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Topological Relationship of Prophage λ to the Bacterial Chromosome in Lysogenic Cells

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Abstract. X irradiation converts bacterial sex factor DNA covalent circles to more slowly sedimenting forms according to first order kinetics. The rate of conversion is greater for sex factors carrying λ prophage than it is for non-lysogenized sex factors. The magnitude of the difference and the absence of covalent circular DNA from the product show that the prophage DNA is linearly inserted into the sex factor, as predicted by Campbell's model for lysogenization.

When a temperate bacteriophage such as *E. coli* phage λ infects a sensitive bacterium, the phage DNA sometimes becomes attached to the bacterial chromosome and thereafter replicates synchronously with it. In this state, the phage DNA is called a prophage and the bacterial cells are said to be lysogenized by λ. The prophage is located at a specific place on the bacterial chromosome, the λ attachment site. Two simple models for this attachment are (1) the prophage DNA is some sort of branch on the bacterial chromosome, or (2) the prophage is linearly inserted into the chromosome. The latter model, predicted by Campbell's hypothesis for the mechanism of lysogenization[1] and supported by the results of genetic mapping,[2, 3] has won general acceptance. Nevertheless, no direct physical evidence for linear insertion has yet appeared. In this paper we report such evidence.

Plan of the experiment: *E. coli* sex factors can be isolated as covalently closed twisted circles of duplex DNA.[4] The introduction of a single polynucleotide chain break converts a twisted circle into an untwisted one and the conversion may easily be detected as a substantial decrease in the sedimentation velocity of the molecule. Single chain breaks may conveniently be introduced into DNA duplexes by irradiation with X rays.[5] As one would expect, the X-ray target size for conversion of twisted circles to untwisted ones is directly proportional to their molecular weight, as has been demonstrated for a series of sex factors with different molecular weights.[6, 7]

Phage λ is able to lysogenize sex factors containing a λ attachment site apparently in the same manner as it lysogenizes the bacterial chromosome itself. If the prophage is attached to the sex factor as a branch, then, except for the special case discussed below, lysogenization should entail no increase in the X-ray target size for conversion of twisted-circular sex factors to untwisted

ones. If instead, the prophage is linearly inserted into the sex factor, the X-ray target size for conversion to untwisted circles should be increased by a factor just equal to the ratio of the molecular weight of λ to that of the sex factor. The experiments described below show the latter expectation to be fulfilled.

Measurements of X-ray target size cannot distinguish branched structures from unbranched ones in the special case that the prophage itself is a twisted circle attached as a branch to a twisted-circular sex factor. An X-ray hit on either twisted circle would convert such compound circles to more slowly sedimenting structures. The target size for conversion would therefore be the same as that of a sex factor containing a linearly inserted prophage. However, the first X-ray breakage product of a single twisted circle is an untwisted one, while an X-ray hit on a compound circle produces a structure still containing a twisted circle. In order to distinguish the two situations, we have subjected X irradiated lysogenized sex factors to ethidium bromide-CsCl equilibrium centrifugation, a sensitive method for detecting twisted-circular DNA.[8] The results show that lysogenized sex factors are simple twisted circles, not compound ones.

Materials and Methods. Conditions for bacterial growth, mating, radioactive labeling, and cell lysis as well as for X irradiation, CsCl, and sucrose gradient centrifugation, and radioactive counting have been described previously.[4, 6, 8, 9] X-ray dosage was measured as the oxidation of $FeSO_4$.[11]

The sex factor employed is the F′ isolated by E. L. Wollman from a derivative of *E. coli* K12 Hfr Cavalli and designated F100 by Adelberg and Low.[12] In addition to the λ attachment site, it is known to carry *gal* and *bio*. We shall refer to K12F′ as strain I. It was lysogenized by infection with λ and the sex factor was transferred to a lysogenic F^- strain to obtain four independent F′ (λ) merozygotes. The presence of the lysogenized sex factor was detected by the ability to transfer prophage λ to sensitive F^- recipients, as determined by zygotic induction. Six sublines of the four merozygotes were studied in these experiments. Strains III and IV descend from one merozygote and strains VI and VII from another. Strains II and V descend from the remaining two merozygotes.

Results. All strains were tested for the presence of F′ by their ability to transfer *gal* and by tests of their sensitivity to the male specific phage R17. They were tested for F′(λ) by zygotic induction. Strain II is insensitive to R17 and unable to transfer *gal;* apparently it arose by loss of the sex factor. Strain III is sensitive to R17 and transfers *gal* but not λ, indicating that its sex factor has lost prophage λ. Strains IV and VII are sensitive to R17 and are able to transfer λ, demonstrating the presence of F′ carrying one or more prophages. These results are summarized in Table 1.

Freifelder and Freifelder[9] have shown that sex factor DNA can be selectively labeled with radioactive thymidine by mixing a culture of an F′ male strain with a heavily irradiated culture of a UV-sensitive thymine-requiring female strain in medium containing radioactive thymine. The sex factor is transferred to the female cells, where it replicates and becomes labeled. Most of the label in such a culture is found in transferred sex factor DNA. Exogenous thymine is utilized mainly by the female strain and the synthesis of its own chromosomal DNA is greatly reduced by the UV irradiation. Upon dodecyl sulfate lysis and zone sedimentation through an alkaline sucrose gradient, five to ten per

cent of the labeled DNA is recovered in the rapidly sedimenting form characteristic of closed circular molecules, an identification confirmed by the three- to fourfold decrease in sedimentation velocity that results from the introduction of single chain breaks by X irradiation.[4, 13]

TABLE 1. *Biological characteristics of male strains.*

Strain	Designation according to biological tests*	Designation according to X-ray target size	Molecular weight of sex factor according to target sizes ($\times 10^{-6}$)
I	F′ (λ⁻)	F′ (standard)	72 (standard)
II	F⁻	—	—
III	F′ (λ⁻)	F′	72
IV	F′ (λ)	F′ (λ)	106
V	F′ (λ)	F′ (λ)	106
VI	F′ (λ)	F′ (λ, λ)	140
VII	F′ (λ)	F′ (λ, λ)	140

* The biological tests we have employed are unable to distinguish singly from multiply lysogenic sex factors. Thus, the designation given above denotes only the presence or absence of prophage λ. However, the F′ in strain V has previously been shown by genetic means to carry only a single prophage.[16]

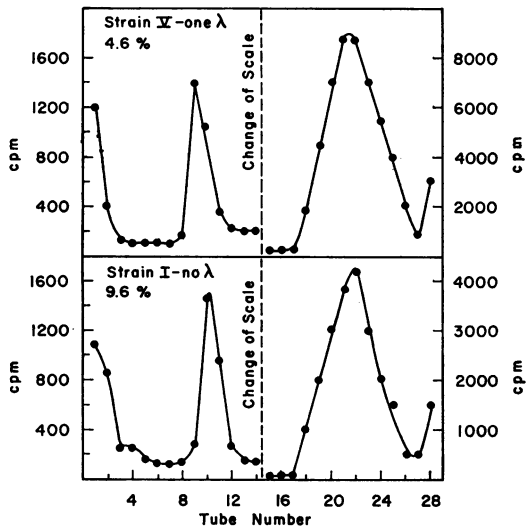

FIG. 1.—Alkaline sedimentation profiles of lysozyme-detergent lysates containing sex factors labeled with H^3-thymidine. Strains V and I are described in Table 1. About 100 μl of the lysate was layered on a 5-ml 5 to 20% alkaline sucrose gradient and centrifuged for 20 min (strain V) or 25 min (strain I) at 40,000 rpm in a Spinco SW65 rotor. Tube number refers to samples obtained by drop collecting. To each sample, cold carrier DNA was added after which it was precipitated by 5% trichloroacetic acid. The total radioactivity found in a rapidly sedimenting fraction (covalent circles) was 4.6 and 9.6% for strains V and I, respectively.

Each of the strains listed in Table 1 was subjected to the above procedure for detecting closed-circular sex factors. The female strain employed was lysogenic for the phage λ*ind*⁻, introduced in order to prevent zygotic induction of transferred prophages. Typical sedimentation distributions of labeled DNA are shown in Figure 1. Closed-circular DNA molecules were detected in all strains except strain II, a result in agreement with the biological tests for the presence of sex factor.

A previous paper[6] reports the measurement of X-ray target size for the conversion to nontwisted forms of several different twisted circular sex factors and of the twisted circular form of the λ chromosome. One of the sex factors studied

there was that from strain I, previously designated strain 57. The ratio of its target size to that of λ was found to be 1:0.45. If target size is simply proportional to the circumference of the DNA circle, λ circles are 0.45 the size of the unlysogenized sex factor.

In the present experiments, the X-ray target sizes of the sex factors from strains III–VII were compared to that of the sex factor from strain I. The results for strains I, V, and VII are shown in Figure 2. The exponential depen-

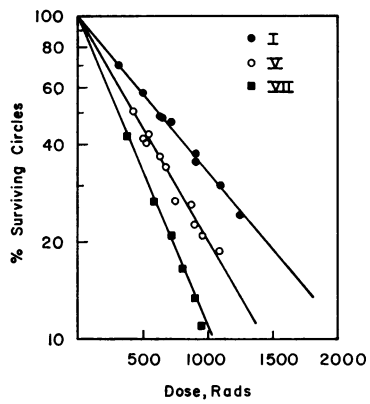

FIG. 2.—Percentage of surviving covalent circles derived from a strain with a nonlysogenic sex factor (strain I) and two strains (V and VII) with lysogenic sex factors—as a function of X-ray dose. Lysates containing covalent circles were X irradiated and sedimented through alkaline sucrose yielding sedimentation patterns such as those seen in Figure 1. Each point represents the result of a single centrifugation run.

dence of survival of twisted circles upon X-ray dose shows that a single X-ray induced "hit" is responsible for the destruction of twisted circles and that each preparation of twisted circles is homogeneous with respect to target size.

The X-ray sensitivity of twisted circles from each of the male strains listed in Table 1 has been studied. The data for strains I and III were found to fall on the upper line of Figure 2; those for strains IV and V on the middle line; and the data for strains VI and VII fall on the lower line. The ratios of the slopes of the upper two lines is 1:1.45. This is just the result to be expected if the sex factor of strain III, like that of strain I, contains no prophage and if the sex factors of strains IV and V contain just one prophage, *linearly inserted.* Furthermore, the slope of the lowest line is 1.45 times steeper than that of the middle one, suggesting that the sex factors of strains V and VII each contain two linearly inserted prophages. These results are all consistent with the biological characteristics of the strains summarized in Table 1.

The three X-ray target sizes found in the present experiments may be expressed in terms of molecular weights. The weight of the sex factor of strain I has previously been determined to be 72×10^6 by comparison of its target size with that of λ circles, taking the molecular weight of the latter as 33×10^6.[15] Accordingly, the three slopes seen in Figure 2 correspond to molecular weights of 72, 106, and 140×10^6 daltons, respectively. The first two values have been checked by direct measurement of the corresponding sedimentation velocity coefficients in alkali under conditions described by Clayton and Vinograd.[14] Using their relation between sedimentation coefficient and molecular weights,

Freifelder found the values 74 and 108 $\times$ 10^6 daltons, respectively (unpublished experiments).

Evidence against compound circles: If the prophage and the sex factor are both twisted circles somehow linked together, a single X-ray induced break should produce a structure containing one untwisted circle and one twisted one. Its density in a CsCl-ethidium bromide density gradient should be intermediate between that of intact compound circles and that of untwisted DNA. Figure 3 shows the density distributions of DNA from irradiated and unirradiated bacteria containing F'(λ). The peak corresponding to twisted circular DNA is located at the density found in other experiments for nonlysogenic sex factors and for λ twisted circles. No evidence is seen for the presence of the production by X rays of structures with intermediate density. We conclude that compound circles are not present.

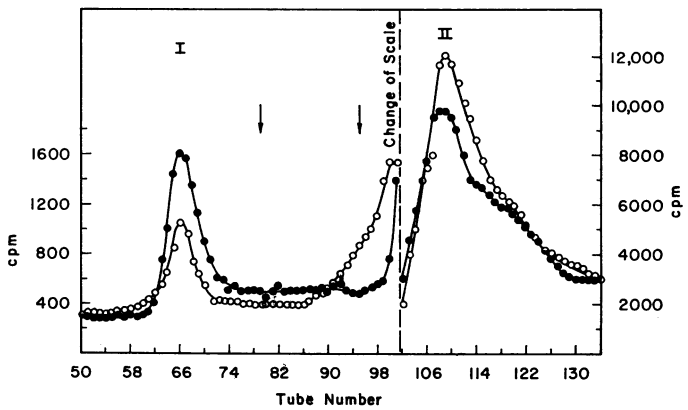

FIG. 3.—Equilibrium centrifugation in CsCl-ethidium bromide of a lysate from unirradiated and X-irradiated strain B583 harboring F' (λ). (This strain is not included in Table 1 and will be described in a subsequent publication. It is not significantly different from strain IV.) One-half milliliter of culture grown to 10^9/ml at 30° for 5 hr in tryptone broth containing 200 μg/ml uridine and 50 μc/ml H^3-thymidine was collected by centrifugation, and resuspended in 0.2 ml iced 0.01 *M* PO_4, pH 7.8, containing 10^{-3} *M* $MgSO_4$ and 10^{-4} *M* $CaCl_2$. Half of this suspension was X irradiated at 0°C with *ca.* 2000 rads. Each sample was then lysed with lysozyme-EDTA and 1%-Na sarkosinate and layered onto a solution containing 3.198 gm CsCl and 1.2 mg ethidium bromide and a total liquid (0.01 *M* Tris, pH 7.5) weight of 3.264 gm (sample included), covered with paraffin oil, and centrifuged at 20°C for 20 hr at 43,000 rpm in a Spinco 65 angle-head rotor. The gradient was fractionated by drop collecting into 180 fractions. ● unirradiated; ○ X-irradiated. Note change of ordinate for fractions numbered 102 and higher. Peak I represents covalently circular molecules; peak II consists of nicked circles and linear molecules. Arrows indicate the expected positions of products if the F' (λ) molecule consisted of two attached circles, as described in text. The high dose used in this experiment is necessitated by the fact that intact *bacteria* were irradiated rather than *lysate*, as was the case in the experiments of Figure 2.

Summary. We have shown that the X-ray target size for conversion of sex factor twisted circles to more slowly sedimenting forms is greater for sex factors carrying prophage λ than it is for nonlysogenized sex factors. The magnitude

of the difference corresponds to the linear insertion into the sex factor of exactly one or, in some cases, exactly two phage chromosomes. This result would also have been obtained if the prophage were attached as a branch to the sex factor and if both elements happened to be twisted circles. However, this possibility is ruled out by equilibrium sedimentation analysis in ethidium bromide—CsCl which shows that X rays convert lysogenized sex factors directly into structures lacking any twisted circular DNA.

We conclude that, as postulated by the Campbell model and suggested by genetic results, prophage λ is linearly inserted in the chromosomes of lysogenic bacteria.

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