Formation of a Cointegrate between Phage $\phi 80$ and a Col,Trp Plasmid

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INTRODUCTION

By crossing the wild-type colicinogenic *Escherichia coli* strain $\kappa 260$ with an *E. coli* $\kappa 12$ derivative, and selecting for transfer of tryptophan-independence, a stable conjugative plasmid was obtained in which the transferred chromosomal segment of $\kappa 260$ remained associated with the Col factor of the donor strain (Fredericq, 1963, 1965). As the chromosomal segment included *att₈₀* as well as *cysB*, *trp* and *tonB*, it appeared that phage $\phi 80$ could integrate into the plasmid. In a previous report (Fredericq, 1969) circumstantial evidence of such an integration was presented, based on the observations that: (i) a strain with a *trp tonB att₈₀* deletion on the chromosome could only be lysogenized if it carried the plasmid; and (ii) there was zygotic induction when the plasmid from the lysogenized strain was transferred by conjugation to a non-immune recipient – the recombination rate was reduced 100-fold compared with crosses in which donor and recipient were both either non-lysogenic or lysogenic for $\phi 80$. In this paper we confirm the integration of phage $\phi 80$ into a Col,Trp plasmid, giving physical and genetical evidence that the prophage is inserted into the continuity of the plasmid molecule.

METHODS

Bacterial strains. The donor strain 62G1 was obtained by transferring a Col,Trp plasmid to strain G43, an *E. coli* $\kappa 12$ derivative with a *trp tonB att₈₀* deletion on the chromosome (obtained from J. P. Gratia). The Col,Trp plasmid, designated pPF102, was derived from the original Col,Trp plasmid (Fredericq, 1963, 1965) obtained from strain $\kappa 260$, but its precise relationship to the latter is not known since it was obtained in several steps. It still carried the transfer genes (derepressed) and colicin determinants (V, B, M) originally carried by the Col factor in strain $\kappa 260$, and a segment of chromosomal genes from $\kappa 260$ including *cysB*, *trp*, *tonB* and *att₈₀*.

The recipient strain 206md was a *metB trpB* strain derived from strain 206, an *E. coli* $\kappa 12$ derivative carrying a defective F agent (obtained from J. P. Gratia). Only *tonA* mutants, selected for resistance to phage T₅, were used as recipients to prevent infection by phage $\phi 80$.

Phage strains. Lysogenic derivatives of both donor and recipient strains were produced by infection with phage $\phi 80$ (Matsushiro, 1963) or with the hybrid phages $\lambda 80$ (*imm λ h80 att₈₀*) or 80 λ (*imm80 h λ att₈₀*), obtained from R. Thomas.

Media and techniques for transduction and marker analysis. All media and techniques were as described previously (Fredericq & Delhalle, 1973, 1974). Transducing lysates were obtained by growing phage P1*kc* (Lennox, 1955) on the lysogenic donor strains. Transfer of the plasmid marker Trp⁺ or of the chromosomal marker Met⁺ from donor to recipient was selected. Efficiency of transduction was defined as the ratio of the number of Trp⁺ transductants to the number of Met⁺ transductants obtained for each combination. From each cross, 1000 Trp⁺ transductants were isolated and tested for their colicinogenic and lysogenic properties.

Electron microscopy. Radioactive plasmid DNA was prepared from bacteria grown in nutrient broth containing [6-³H]thymidine (1 μ Ci ml⁻¹, 28 Ci mm⁻¹; I.R.E., Fleurus, Belgium) and adenosine (250 μ g ml⁻¹). The labelled cultures were lysed and plasmid DNA was prepared from the lysates essentially as described by Grinstead *et al.* (1972). Grids for electron microscopy were prepared by the micro-version of the spontaneous adsorption method of Lang & Mitani (1970). The grids were rotary shadowed with platinum at an angle of 7 to 10° with a Balzers BAE120 evaporator and examined using a Philips EM300 electron microscope. Unambiguously open circular molecules were photographed, enlarged, traced on to paper and measured with a map measurer. Molecular weights were calculated using plasmid RP1 as a standard (38 Mdaltons) (Stanisich, Bennett & Ortiz, 1976).

RESULTS AND DISCUSSION

Physical evidence

Plasmid DNA was isolated from the pPF102⁺ strain 62G1 and from derivatives of it that had been lysogenized by ϕ 80 or one of the ϕ 80- λ hybrids (80 λ or λ 80). Contour length measurements of open circular plasmid molecules showed that in all strains only a single species of DNA was present. That from the pPF102⁺ strain was 112 ± 4 Mdal whereas that from the pPF102⁺(ϕ 80)⁺ lysogen was 142 ± 4 Mdal. Since the latter represents an increase in the size of pPF102 of 30 Mdal, a size comparable to that estimated for the ϕ 80 genome (Yamagishi, Yoshizawa & Sato, 1966), it is likely that in the host strain lysogenization occurred by the insertion of ϕ 80 DNA into pPF102. This is further supported by the observation of a similar increase in the size of pPF102 following lysogenization of the host strain with λ 80 or 80 λ (136 ± 8 and 148 ± 6 Mdal, respectively).

Genetical evidence

In Table 1, linkage relationships obtained by transducing Trp⁺ from a lysogenized donor, into non-immune or immune recipients are compared with the values obtained in a control experiment, where both donor and recipient were non-lysogenic.

When the recipient was not immune, the efficiency of transduction was decreased about 10-fold, probably due to zygotic induction of the transduced prophage. This decrease was accompanied by a marked reduction in all classes of transductants receiving ColM, and eventually ColB genes, pointing to their linkage with the prophage genes. Transfer of ColV genes was little affected.

When the recipient was carrying the same prophage as the donor and was therefore immune, zygotic induction was prevented and the efficiency of transduction was normal or even elevated. Cotransfer of ColM with Trp reappeared but the ColV⁺Trp⁺ColM⁺ and the Trp⁺ColM⁺ColB⁺ classes were still missing, indicating that the distance between Trp and ColM had been extended by insertion of the prophage and that segments extending from ColV to ColM or from Trp to ColB were now too large to be included in one transducing particle.

When the recipient was carrying 80 λ , a homo-immune prophage with a different host range, the results were essentially the same but transfer of the donor prophage could also be scored. The only transductants which received the donor prophage were found among those receiving Trp⁺ alone (60%) or Trp⁺ and ColM (91%), indicating its location between these two markers. All ColB⁺ and some rare ColM⁺ transductants were, however, non-lysogenic for ϕ 80, which might be explained by transducing particles originating in spontaneously cured donor cells. The rare ColM⁺ transductants observed in the cross with a non-immune recipient may have the same origin.

These results demonstrate that the prophage ϕ 80 was inserted between Trp and ColM in the sequence ColV-Trp- ϕ 80-ColM-ColB. Such linear insertion of a prophage into the plasmid molecule should modify transductional linkage values for three reasons. (i) If the recipient is not immune, zygotic induction should reduce the total number of transductants and particularly those receiving Col genes separated from *trp* by the prophage,

Table 1. *Transduction from non-lysogenic and lysogenic donors (D) to non-lysogenic and lysogenic recipients (R)*

Classes of transductants				D = (80) ⁻	D = (80) ⁺		
ColV	Trp*	ColM	ColB	R = (80) ⁻	R = (80) ⁻	R = (80) ⁺	R = (80λ) ⁺
—	+	—	—	670	957 (0)†	947	956 (574)†
+	+	—	—	15	37 (0)	22	15 (0)
+	+	+	—	70	4 (0)	1	3 (0)
—	+	+	—	25	0 (0)	29	23 (21)
—	+	+	+	220	2 (0)	1	3 (0)
Total				1000	1000 (0)	1000	1000 (595)
Efficiency of transduction‡				1	0.1	2	1.5

* Trp⁺ was the selected marker.

† No. which received (80)⁺ in parentheses.

‡ See Methods.

that is ColM ColB if the order is ColV-*trp-att*₈₀-ColM-ColB. (ii) Insertion of the prophage at the *att*₈₀ site, near *trp*, should extend the distance separating either ColV or ColM ColB from *trp*, according to the orientation of the *trp att*₈₀ segment, and consequently reduce cotransfer of the Col genes located nearest to the prophage. (iii) The presence of a prophage in both donor and recipient should extend the region of homology between donor plasmid and recipient chromosome and favour rescue of *trp* genes by crossing over. This should increase the total number of transductants but not the number of colicinogenic transductants; thus the latter should be proportionately reduced.

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