EFFECT OF PHAGE-CONTROLLED RESTRICTION ON GENETIC LINKAGE IN BACTERIAL CROSSES

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Arber and Dussoix (J. Mol. Biol. **5**:18, 1962) demonstrated that lambda-bacteriophage, when grown on *Escherichia coli* K-12, plates with an efficiency of 1 on *E. coli* K-12 but with an efficiency of only 2×10^{-5} on *E. coli* K-12 (P_1) which is lysogenic for bacteriophage P_1. Furthermore, they showed that the low efficiency of plating is a result of the breakdown of the lambda-deoxyribonucleic acid (DNA) injected into the P_1 lysogenic host. This phenomenon has been termed restriction. The lambda-bacteriophage that is recovered from the *E. coli* K-12 (P_1) host now plates with an efficiency of 1 on strains that are either lysogenic or nonlysogenic for bacteriophage P_1. This effect has been termed modification. Schell and Glover (Proc. Intern. Congr. Genet. 11th, 1963) showed a 100-fold reduction in the recovery of lac^+ recombinants when a P_1 sensitive F′ strain carrying *F-lac* is crossed with a P_1 lysogenic recipient, instead of with a nonlysogenic recipient. They also showed that if the donor is P_1 lysogenic then the recovery of lac^+ recombinants is the same with either P_1 lysogenic or P_1 nonlysogenic recipient strains.

The present paper reports some studies of recombination between a P_1 sensitive Hfr, AB2229, and a P_1 lysogenic recipient, AB2147, in which two effects of restriction on the recovery of recombinants were observed. (i) The frequency of recovery of recombinants for the proximal markers xyl^+ and mal^+ from the Hfr male was reduced six- to tenfold in the restrictive cross by comparison with a similar cross involving the same male and nonlysogenic recipient. A more dramatic reduction was seen in the case of the terminal marker *ilv* where the reduction was as high as 300-fold. (ii) When mal^+ recombinants were examined for the inheritance of the proximal markers xyl^+ and tpa^+, a great decrease in the linkage between these markers was observed in the case of the restrictive cross.

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Strain AB2229 is an Hfr strain which transfers markers in the sequence tpa^+ xyl^+ mal^+ ilv^+ sex-factor. Recombinants receiving the tpa^+ gene are first recovered after about 5 min of mating, xyl^+ after 15 min, mal^+ after 23 min, and *ilv* as a terminal marker linked to sex-factor after 100 min or more. Strain AB2229 is nonlysogenic for bacteriophage P_1. Strain AB2154 is a P_1 lysogenic derivative of strain AB2229 obtained by infection with bacteriophage P_1. Strains AB1450 and AB2147 are xyl^- mal^- ilv^- females. Strain AB2147 is also tpa^-. Strain AB1450 is nonlysogenic for bacteriophage P_1 whereas strain AB2147 is P_1 lysogenic. Recombination experiments were carried out by use of the techniques described by Adelberg and Burns (J. Bacteriol. **79**:321, 1960). Samples from the mating mixture were plated for recombinants after 120 min of mating. The results of these experiments are shown in Table 1. It can be seen that, when the male strain is lysogenic for P_1 (crosses III and IV), the overall recombination frequency for either mal^+ or ilv^+ is not influenced by the nonlysogenic state of the recipient. On the other hand, when the male strain is nonlysogenic for bacteriophage P_1 (crosses I and II), the recombination frequencies for both mal^+ and ilv^+ are reduced when the recipient is P_1 lysogenic. An even more striking difference is observed in the linkage of mal^+ with xyl^+ and tpa^+. When both strains are nonlysogenic for P_1 (cross I), 90% of the mal^+ recombinants are also xyl^+. However, in the restrictive cross (cross II), in which the male is nonlysogenic and the female is lysogenic for P_1, only 10% of the mal^+ recombinants are also xyl^+. In crosses III and IV, in which the male donor is lysogenic for P_1, the recovery of xyl^+ in the mal^+ class is 82 and 80%, respectively. Similarly, only 8% of the mal^+ recombinants from the restrictive cross are also tpa^+, whereas in the cross using the same recipient with a P_1 lysogenic male 68% of the mal^+ recombinants are also tpa^+. The most likely explanation of the drastic reduction of linkage found in cross II is that the DNA which is transferred from the nonlysogenic donor is

TABLE 1. *Number of recombinants formed in crosses between lysogenic (P_1) and nonlysogenic strains and recovery of xyl^+ and tpa^+ in the mal^+ recombinants**

Cross	Male × female	No. of mal^+ recombinants	No. of ilv^+ recombinants	Per cent mal^+ recombinants that are	
				xyl^+	tpa^+
I	AB2229 × AB1450	6×10^6	1×10^5	90	Not done
II	AB2229 × AB2147 (P_1)	1×10^6	3×10^2	10	8
III	AB2154(P_1) × AB2147(P_1)	3×10^6	5×10^4	82	68
IV	AB2154(P_1) × AB1450	7×10^6	1×10^5	80	Not done

* Abbreviations: *mal*, maltose; *lac*, lactose; *xyl*, xylose; *ilv*, isoleucine and valine; *tpa*, tryptophanase; (P_1) lysogenic for bacteriophage P_1.

broken down into smaller pieces in the zygotes before integration has occurred. Thus, the physical linkage between mal^+ and the two proximal markers, xyl^+ and tpa^+, is destroyed in the restrictive cross. Boyer (Bacteriol. Proc., p. 31, 1964) also observed a reduction in linkage, as a result of restriction, when *E. coli* B/r is crossed with *E. coli* K-12.

IMMUNOFLUORESCENCE TECHNIQUES IN RETROSPECTIVE DIAGNOSIS OF HUMAN LISTERIOSIS

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Listeria monocytogenes was first reported to be the etiological agent of infections in animals (Murray, Webb, and Swann, J. Pathol. Bacteriol. **29**:407, 1926). The first recognized case of human disease resulting from infection with this organism was published 3 years later (Hyfeldt, Compt. Rend. **101**:590, 1929). Listeric infection resulting in stillbirth or abortion is not common in this country and, since organs and tissues obtained from fetal or neonatal death are not routinely examined bacteriologically, specimens are not always submitted to the clinical laboratory. However, the diagnosis of listeriosis cannot be made without a definitive identification of the organism. Although the physical and physiological characteristics of *Listeria* are clearly defined, the organism may be confused with other related species, especially when observed in histological preparations where *L. monocytogenes* may occur singly, in groups of short chains, or as coccoidal rods in palisade arrangement. Bacteriological identification depends upon recovery of the pathogen from the clinical specimen, a procedure which may be extremely difficult. The organism is serologically type-specific, but its identification requires isolation in pure culture and preparation of suitable suspensions for performance of agglutination tests. Therefore, a rapid, sensitive, highly specific test which does not require viable organisms would facilitate the laboratory identification of *L. monocytogenes* in tissue imprints or sections prepared at necropsy.

The globulin fractions of *L. monocytogenes* antiserums prepared against serotypes I, II, III, IV A, and IV B were labeled with fluorescein isothiocyanate (Riggs et al., Am. J. Pathol. **34**:1087, 1958) according to a modification of the method of Coons and Kaplan (J. Exptl. Med. **91**:1, 1950). The reagents (conjugates) were tested for specificity by (i) staining heterologous species of gram-negative and gram-positive bacteria, (ii) staining heterologous serotypes of *L. monocytogenes* with type-specific conjugates, (iii) blocking the fluorescence reaction by the use of the one-step inhibition test (Goldman, J. Exptl. Med. **105**:557, 1957), and (iv) treating the organisms with conjugated normal rabbit globu-