Genetic Transfer in *Mycobacterium phlei*

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(Accepted for publication 4 November 1970)

Transfer of genetic information in the genus *Mycobacterium* has been reported (Juhasz, 1960; Redmond, Ward & Wright, 1967) but the role of phage in such transfers could not be established. We would like to report on genetic transfer in *M. phlei* in which transducing phage Bo 2 plays a major role.

The donor strain, *Mycobacterium phlei* SN109, obtained from the Borstel Culture Collection, Borstel, West Germany, could grow on D-xylose (*xyl*⁺) when this sugar was present as the sole carbon source, and was sensitive to 1.0 µg./ml. streptomycin (*str-s*). The recipient strain, a mutant of *M. phlei* F89 resistant to 100 µg./ml. streptomycin (*str-r*), could not utilize D-xylose (*xyl*⁻) as a carbon source. Original *M. phlei* F89 was obtained from S. Froman, Olive View Hospital, Olive View, California. The phage employed was mycobacteriophage phlei Bo 2, originally designated B2, isolated from dung and propagated on *M. phlei* F89 (Juhasz & Bönicke, 1965). The selective medium chosen for these experiments was a minimal medium (Gordon & Mihm, 1959) containing 1% D-xylose (Pfanstiehl), to which 50 µg./ml. FeCl₃ and 50 µg./ml. streptomycin sulphate (Sigma) were added. Several controls were employed. These consisted of bacteria treated with (i) heat-killed phage (autoclaved at 15 lb. pressure for 15 min.); (ii) phage and DNase (Calbiochem); (iii) heat-killed (autoclaved) phage and DNase; and (iv) sterile heart infusion broth (Difco) in place of phage.

In our experiments phage Bo 2 was propagated on the donor strain *Mycobacterium phlei* SN109 (*xyl*⁺, *str-s*), harvested, filtered and tested for bacterial sterility. Sterile phage preparations were then used to infect the recipient *M. phlei* F89 (*xyl*⁻, *str-r*). Various multiplicities of infection were tried. Positive results were obtained with multiplicities of infection of 1.0 and 2.0, the results being proportional to the phage concentrations employed. However, if the phage concentration was too high, background bacterial growth, stemming from nutrients present in the phage preparation itself, made isolation of *xyl*⁺ transductants difficult. The phage-bacterium mixture was incubated at 37° for 48 h. and subsequently inoculated on to the selective medium. Growth was recorded after 4 weeks. In order to insure that mutants could in fact utilize xylose and that bacterial growth had not been due to nutrients in the phage inoculum, randomly selected colonies from each experiment were washed twice in physiological saline and reinoculated on to the selective medium. All the colonies thus tested were able to grow on xylose as sole carbon source. Phage conversion did not seem to account for the emergence of the *xyl*⁺ character since infection with phage Bo 2, which has been propagated previously on F89 (*xyl*⁻, *str-r*) did not convert F89 (*xyl*⁻, *str-r*) to *xyl*⁺.

Transfer of the *xyl*⁺ marker by *transduction* is firmly supported by the data from four transfer experiments summarized in Table 1. The larger number of prototrophic colonies obtained with live as opposed to heat-killed phage, the proportionality of the number of such colonies to the number of phage employed, and, finally, the transfer activity retained by DNase-treated phage are major characteristics of the known transduction systems. The peculiarity of the transfer system presented in this paper was,

Table 1. *Transfer of the xyl*⁺ character to *Mycobacterium phlei* F89 (*xyl*⁻, *str-r*)

Experiment*	A	B	C	D
Phage input/ml. (plaque-forming units)	1.2 × 10 ⁸	3 × 10 ⁸	8 × 10 ⁸	4 × 10 ⁸
Multiplicity of infection	1.2	1.0	2.0	1.0
1. Bacteria and phage mixture	217	481	1031	451
2. Bacteria and phage and DNase mixture	—	411	—	367
3. Bacteria and heat-killed phage mixture	—	84	343	108
4. Bacteria and heat-killed phage and DNase mixture	—	—	56	—
5. Bacteria and sterile heart infusion broth	13	—	38	—

* Numbers in columns refer to the number of colonies obtained on 20 plates containing 0.1 ml. amounts of the incubation mixture/plate (in the presence of xylose as sole carbon source).

however, that heat-killed phage preparations retained part of the original transfer activity. Upon treatment with DNase, this residual activity of heat-killed phage disappeared and the number of spontaneous revertants was comparable with that found when heart infusion broth replaced phage. Since the heat-killed phage appeared to retain the transfer activity lost when the phage preparation was treated with DNase (sum of lines two and three of Table 1 compared with line one) one can hardly escape the conclusion that the observed residual activity was due to transformation by bacterial DNA. Direct evidence for transformation to *xyl*⁺ of *Mycobacterium phlei* F89 will be obtained only upon isolation of biologically active DNA from either donor SN109 or from phage Bo2 which has been propagated previously on SN109.

ADDENDUM

After this paper was submitted for publication an article appeared in *Nature, London*, on 'Transduction in *Mycobacterium smegmatis*'. (C. V. Sundar Raj & T. Ramakrishnan, 1970, *Nature London* **228**, 280.)

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