TRANSFORMATION OF BACTERIAL MARKERS AND TRANSFER OF PHAGE MARKERS WITH DNA ISOLATED FROM A λ-Ø80 HYBRID PHAGE CARRYING THE TRYPTOPHAN GENES OF *E. coli**

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The discovery by Matsushiro (1961) of a temperate transducing phage, Ø80, which is capable of carrying the tryptophan genes of *E. coli*, has made it possible to attempt transformation with isolated DNA in a manner analogous to that described by Kaiser and Hogness (1960) for DNA from λdg. Studies by Matsushiro (1963) have established the following: 1) The prophage site of Ø80 is near the tryptophan genes on the *E. coli* chromosome; 2) after UV induction of bacteria lysogenic for Ø80, lysates are produced which show low frequency transduction of tryptophan markers; 3) transduction with such LFT lysates results in the formation of heterogenotes which on induction give HFT lysates; 4) the HFT lysates may contain either defective or non-defective transducing particles.

In this preliminary communication we report the transfer of phage markers and transformation of bacterial genes with DNA extracted respectively from Ø80 and from a λ-Ø80 phage hybrid which carries a segment of the cluster of bacterial tryptophan genes.

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MATERIALS AND METHODS

The bacteriophage and bacterial strains used in this study are described in Table I.

TABLE I

Bacteriophage and Bacterial Strains Used

(a) Bacteriophage	Description	Use
Ø80	Temperate phage, transduces tryptophan genes. (Matsushiro, 1961, 1963)	Source of DNA, helper phage
$i^{\lambda}h^{Ø80}$tryp A^+B^+	Non-defective λ-Ø80 hybrid carrying the A and B tryptophan genes. (N. C. Franklin, Personal communication)	Source of DNA
i^{434}	434-λ hybrid, has 434 immunity marker. (Kaiser and Jacob, 1957)	Helper phage
i^{434}c sus QR	Clear plaque 434-λ hybrid with QR suppressor-sensitive (sus) markers. (A. Campbell, 1961; A. D. Kaiser, Personal communication)	Helper phage
λb2c	Clear plaque density mutant of λ.	Helper phage
(b) Bacterial Strains		
W1485	Wild-type K12, Pm^{+1} for sus mutants QR.	Preparation of lysates; recipient for DNA
W1485 A23	Tryptophan auxotroph	Transformation recipient
W1485 A23(Ø80)	Lysogenic auxotroph	Transformation recipient
B185(i^{434})T_1^r	Pm^{+1}, T_1^{r2}; lysogenic for i^{434}, derived from C600. (A. D. Kaiser, Personal communication)	Selective indicator immune to i^{434} and resistant to Ø80
Y mel	K12 stock, lysogenic for λ.	Selective indicator immune to λ.

[1] Pm^+, Pm^- = Permissive, non-permissive (for sus mutants).
[2] Phage with the host range of Ø80 are unable to form plaques on T_1^r bacteria.

Preparation of lysates: Ø80 and $i^{\lambda}h^{Ø80}$tryp A^+B^+ lysates of 1-2x10^{11} phage/ml were prepared by infecting 100 ml of an early log phase culture

of strain W1485 in L-broth (Lennox, 1955) at a multiplicity of 0.1. Cultures were incubated with shaking at 37° C for 6 hours and the cell debris was removed by centrifugation. The lysates were titered for infective phage on soft-agar tryptone plates with W1485 as indicator, or for transducing ability with tryptophan auxotrophs W1485 A23 or W1485 A23(ø80) on minimal agar. All phage used either as helper or for DNA isolation were purified by differential centrifugation followed by banding in CsCl. DNA was prepared from purified phage by extraction with phenol saturated with 0.01M phosphate buffer, pH 7.0 (Grossman et al., 1961). The aqueous layer containing the DNA was removed and dialyzed for 48 hours against repeated changes of 0.1M Tris, pH 7.8, 0.001M $MgCl_2$ or 0.01M Tris pH 7.8, 0.001M EDTA to remove phenol.

Transformation procedure

The procedure employed in transformation experiments was similar to that described by Radding and Kaiser (1963). A culture of the recipient bacterium was grown to a density of $1\text{-}2\times10^9$/ml in a minimal medium (Vogel and Bonner, 1956) supplemented with glucose and, where necessary, with tryptophan. The bacteria were sedimented and resuspended in half the original volume of 0.01M Tris, pH 7.8, 0.001M $MgCl_2$ at 0° C. Helper phage were added at a multiplicity of 5. The mixture was chilled at 4° C for 10 minutes, incubated for 15 minutes at 37° C, and rechilled for 5 minutes. This treatment was followed by sedimentation and resuspension of bacteria in cold 0.01M Tris pH 7.8 buffer containing 0.01M $CaCl_2$ and 0.01M $MgSO_4$ (TCM). Various dilutions of a DNA solution were added to aliquots of a suspension of helper-infected bacteria. This mixture was incubated without shaking for 30 minutes at 37° C. For phage marker transfer an appropriate dilution was added to 2 ml of soft agar containing 0.2 ml of the indicator strain and the mixture poured over tryptone agar. For bacterial marker transformation the appropriate dilution was spread directly on minimal agar.

RESULTS AND DISCUSSION

Gene transfer with phage DNA

When DNA from phage Ø80 was mixed with suspensions of strain W1485 infected with helper phage i^{434}c sus QR and the mixture plated on B185(i^{434})T_1^r, progeny phage were recovered with $_i$Ø80, the Ø80 immunity marker. The recovery of markers of the phage used as the source of DNA was dependent on the presence of DNA (Figure 1) and helper phage (Table II), and was susceptible to treatment with DNase (Table II).

TABLE II

Transfer of Ø80 Immunity Gene with Ø80 DNA

Additions to W1485	No. plaques observed on B185(i^{434})T_1^r/plate	Progeny carrying $i^{Ø80}$/ml mixture
Ø80 DNA + helper	200-300	7.6×10^7
Ø80 DNA, no helper	2	20
Helper, no Ø80 DNA	0	10
No helper, no Ø80 DNA	0	10
DNase-treated Ø80 DNA and helper phage	0	10

The DNA solution employed had an O.D. of 2.61 at 260 mu. W1485 mixed with DNA and/or helper was plated on B185(i^{434})T_1^r as selective indicator. Neither i^{434} nor Ø80 can form plaques on this strain since it is lysogenic for i^{434} and is T_1-resistant (does not adsorb Ø80). The plaques are therefore due to phage carrying the immunity of Ø80, derived from the Ø80 DNA. The DNase treatment was performed by incubating 0.1 ml of DNA solution with 20 γ/ml DNase for 30 minutes at 37° C in 0.01M Tris pH 7.8 containing 0.01M $MgCl_2$. The efficiency of transfer was 8×10^{-5} based on the number of phage DNA equivalents added, using the relationship 10 O.D. units = 4×10^{12} plaque forming units/ml.

Other helper phage (λb2c and Ø80) were also tested in phage marker transfer experiments, with DNA from Ø80 and from $i^{\lambda}h^{Ø80}$. A variety of recombinants carrying the markers of the helper and the input DNA were observed. In experiments where DNA from Ø80 and Ø80 mutants (in the absence of helper phage) was added to protoplasts of non-lysogenic bacteria, infective phage were recovered which were indistinguishable from the phage used as the source of DNA. In these experiments omission of

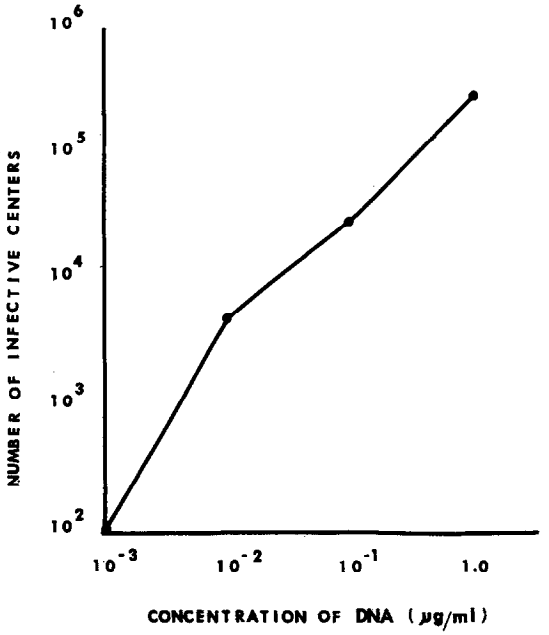

Fig. 1. Recovery of phage with immunity of ø80 as a function of DNA concentration. The assay procedure is described in the legend to Table II.

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either the DNA, the protoplasts, or both, or treatment of the DNA with DNase, prevented the appearance of phage with the markers of the input DNA.

Transformation of bacterial markers

W1485 A23 and W1485 A23(ø80) were used as recipients in transformation experiments with DNA from the non-defective transducing phage $i^{\lambda}h\text{ø}80$tryp A^+B^+. Table III presents the results of a typical experiment in which ø80 was used as helper.

The efficiency of $tryp^+$ transformation ($tryp^+$ transformants/phage DNA equivalents) was 1.8×10^{-5}. When ø80, i^{434} or λ were tested as helper, the efficiency of transformation was greatest with i^{434}. All transformants picked in experiments with W1485 A23 as recipient and ø80 as helper segregated $tryp^-$ cells at a high frequency; i.e., approximately 30% of the cells in an overnight culture grown in minimal medium were $tryp^-$. Twelve transformants (W1485 A23 as recipient) were tested for immunity

TABLE III

Transformation of W1485 A23 and W1485 A23(Ø80) to Prototrophy with $i^{\lambda}h^{\emptyset 80}$tryp A^+B^+ DNA

Incubation mixture	No. of $tryp^+$ colonies/ml DNA sol.	
	Recipient A23	Recipient A23(Ø80)
Recipient alone	0	1
Recipient + DNA from $i^{\lambda}h^{\emptyset 80}$tryp A^+B^+	0	2
Recipient + helper phage	0	5
Recipient + DNA from $i^{\lambda}h^{\emptyset 80}$tryp A^+B^+ + helper phage	1.9×10^7	1.3×10^7
Recipient + DNase-treated DNA + helper phage	18	10

Ø80 helper phage were added to W1485 A23 or W1485 A23(Ø80) at a multiplicity of five. Appropriate dilutions of DNA ($O.D._{260} = 7.0$) were added to 0.2 ml recipient bacteria (2×10^9 bact./ml), the mixture incubated 30 minutes at 37° C and 0.1 ml aliquots plated on minimal agar.

to Ø80, and λ, and all were found to segregate λ and Ø80-sensitive bacteria. This instability of Ø80-helped transformants was also reflected in the appearance of the original $tryp^+$ colonies on the agar plates: the colonies were surrounded by areas of lysis. Lysates prepared from these $tryp^+$ isolates were characteristically HFT, and contained wild-type Ø80 and $i^{\lambda}h^{\emptyset 80}$tryp A^+B^+ phage at a ratio 10:1. When i^{434} was used as helper, the $tryp^+$ transformants were stable. In addition, they were invariably stably lysogenic for i^{434} and λ. Induced lysates from these stable transformants were either HFT or LFT. Various phage recombinants were found in the lysates; however, transduction was observed only with $i^{\lambda}h^{\emptyset 80}$ phage. Further work is required to establish whether the different degrees of stability observed are the result of recombinational events at the prophage site or of recombination with the tryptophan genes on the bacterial chromosome. In any event, the experiments described in this report establish that transformation can be accomplished with DNA corresponding to bacterial genes that code for the structure of a well characterized protein (Yanofsky et al., 1964).

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REFERENCES

Campbell, A., Virology 14, 22 (1961).
Grossman, L., Levine, S. S., and Allison, W. S., J. Mol. Biol. 3, 47 (1961).
Kaiser, A. D., and Hogness, D. S., J. Mol. Biol. 2, 392 (1960).
Kaiser, A. D., and Jacob, F., Virology 4, 509 (1957).
Kellenberger, G., Zichichi, M. L., and Weigle, J., J. Mol. Biol. 3, 399 (1961).
Lennox, E. S., Virology 1, 190 (1955).
Matsushiro, A., Bikins J. 4, 133 (1961).
Matsushiro, A., Virology 19, 475 (1963).
Radding, C. M., and Kaiser, A. D., J. Mol. Biol. 7, 225 (1963).
Vogel, H., and Bonner, D. M., Microb. Genetics Bull. No. 13, 43 (1956).
Yanofsky, C., Carlton, B. C., Guest, J. R., Helinski, D. R., and Henning, U., Proc. Natl. Acad. Sci. U. S. 51, 266 (1964).