MUTANT MALE STRAINS WITH AN ALTERED NUCLEIC ACID PUMP+

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The very different male-specific bacteriophages f1 and f2 both adsorb to F-pili but at different sites - f1 to the tip and f2 to the sides (Caro and Schnös, 1966; Ippen and Valentine, 1966). Although adsorbing at different sites, the two viruses appear to use a similar mechanism for injecting or penetrating their nucleic acid into the male cell (Ippen and Valentine, 1966). We have speculated (Ippen and Valentine, 1966) that the male bacterium possesses a type of "nucleic acid pump" responsible for penetrating the viral nucleic acid into the cell.

Recently we have reported the isolation of male mutants which may have an altered pump (Fig. 1). The DNA of f1 penetrated these mutants, but f2 RNA did not. It was of some interest that Qβ RNA penetrated the mutants (Silverman et al., 1967).

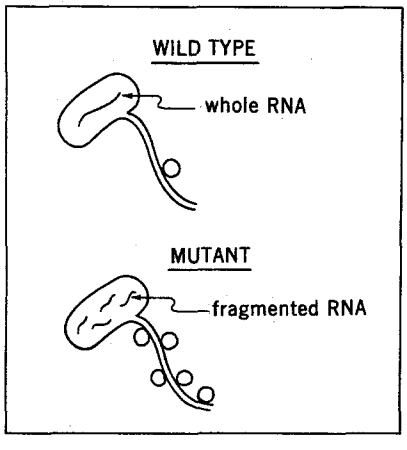

Fig. 1. At high phage concentrations, mutant males may take up noninfectious segments of f2 RNA.

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We have continued to study the defect in these male mutants and find: a) f2 RNA injection is inhibited in the mutants, b) It is possible to "force" penetration using high virus multiplicity, c) Most of the f2 RNA entering the mutant at high multiplicity is noninfectious.

Results and Experimental

The Male Mutants. Several more mutant male strains sensitive to f1 but resistant to f2 were isolated (Silverman et al., 1967). The plating efficiencies of different male-specific viruses on these strains are listed in Table 1. Note from the table that M127 plates RNA phage Qβ with much lower efficiency than the other strains; f1 also plates less well on this strain. Although M127 may represent another mutant class it is also possible that secondary mutations may be responsible for its altered phage plating pattern. The similar phage plating pattern of the other mutants suggests but does not prove that the same gene may be affected in each mutant.

Table 1

Plating Efficiencies of Male-Specific Phages on Mutant Males*

Phage Used	Plating Efficiencies							
	HfrC	Mutant 1	27	52	66	127	129	133
f1	1.0	0.8	0.7	0.85	1.0	0.5	0.9	0.8
Qβ	1.0	1.25	0.9	1.1	0.65	0.1	1.1	1.16
f2	1.0	$<10^{-9}$	$<10^{-9}$	$<10^{-9}$	$<10^{-9}$	$<10^{-9}$	$<10^{-9}$	$<10^{-9}$

*Mutant male strains isolated from E. coli HfrC as described earlier (Silverman et al., 1967).

RNA Injection is Inhibited in the Mutants. We had previously shown, using radioactive phage assays, that f2 adsorbs normally but does not penetrate its RNA into the mutant males (Silverman et al., 1967). We have now carried out a type of phage "eclipse" experiment (Table 2) which shows that the mutant is blocked at the RNA injection stage of infection.

Presumably the injection of the RNA into the core of the pilus is retarded in the mutant. Note from Table 2 that mutant strain M27 is unable to eclipse f2 particles. The phage-cell complexes do not lose their infectivity in the presence of chloroform, which disrupts the complexes. The phage appear to remain adsorbed to the surface of the F-pilus of the mutant and apparently have not begun injection since infectious particles can be recovered. We have previously shown that simple adsorption of the phage to the F-pilus does not destroy infectivity (Valentine and Strand, 1965). We have also found that f2-M27 complexes are neutralized by anti f2 antibody while plaque forming units in the wild type strain are resistant and that f2 competes poorly with f1 penetration in M27 (Ippen and Valentine, 1966). Both of these observations support our contention that the injection phase of f2 penetration is affected in mutant male cells.

Table 2

Mutant Cells Do Not Eclipse f2

Time After Infection (min)	% Phage Remaining*	
	M27	W1895
0	100	100
5	90.5	73.5
10	100.5	39.5

*Ave. of 2 experiments.

Mutant male strain and parent (HfrC) infected with 0.1 phage per cell. Eclipse of phages was measured by removing infected cells and adding chloroform at times indicated and plating for phages which had not injected. Note lack of phage eclipse by mutant.

Forcing RNA Penetration. We have observed in single burst experiments that about 0.1% of the mutant male cells produce a normal burst of phage particles. The cells which became infected were not revertants. These experiments suggested that intact phage RNA could penetrate the mutant cells at a low frequency. Using radioactive phage we have assayed penetration at varying multiplicities of infection to determine whether phage RNA could

be forced into these mutants. Fig. 2a shows that at a multiplicity of 100 the mutant cells take up phage RNA about half as well as the wild type parent, while at a multiplicity of one the mutant cells are almost impenetrable.

Since the radioactive assay does not distinguish infectious RNA, we assayed infective centers over the same range of multiplicities as in the penetration experiment. Fig. 2b demonstrates that even at a multiplicity of 100 the mutant cells give only 1-2% the number of infective centers found in wild type cells.

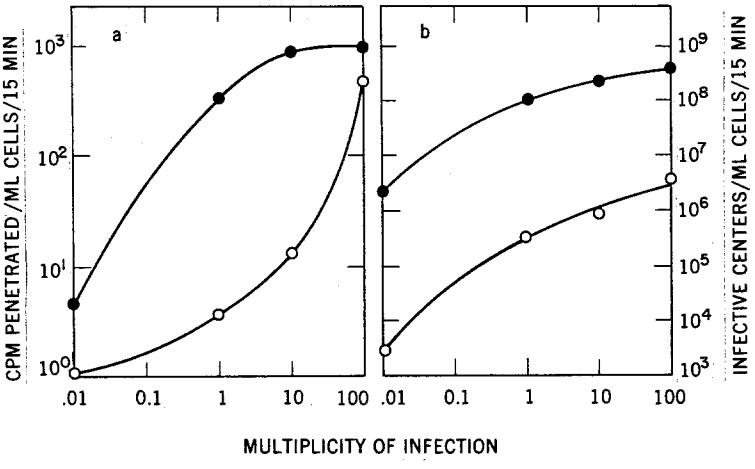

Fig. 2a. Penetration of radioactive RNA into mutant and wild type male cells of E. coli as a function of multiplicity of infection. The penetration assay has been described (Valentine and Wedel, 1965).

Fig. 2b. Formation of infective centers in mutant and wild type male cells of E. coli as a function of multiplicity of infection. Cells were infected at desired multiplicity and 15 min after infection f2-antibody was added to neutralize free phage. Aliquots were diluted and plated on a suitable indicator strain of E. coli.

We repeated these experiments assaying total yield of phage. Since infected mutant cells give a normal burst of phage (Silverman, unpublished), the yield of viable phage per radioactive RNA equivalent penetrated gives the fraction of penetrated RNA which is infectious. These results are shown in Table 3. The infective center data are included for comparison.

The agreement between the two methods is good and each demonstrates that at a high multiplicity when the mutant cells take up phage RNA almost as well as wild type cells, more than 90% of this RNA is not infectious, i.e., yields netiher infective centers nor viable phage particles.

Table 3

Penetration of Noninfectious RNA*

Strain	Burst/Radioactive RNA Equiv. Penetrated**		Infective Centers/ml Cells	
	m.o.i. = .01	100	.01	100
M27	6.6	113	3.4×10^3	4×10^6
HfrC	4100	3260	2.2×10^6	2.5×10^8

*Data from Fig. 2.

**Average burst size calculated from total phage yield and number of RNA molecule equivalents penetrating the cells calculated from radioactivity measurements.

We conclude that RNA can be forced into mutant cells by raising the multiplicity, but that most of this RNA is noninfectious. It is possible that this RNA has been fragmented while penetrating the mutant cell. The cellular fate of this RNA is presently being studied.

Discussion

In an earlier communication (Ippen and Valentine, 1966), we introduced the notion of a viral "nucleic acid pump" to account for observations on the penetration of male-specific phage nucleic acids into male cells. We stressed the active role of the male cell in this process and the function of the F-pilus in nucleic acid transport. It is of some interest that even fragments of viral nucleic acid enter the cell by this mechanism (Fareed et al., 1966). We proposed that the male cell has a common mechanism for penetration of all male-specific viral nucleic acid. It is this mechanism of transport in which we are presently interested.

A study of more mutants may shed some light on the nature of this pump. The mutants studied in this and in a previous communication (Silverman

et al., 1967) appear to be affected in an early stage of f2 penetration, the injection of RNA into the pilus. Of interest is the finding that Qβ infection is not detectably affected by a mutation which essentially precludes f2 infection (Silverman et al., 1967). We feel that this fact argues for a type of specificity in the pump, probably at some early extracellular stage of infection (Valentine and Wedel, 1965). While the specificity of the pump seems to be altered in this set of mutants, its function seems to be largely, but not completely, intact. Qβ penetrates normally and f2 can be made to penetrate in near normal amounts by raising the multiplicity of infection. However, the fact that most of the f2 which penetrated was not infectious suggests that the function of the pump has been somewhat altered. The role of the sex hair or F-pilus in the pump is of considerable interest. We speculate that the F-pilus is a vital part of the pump and that the mutants described have altered F-pili. With the information presently available it may be possible to settle this question by direct isolation of pili-nucleic acid complexes.

Acknowledgment

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