Transduction of *pro*⁺ to *Salmonella typhimurium proAB47* by Phage P22

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SUMMARY

The multisite mutant *Salmonella typhimurium proAB 47* was infected with phage P 22 from a suspension mediating restricted transduction of proline markers. Evidence was obtained which supports the conclusion that *proAB 47* forms only a few colonies by the mechanism of restricted transduction, and that the near absence of this class of transductants is responsible for the low frequency of incorporation of the *pro*⁺ marker by this strain.

INTRODUCTION

Miyake & Demerec (1960) reported, in transductions from a wild-type donor to *Salmonella typhimurium proB* mutants using phage P 22 as vector, that the wild-type marker was incorporated in the multisite mutant *proAB 47* (and also *proAB 126*) significantly less frequently than in other strains. They proposed that in *proAB 47* one or both of the regions between the ends of the deletion and the ends of the relevant transduction fragment was shortened, lowering the chance of incorporation by recombination. An alternative explanation, for which we present evidence in this paper, is that this effect results from deletion of the P 22 prophage attachment site in *proAB 47* (Smith & Levine, 1965).

Smith-Keary (1966) showed that a proline-requiring mutant could be converted to proline-independence either by the attachment of a phage fragment at the P 22 prophage site (Young & Hartman, 1966), analogous to the transduction of galactose markers by phage lambda in *Escherichia coli* (Morse, Lederberg & Lederberg, 1956), or by recombination with a donor fragment as in general transduction. If a transductant of the former type also harboured a normal P 22 prophage, the phage fragment could replicate and, after lysis of the cell, convert numerous auxotrophic cells in the background to *pro*⁺ satellite colonies; a cell-free suspension of such a colony, termed a 'satellited transductant', could transduce *pro*⁺, but no other markers, at high frequency.

We propose that the diminished numbers of colonies found in *proAB 47* crosses results from the absence of the class of *pro*⁺ transductants in which a phage fragment is attached at the P 22 prophage site, which is adjacent to the proline genes. If this is correct, it is predicted that *proAB 47* should be unable to incorporate a wild-type marker transduced by particles from a restricted transducing phage suspension.

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METHODS

The nomenclature, materials and methods were essentially those described by Smith-Keary (1960, 1966). The symbol used to represent a transduction is $a(\times)b$, where a is the recipient and b the donor.

RESULTS AND DISCUSSION

proAB 47 was infected with sterile satellited transductant suspensions and also with phage P 22 reared on a wild-type donor; the multisite mutant *proB 25*, in which the deleted region is known not to extend as far as the P 22 prophage attachment site (Miyake & Demerec, 1960) was infected as a control. Table 1 shows that: (a) phage reared on a wild-type donor produced less colonies on *proAB 47* than on *proB 25*, confirming the observation of Miyake & Demerec (1960). (b) The satellited transductant suspensions (e.g. *ES 5*) produced significant numbers of colonies on *proB 25* but few on *proAB 47*. Also, in contrast to *proB 25*, very few of the *proAB 47* transductants (not more than one per plate) were surrounded by satellites; the latter were wild type. This confirms the prediction that *proAB 47* is rarely converted to wild type by restricted phage suspensions.

Table 1. *Infection of multisite mutants with satellited transductant suspensions*

Recipient bacteria	Donor*		Revertants*
	<i>ES 5</i>	<i>WT</i>	
<i>proAB 47</i>	91	123	68
<i>proB 25</i>	338	4800	101
	<i>SC 47-5</i>	<i>WT</i>	
<i>proAB 47</i>	17730†	209	74
<i>proB 25</i>	13950	14940	171

* Numbers of colonies (larger figures approximate) on 4 enriched minimal medium plates (Howe & Dawson, 1968) after 10 days incubation; $c. 5 \times 10^7$ surviving bacteria per plate. *ES 5* and *SC 47-5* are satellited transductant suspensions from an independent proline-requiring mutant (*proB 401*) and from *proAB 47* respectively; *WT*, phage P 22 reared on a wild-type donor. Multiplicity of infection, 0.05 for *ES 5* and *SC 47-5* and 0.5 for *WT*. Each experiment was repeated with independent satellited donors and gave similar results.

† 119 of these 17730 colonies were conspicuously larger than the majority.

proAB 47 and *proB 25* were infected with cell-free suspensions of the rare satellited transductants formed by *proAB 47*. Fragments of donor genomes were now transduced to both auxotrophs at high frequency (e.g. *SC 47-5*, Table 1). A few of the colonies on the *proAB 47* ($\times$) *SC 47-5* plates were conspicuously larger than the majority; these large colonies, and also the *proB 25*($\times$)*SC 47-5*, *proAB 47*($\times$)wild-type and *proB 25*($\times$)wild-type colonies, all grew as stable wild-type clones when subcultured on minimal medium. The smaller colonies from the *proAB 47*($\times$)*SC 47-5* plates, however, segregated mainly auxotrophs and a few slow-growing cells on subculture; the latter, on further subculture, segregated auxotrophs at high frequency. This indicates that the phage from these rare satellited colonies was unable to convert *proAB 47* cells to stable *pro*⁺ transductants, supporting the proposal that the P 22 prophage attachment site is at least partially deleted in this mutant, and consistent with the finding of Smith & Levine (1965) and recent data of Rao & Smith (1968) showing that P 22 does not

lysogenize at its normal attachment site in *proAB* 47. A possible explanation of the large number of unstable transductants is that the satellited donors (e.g. *SC* 47-5) liberated populations of transduction fragments which, while unable to be integrated into the *proAB* 47 genome, could compensate for the deletion in the latter.

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