The Nature of the Transfer Inhibitor of Several F-like Plasmids

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Summary. A model is proposed for the nature of the transfer inhibitor of F-like plasmids, and is shown to be applicable to six of these. In this model, the transfer inhibitor has two components. One, the product of a gene called \( \text{fin} \) (previously \( \text{fi} \) or \( \text{i} \)) is relatively non-specific. The other, called a \( \text{P} \) product, can be recognised by its slow synthesis or action in a newly-infected cell, and is relatively specific. An approximate location for \( \text{traP} \), the cistron coding for the \( \text{P} \) product of Flat, has been found.

There are a number of resistance transfer factors (R factors) and colicinogenic factors (Col factors) which appear to be related to F in that strains carrying them will act as hosts for F-specific phages (Lawn et al., 1967; Meynell et al., 1968). Such plasmids have been called F-like (Meynell et al., 1968). Some F-like plasmids transfer themselves less efficiently than F and are also able to reduce the transfer frequency of an F factor coexisting with them (Watanabe and Fukasawa, 1962; Puig and Nagel de Zwaig, 1964; Meynell et al., 1968). The gene responsible has previously been written as \( \text{i} \) (Egawa and Hirota, 1962) or \( \text{fi} \) (Watanabe et al., 1964); in this paper we shall use the symbol \( \text{fin} \) (for fertility inhibition), in accordance with the recommendations of Demerec et al. (1966).

Finnegan and Willetts (1971) described mutants of Flac in a gene called \( \text{traP} \), also involved in transfer inhibition. Transfer of an Flac \( \text{traP}^- \) mutant was not inhibited by the \( \text{fin}^+ \) R factor R100 in established strains carrying the two plasmids. However, when an Flac \( \text{traP}^- \) mutant was transferred to cells carrying R100 and Fhis, to form a transient population of \( \text{traP}^+/\text{traP}^- \) heterozygous cells, its (immediate) retransfer to an F− R− recipient strain was inhibited. This recessiveness of \( \text{traP} \) mutations showed that \( \text{fin}^+ \) specifies a product required, in addition to the \( \text{fin}^+ \) product of R100, for inhibition of F transfer. Other Flac factors were described which were presumed to be mutant in the site of action of the transfer inhibitor (\( \text{traO} \)); their retransfer from \( \text{traO}^+/\text{traO}^- \) heterozygous cells was not inhibited by R100.

Finnegan and Willetts (1971) further found that when wild-type Flac was transferred to cells carrying R100, its retransfer was not inhibited. However it was inhibited if the cells carried Fhis as well as R100. This again indicated that inhibition of F transfer requires a product specified by F, and moreover, that this is either slowly synthesized or acts slowly. We presume, but cannot prove, that

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this product, designated PF, is specified by the gene traP. The approximate location of traP on the map of Flac (Ippen et al., 1972) is reported in this paper.

On the basis of the results for inhibition of Flac transfer summarised above, we derived the following model for transfer inhibition. Firstly, the transfer inhibitor is formed by the interaction of a non-specific component (the fin+ product) with a plasmid-specific component (the P product). Secondly, the P product is slowly synthesized or interacts slowly with the fin+ product after transfer of the plasmid to a cell not previously carrying it. Thirdly, the transfer inhibitor prevents synthesis or function of (at least) the traJ product, which is essential for transfer; the several possible mechanisms for this have been enumerated previously (Finnegan and Willetts, 1971). We have now tested the applicability of this model for the transfer inhibitor to several other F-like plasmids. Three naturally-occurring Fin− plasmids, F, ColV–K94 (Kahn and Holinski, 1964; MacFarren and Clowes, 1967) and ColVBrp (Fredericq, 1969) and three mutant Fin− plasmids, R1−19 (Meynell and Datta, 1967), R100−1 (Egawa and Hirota, 1962) and R136i−1 (Grindley et al., 1971) were chosen for investigation. The fin+ plasmids tested were ColB–K98 (Puig and Nagel de Zwaig, 1964), R136 (Meynell and Cooke, 1969), R1 and R100.

Our results indicated that in general the fin+ products of the plasmids tested were functionally indistinguishable, although there was one possible exception. Each Fin− plasmid specified a slowly synthesized or slowly acting P product required for inhibition of its transfer, and four functionally distinct groups of these were identified.

Materials and Methods

**Bacterial Strains.** These have been described (Finnegan and Willetts, 1971; Ippen et al., 1972). Relevant phenotypes are: JC3051, His− Trp− Lac− T6 R Str1−; JC5455, His− Trp− Lac− T6 R Str8 Spc8; JC9255, Trp− Lac− T68 Str8 Spc8.

**Phage Techniques.** Sensitivities to the F-specific phages f1, f2, Qβ, M12, and MS2 were determined by spot tests as described by Achtman et al. (1972).

UV irradiated high titre T6 lysates were prepared as described by Achtman et al. (1972). ColVBrp was obtained from Dr. C. Yanofsky, ColV–K94 from Dr. R. C. Clowes, ColB–K98 from both Dr. E. E. M. Moody and Dr. P. Fredericq, R1 and R1−19 from Dr. E. E. M. Moody, R1−19, R100, and R100−1 from Dr. E. Meynell, R136, R100 Tet8 (a tetracycline-sensitive segregant of R100) and R136i−1 from N. D. F. Grindley, and R386 from S. Dennison.

**Media.** These have been described previously by Finnegan and Willetts (1971).

**Mating conditions.** Most of these have been described (Finnegan and Willetts, 1971).

The transfer of plasmids from established strains was measured by mixing 0.2 ml of an exponential phase culture of the donor strain containing 2 × 10^6 cells/ml with 1.8 ml of a similar culture of JC3051. After 30 min, dilutions were plated on the appropriate selective media. The donor population was tested to determine what proportion carried the appropriate plasmid(s) (usually > 96%) and the results corrected accordingly.

Progeny carrying Flac, Fhis or ColVBrp were selected by means of the Lac+, His+, or Trp+ phenotypes which they conferred, respectively. Progeny carrying R100, R100−1, or R136 were selected by their resistance to tetracycline (20 μg/ml); R1 or R1−19 by their resistance to ampicillin (25 μg/ml); and R100 Tet8 by their resistance to chloramphenicol (100 μg/ml). R100 and R136 do not determine ampicillin resistance, and R1 does not determine tetracycline resistance. Transfer of ColB–K98 was measured by plating the mating mixture on nutrient agar containing streptomycin (to which the donor strain was sensitive). After overnight growth, the cells were killed by exposure to chloroform vapour, and those