

## The restriction of phage T3 by certain strains of *Escherichia coli*

By J. SCHELL,\* S. W. GLOVER, K. A. STACEY,  
P. M. A. BRODA AND N. SYMONDS

*Medical Research Council, Microbial Genetics Research Unit,  
Hammersmith Hospital, London, W.12.*

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The efficiency of plating (e.o.p.) of phage T3 is equal on the *E. coli* strains B and C, and also on most F<sup>-</sup> strains of K12 (afterwards called K). However, in strains of K which carry the F episome its e.o.p. is greatly reduced. On most F<sup>+</sup> strains of K its e.o.p. is about 10<sup>-5</sup>. The phages which do multiply in the F<sup>+</sup> bacteria are not modified; they still have an e.o.p. of 10<sup>-5</sup> when replated on the same host. The actual fraction of cells which accept T3 phages is approximately 10<sup>-2</sup>, and these cells yield a small burst of unmodified T3. It seems to be this combination of restriction coupled with a small burst which defines the level of the e.o.p. and produces the typical small plaques which are observed on F<sup>+</sup> strains.

As a result of screening a large number of male and female strains of K two striking exceptions were found to the observation stated above. The F<sup>+</sup> strain W1485 was found to plate T3 efficiently, while some F<sup>-</sup> derivatives of 58-161 plate T3 with a low e.o.p. In an effort to elucidate this situation a series of conjugation experiments was performed to establish the relationship between F and T3 restriction. The results of these experiments are presented in Table 1.

The first three crosses show that the property of T3 restriction is transferred with F, is not transferred in the absence of F transfer, and is not transferred from F<sup>-</sup> to F<sup>+</sup> cells. Cross 4 demonstrates that the episome F-lac (Jacob & Adelberg, 1959) transfers T3 restriction in a similar way to F. The crosses 5 and 6 show that T3 restriction can be transferred into *coli* C and *coli* B. In *coli* C, however, the e.o.p. of T3 is reduced only to 10<sup>-1</sup> and not to 10<sup>-5</sup> as in F<sup>+</sup> strains of K. The fact that this reflects some property of the host is demonstrated by cross 7, in which the F factor from *coli* C is transferred back into F<sup>-</sup> cells of K when the e.o.p. of the KF<sup>+</sup> recipients is again 10<sup>-5</sup>. It was not possible to demonstrate the transfer of F to the B cells that restrict T3 by testing with the male-specific phage, nor, as shown by cross 8, could the T3 restriction be transferred back to K.

The next two crosses involve W1485, which is the F<sup>+</sup> strain which does not restrict T3. It can be seen (cross 9) that T3 restriction can be fully expressed in this strain if infected with the F from a normal F<sup>+</sup> strain. And, conversely (cross 10), when F is transferred from W1485 F<sup>+</sup> to a normal K strain, none of the recipient F<sup>+</sup> cells restrict T3.

Although the last two experiments indicate that the property of T3 restriction may be separated from F, other experiments confirm that normally the association is close. If F<sup>+</sup> strains are 'cured' of their F episome by incubation in acridine orange (Hirota, 1960), the ability of these strains to restrict T3 is simultaneously lost. Also in crosses between Hfr Cavalli (Cavalli, 1950) and W945F<sup>-</sup>, it was only those recombinants that had received a late marker that had the ability to restrict T3; and all these recombinants were male.

\* Present address: Laboratory for Microbiology, Faculty of Sciences, State University, Ghent, Belgium.

One explanation of these findings is that the ability of F<sup>+</sup> strains to restrict T3 is controlled by a gene in the F episome. On this view male strains which do not restrict T3 must carry a mutant form of F, and female strains which restrict T3 must have retained the gene controlling restriction while losing the other recognizable F properties. Alternatively, T3 restriction is controlled by another cytoplasmic factor present in most male strains and absent from most female strains, and which cannot mediate its own transfer.

Table 1. *Approximately equal numbers of donor and recipient bacteria were mixed together and incubated at 37° with gentle aeration for one hour. After dilution aliquots were plated on media selective for the recipient cell type. Purified colonies were tested for the presence of F with the male-specific phage  $\mu$  (Dettori et al. 1961). The symbol T3<sup>r</sup> denotes strains which have reduced e.o.p. for phage T3; T3<sup>-</sup> denotes strains which plate T3 with an e.o.p. of one.*

| Cross | Type of cross   | Donor                   | Recipient           | Number of exconjugant recipients tested | Per cent F <sup>+</sup> | Per cent conjugants which restrict T3 |                |
|-------|---|-------------------------|---------------------|---|-------------------------|---------------------------------------|----------------|
|       |   |                         |                     |   |                         | F <sup>+</sup>                        | F <sup>-</sup> |
| 1     | KF <sup>+</sup> T3 <sup>r</sup> × KF <sup>-</sup> T3 <sup>r</sup> | 58-161F <sup>+</sup>    | W945F <sup>-</sup>  | 60                                      | 87                      | 100                                   | 0              |
| 2     | KF <sup>-</sup> T3 × KF <sup>-</sup> T3 <sup>r</sup>              | 58-161F <sup>-</sup>    | C600F <sup>-</sup>  | 52                                      | 0                       | —                                     | 0              |
| 3     | KF <sup>-</sup> T3 <sup>r</sup> × KF <sup>+</sup> T3 <sup>r</sup> | 58-161F <sup>-</sup>    | W1485F <sup>+</sup> | 56                                      | 100                     | 0                                     | —              |
| 4     | KF-lac T3 <sup>r</sup> × KF <sup>-</sup> T3 <sup>r</sup>          | W1845F-lac <sup>+</sup> | C600F <sup>-</sup>  | 60                                      | 100                     | 100                                   | —              |
| 5     | KF <sup>+</sup> T3 <sup>r</sup> × CF <sup>-</sup> T3 <sup>r</sup> | 58-161F <sup>+</sup>    | CF <sup>-</sup>     | 60                                      | 100                     | 100                                   | —              |
| 6     | KF <sup>+</sup> T3 <sup>r</sup> × BF <sup>-</sup> T3 <sup>r</sup> | 58-161F <sup>+</sup>    | BF <sup>-</sup>     | 56                                      | 0                       | 0                                     | 30             |
| 7     | CF <sup>+</sup> T3 <sup>r</sup> × KF <sup>-</sup> T3 <sup>r</sup> | CF <sup>+</sup>         | C600F <sup>-</sup>  | 42                                      | 7                       | 100                                   | 0              |
| 8     | B T3 <sup>r</sup> × KF <sup>-</sup> T3 <sup>r</sup>               | BT3 <sup>r</sup>        | C600F <sup>-</sup>  | 50                                      | 0                       | —                                     | 0              |
| 9     | KF <sup>+</sup> T3 <sup>r</sup> × KF <sup>-</sup> T3 <sup>r</sup> | 58-161F <sup>+</sup>    | W1485F <sup>-</sup> | 20                                      | 50                      | 100                                   | 0              |
| 10    | KF <sup>+</sup> T3 <sup>r</sup> × KF <sup>-</sup> T3 <sup>r</sup> | W1485F <sup>+</sup>     | C600F <sup>-</sup>  | 20                                      | 40                      | 0                                     | 0              |

It is not yet resolved whether the restriction of T3 discussed in this paper, which is recognized by a reduced e.o.p. of T3 on male strains, is due to a reduced ability of the phage to absorb or inject its DNA into male bacteria, in which case it would be due to a difference in the properties of the cell surface between male and female strains (an explanation proposed by Dettori *et al.* (1961) for the reduced e.o.p. of the phage  $\phi$  on F<sup>+</sup> strains); or whether it is due to restriction in the sense described by Arber & Dussoix (1962), in which the T3 DNA would successfully enter the male cells, but would then in some way be prevented from multiplying.

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