered - (i) are they true dimers, catenated dimers (i.e., two linked monomers), or figure-8 molecules (such as observed with phages ϕ X174, S13, and G4); (ii) are they a result of the interaction of two monomers (as opposed, for example, to a Red-mediated accident not on the recombination pathway). Point (ii) is important since a true dimer indicates a reciprocal exchange and the work from Stahl's and Meselson's laboratories suggest that Red-recombination is mostly nonreciprocal (see item 4 in this report for further discussion of this point). Dorothy Freifelder has investigated both of these questions in the following way. If the material sedimenting as a dimer is given a small dose of x-rays sufficient to produce one single-strand break in 50% of the molecules, both catenated dimers and figure-8 molecules should shift in an alkaline sucrose gradient to a position intermediate between that of a supercoiled monomer and supercoiled dimer circle; the true dimer will shift to the position of a single-stranded circle. No material in the intermediate position was seen indicating that the structure is probably a true dimer. To investigate the second question, cells were infected with a mixture of equal numbers of λ int- and λ b221, a phage which has a large deletion of the b2 att int region and is roughly 20% smaller than normal λ . The int-mutation was included to exclude Int-mediated dimers. Due to the size difference of the two DNA's the monomers form distinct bands when centrifuged in an alkaline sucrose gradient. Using the high resolution methods in our laboratory, we know that we can distinguish the homogeneous dimers, ($\lambda+\lambda$) and (λ b221, λ b221) and the mixed dimer ($\lambda+\lambda$ b221). If the dimers are a result of the random interaction of two DNA molecules, one would expect 25% ($\lambda+\lambda$), 25% (λ b221+ λ b221) and 50% ($\lambda+\lambda$ b221). However, no mixed dimers were seen. This surprising result indicates that the dimers (which by the way can only be formed when replication is allowed) are not the result of the interaction of two DNA molecules but are the result of a Red-mediated replicative conversion of a monomer to a dimer. How this occurs is a matter for future study. This work is being prepared for publication.

5. Reciprocity of λ Red-mediated recombination. The above experiment eliminated the single bit of evidence we have for years claimed to support the idea of reciprocity in Red-mediated recombination. However, the idea is not excluded by the result. Statistical analyses of single-burst experiments from various laboratories have consistently shown that Red is at best only rarely reciprocal - i.e., linear correlation coefficients for reciprocal products in single bursts have been on the order of 0.2. Values of 0 and 1.0 would mean no or total reciprocity, respectively. The number 0.2 has been tantalizing (since it is not zero), although it might represent violations of the assumptions needed in the calculations. Since we had shown last year that only a single phage particle is maturable from a dimer circle, it seemed possible to us that Red recombination might be physically reciprocal yet not be detectable due to non-reciprocity of the maturation system. Our hypothesis was that if maturation was delayed so that a recombination intermediate could replicate extensively, the reciprocal products might be detectable. We had in our laboratory a reversibly temperature-sensitive mutant in gene A - at 42° maturation is prevented yet upon restoring the infected cell to 34°, maturation ensues. Using this trick Donald Ross, as part of his Ph.D. Thesis, did two single-burst experiments - in one case the cells remained at 34° throughout the phage cycle, in the other the cells were held at 42° for 30 minutes before lowering the temperature. The result of this massive experiment was that the linear correlation coefficients did not differ significantly - that is, delay in maturation does not make evident reciprocal recombination. Hence we conclude that the λ Red system is rarely reciprocal. This work is being prepared for publication.

6. Substitution of λ Red genes for bacterial Rec genes. The *E. coli* Rec genes are involved in both genetic recombination and repair of ultraviolet damage. Because of a similarity between the activities of the λ Red exonuclease and the *E. coli* RecBC nuclease, it seemed possible that these enzymes might substitute for one another. It was known though that the RecBC enzyme possessed certain activities detrimental to λ replication and that it cannot substitute for λ exo. However, the reverse is not the case; the λ exo gene can substitute for the RecBC enzyme in both repair and in recombination (in an Hfr mating). The observation is the following. A recBC- cell lysogenic for λ cI857 (a mutation conferring reversible temperature sensitivity on the λ cI repressor) is briefly heated to 42° to initiate synthesis of λ proteins and then restored to 34° to prevent synthesis of phage functions which kill the cell. These cells are either UV-irradiated or used as a recipient in a cross with an Hfr. If the phage is red+, the recBC- defect is overcome; if it is red-, it is not. No difference was seen between mutations in the λ Red exo or λ Red beta genes. The λ genes could not substitute for a recA- defect. It was also observed that whereas active λ O and P genes are required for Red-mediated λ recombination, that is not the case for recBC substitution. This work has been submitted for publication.

II. Progress on problems not described in previous contract application.

1. Role of DNA synthesis in UV induction of prophages.

R. Sussman has recently proposed that induction of prophages is generally caused by single-stranded DNA in the bacterium binding the λ repressor and allowing λ transcription. The role of UV would be to produce single-strand gaps resulting from attempts to replicate across unexcised thymine dimers. A prediction of this hypothesis is that if DNA synthesis is inhibited in a mutant that cannot excise thymine dimers, induction would be inhibited. Andrew Braun prepared a double mutant *dnaAuvrB*, the *dnaA* mutant being defective in initiation of DNA synthesis and *uvrB* lacking the UV-endonuclease. At 42° this mutant completes a round of DNA replication but cannot re-initiate. It was found that following such a protocol, including appropriate controls, UV induction cannot occur until DNA synthesis is permitted, thus supporting the original hypothesis. This work has been submitted for publication.

2. Membrane binding of replicating DNA.

For many years it has been proposed that replicating DNA is membrane bound and DNA pulse-labeled with H^3 -thymidine is found to be mostly associated with very rapidly sedimenting material. Manuel Valenzuela checked this hypothesis directly by infecting cells with λ , isolating the rapidly sedimenting DNA and observing this DNA by electron microscopy. Replicating theta-type molecules were found in agreement with the widespread belief. Their presence was eliminated in the rapidly sedimenting fraction by treatment with a lipase. This work is in press.

3. Observation of recombination intermediates by electron microscopy.

In the course of studying replicating λ DNA by electron microscopy, M. Valenzuela observed molecules with novel junctions consistent with the Holliday model of genetic recombination. These are formed in the absence of all known *E. coli* and λ recombination genes and probably represent a very early step in recombination. This work has been published.

4. A textbook on physical biochemistry.

During the past year I have written a 500 page undergraduate textbook entitled "Physical Biochemistry - Applications to Molecular Biology and Biochemistry". This book is in press with W.H. Freeman and Company and will be published in July, 1976.

5. Dimer λ DNA circles in superinfected λ lysogens.

λ DNA has complementary single-strand termini which can join in vitro to form circular monomers, linear and circle dimers, catenated dimers, and higher polymers. At high concentrations in vitro the polymeriza-

tion is favored. The appropriate range of concentrations corresponds to that found within infected cells. We have determined whether such polymerization occurs in vivo (i) to determine whether there exists a mechanism to protect the cohesive termini and to guarantee monomerization and (ii) since it was proposed (Melechen and Hudnik-Plevik, Proc. Nat. Acad. Sci., 1973) that such dimerization might be a precursor to recombination. By infecting lysogens with phages labeled with H^3 -thymidine and analysis by sedimentation through alkaline sucrose gradients, we have shown that supercoiled dimers and catenanes exist, that they are not made by the λ Red or Int systems or the bacterial Rec system, and that they are probably a result of cohesive end joining. This work has been submitted for publication.

6. New methods for preparing Hershey circles and covalent circles. Hershey circles can be prepared rapidly and with high efficiency by annealing at pH 10.6. Covalent circles of any λ mutant can be prepared by superinfecting a lysogen, lysis by freezing and thawing and pelleting the chromosome. Ca. 50% of the supercoils are found in the supernatant. This work has been submitted for publication.

7. Establishment of a tissue culture laboratory. Our work on E. coli phage λ has gradually led us to an interest in transformation of animal cells by viruses and chemical carcinogens. This has required setting up a cell culture laboratory. I have not done tissue culture work for 15 years, but with the help of a few people at Brandeis now have a functional cell culture room.

Effort Devoted by Principal Investigator

I devoted ca. 25% of my time to this contract, the remainder to projects supported by other government agencies and to teaching.

Publications Resulting from Work Described in This Report

1. Braun, A., Host DNA Synthesis or Excision Repair is Required for the UV Induction of Bacteriophage λ , submitted for publication.
2. Braun, A., Low, K.B. and Gluck, D. Substitution of the E. coli Rec BC Gene by the λ Red Gene in Recombination and the Repair of UV Damage, submitted to J. Bacteriol.
3. Freifelder, D. Preparation of Covalently Closed and Open Circular DNA Molecules of Phage λ , submitted to Biochem.Biophys.Acta.
4. Freifelder, D. Physical Biochemistry - Applications to Molecular Biology and Biochemistry, W.H. Freeman and Company, in press (Textbook).
5. Freifelder, D. Formation of Cyclic DNA Dimers and Catenanes of λ in the Absence of Known Recombination Systems, submitted to Virology.

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Publications (continued)

6. Freifelder, D., Baran, N., Folkmanis, A., and Freifelder, D.L.R. Circular Dimers in E. coli Infected with λ , in preparation.
7. Freifelder, D. and Tobinick, E., Curing of E. coli Lysogenic for λ by Heteroimmune Infection, submitted to Virology.
8. Ross, D.G. and Freifelder, D. Maturation of a Single Phage Particle from a Dimeric Circular λ DNA, submitted to Virology.
9. Ross, D.G. and Freifelder, D. Lack of Reciprocity in Red-Mediated Recombination in Coliphage λ , submitted to Virology.
10. Valenzuela, M., Freifelder, D. and Inman, R., Lack of a Unique Termination Site for the First Round of λ DNA Replication, J. Mol. Biol., in press.
11. Valenzuela, M. and Inman, R. Visualization of a Novel Junction in Bacteriophage λ DNA, Proc. Nat. Acad. Sci. 72, 3024-3028 (1975).
12. Valenzuela, M. Intermediates of the First Round of λ DNA Replication are Preferentially Found in a Rapidly Sedimenting Complex, submitted to J. Mol. Biol.