THE NUCLEIC ACID PUMP OF THE MALE BACTERIUM*

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One of the interesting features of the sex factor of E. coli is that it directs the synthesis of receptor sites and an active penetration system for the nucleic acids of certain male-specific viruses (Valentine, 1966). These tiny bacteriophages appear to possess a relatively primitive type of injection mechanism and must rely heavily on the male bacterium for injection of their nucleic acids. Aged, chilled, or drug inhibited male cells adsorb but do not penetrate phage nucleic acid. It has been proposed that the sex hairs (F-pili) of male cells may serve as "tails" for male-specific phages (Valentine and Wedel, 1965). Recent evidence supports this view and suggests that the sex hairs (F-pili) serve as receptor sites for both classes of male phage--the spherical RNA phages (Valentine, Wedel, and Ippen, 1965) and the thread-shaped DNA phages (Caro and Schnös, in Press, 1966). F-pili are also thought to play an active role in the penetration of the viral nucleic acid, although no direct evidence is yet available on this point.

In this communication we show that the two morphologically distinct classes of male phages--the spherical and thread-shaped viruses--share a common pathway for penetration of their nucleic acids across the male cell membrane. The essential experiments involved the blocking or competition of penetration of one form of viral nucleic acid with the other. Penetration or injection but not adsorption was competitive between the two viruses. In this connection it is interesting that the two viruses

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appear to adsorb initially to different portions of the F-pilus--f2 to the sides (Valentine and Strand, 1965) and f1 to the end or lip of the pilus (Caro and Schnös, 1966). Injection of the rod-shaped phage f1 took place normally if penetration of the competing RNA phage was prevented by addition of RN'ase.

These experiments have led us to speculate that the male cell possesses a type of active transport system or "nucleic acid pump" for concentration of viral nucleic acids.

Results and Experimental

Methods. Male specific bacteriophages f1 and f2 were grown on a suitable E. coli K12 male strain (C-600). Radioactive (P^{32}) phages with an initial specific activity of about 1 cpm per 10^5 viable particles were prepared and purified as described previously (Valentine and Strand, 1965). The purification procedure for f1 was similar to that for f2 except for the final CsCl steps in which f1 bands at a buoyant density of about 1.29. The methods for measuring the extracellular stages of f2 infection and determination of F-pili were described in previous papers (Ippen and Valentine (1965); Valentine and Wedel (1965); Valentine, Wedel and Ippen (1965)). The methods described by Tzagoloff and Pratt (1964) for measuring adsorption and penetration of phage M13 (f1) were modified for use with radiophosphorus-labeled virus. Because of the filamentous nature of f1, adsorption could not be followed by the filtration technique used for f2. Instead, adsorption of radioactive phage to male cells was measured by determining the radioactivity sedimenting with the male cells during centrifugation. One centrifugation cycle of the male cells appeared to lower the background sufficiently for meaningful measurements. Since adsorption continues even at $0^{\circ}C$, adsorption was terminated by rapid centrifugation of the male cell suspension. After careful draining, the packed cells were suspended in 5% TCA, filtered on glass membrane pads, and counted for adsorbed

radioactivity.

Penetration of f1 DNA into the cell was measured by a procedure similar to that described for f2 (Valentine and Wedel, 1965). Radioactive virus was incubated with male cells in broth at 37°C to allow penetration to occur. Samples were then chilled at 0°C to prevent further penetration, and centrifuged to remove unadsorbed particles. The cell pellet was resuspended in saline solution and blended for 1 min at approximately 1/2 maximum speed on the Servall "Omni-mixer" to remove adsorbed particles which had not yet injected their DNA. The blended cells, freed of phage adsorbed to F-pili, were collected by centrifugation and counted for radioactivity as above.

f1 and f2 Both Adsorb to F-pili but at Different Sites. The first experiments were designed to test whether F-pili were required for adsorption of f1 to male cells. Tzagoloff and Pratt (1964) had earlier shown that the primary adsorption sites for M13 (f1) were highly susceptible to shear and were readily removed by blending of the male cells. We have repeated these experiments using radioactive f1 (Fig. 1) and found that blending of male cells for 1 min removed more than 95% of the f1 receptor sites--a result similar to that observed for RNA phage f2 (Valentine, Wedel and Ippen, 1965). Caro and Schnös (1966) have recently shown by electron microscopy that free F-pili present in the supernatants of male cultures adsorb f1. From these data it can be argued that F-pili serve as the receptor sites for both classes of male phages.

It is also apparent from the electron micrographs of Caro and Schnös (1966) that the filamentous phage f1 and the RNA phage f2 adsorb to different portions of the F-pilus--that is, these two male-specific viruses, while both adsorbing to F-pili, do not actually share the identical attachment or receptor sites on the F-pilus. We have carried out a simple competition experiment to test this idea. It was reasoned

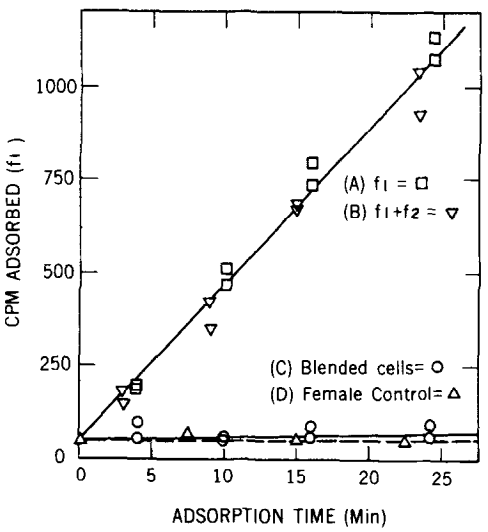

Fig. 1. Adsorption of radioactive (P^{32}) f1 to male cells--F-pili requirement and lack of competition with RNA phages. See methods section for description of adsorption assay. Radioactive f1 (4000 cpm per ml culture) was added to a male culture chilled to 0°C, at a cell density of approximately 10^9 per ml. Duplicate aliquots of 4 ml of infected cells were taken at the times shown and centrifuged immediately to stop further adsorption. Curve A) Normal adsorption curve. Curve B) Nonradioactive f2 added (5×10^{10} f2/ml); note that f2 did not block f1 adsorption. Curve C) Male cells blended for 1 min to remove F-pili; note drastic reduction in f1 adsorption. Curve D) Female control.

that if both f1 and f2 shared the same receptors on the F-pilus a large excess of nonradioactive f2 would compete for the sites and effectively block radioactive f1 particles from adsorbing. As shown in Fig. 1 (compare curves A and B), even with a large excess of "cold" f2 present, radioactive f1 adsorbed normally concurring with the idea that the viruses have different receptors on the F-pilus. In contrast to this lack of competition for adsorption sites we will show below that strong competition occurs between the two viruses at the nucleic acid penetration level, suggesting that the same mechanism operates in the transport of both types of nucleic acid into the cell.

Competition at the Transport Level--Radioactive f1 DNA by Cold RNA. The "F-pilus Theory" (Brinton, 1965) for active transport of

nucleic acids by the male cell suggests that F-pili play a direct role in movement of nucleic acids across the cell membrane. Since both viral RNA and DNA molecules appear to be actively "concentrated" by the male cell, we reasoned that the two forms of viral nucleic acid should compete with each other for transportation across the membrane. As demonstrated in Fig. 2a and 2b, strong competition did occur between the two forms of viral nucleic acids.

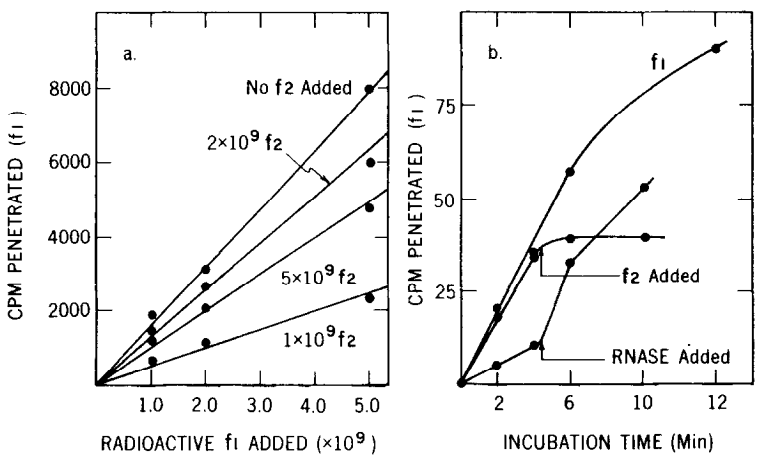

Fig. 2a. Competition at the Penetration Stage--f2 Interference with f1. See methods section for penetration assay. Radioactive f1 was added in the quantities shown to a volume of 5 ml of male cells (6×10^8 cells per ml). Note marked interference by nonradioactive f2 added in various concentrations as shown on the curves.

2b. Release of f2 Inhibition by RN'ase. For each curve, 2×10^9 radioactive f1 [this particular radioactive preparation had a much lower specific activity than for curve A] was added to 15 ml of cells (2×10^8 per ml) and 3 ml aliquots were removed at times indicated. About 10^{11} nonradioactive f2 particles (per ml) were added to block f1 penetration at the time indicated (arrow). Note in the lower curve (f2 added simultaneously with f1) that addition of RN'ase (arrow) allowed f1 penetration to resume.

As shown in Fig. 2a, RNA of phage f2 effectively blocked the transport of radioactive f1 DNA into the male cell. For example, addition of 1×10^{10} f2 inhibited penetration of f1 approximately 67%. It was of interest that f2, added even some minutes after f1, rapidly blocked

f1 penetration (Fig. 2b); it should be noted that a large number of f1 phage should have adsorbed prior to the addition of f2, again indicating that f2 RNA enters by a "side" route and does not compete by blocking f1 adsorption. Addition of RN'ase (Fig. 2b; lower curve), shown previously to completely block RNA penetration (Valentine and Wedel, 1965), markedly reduced the inhibitory effect of f2 and allowed f1 DNA to enter normally. Again it should be pointed out that RN'ase has no effect on f2 adsorption but strongly inhibits the injection of viral RNA (Valentine and Wedel, 1965). The effect of RN'ase then is to totally prevent penetration of RNA into the cell apparently by degrading the viral strand while it is briefly exposed during the injection process. It appears that once the penetrating viral RNA strand has broken down, viral DNA penetration can proceed.

In order to rule out the possibility that f2 blocked f1 by producing some type of "immunity substance" in the cell, the above competition experiments were repeated in the presence of chloramphenicol to prevent induction of any virus-specific proteins (immunity substances) by f2 RNA. The results of these experiments were essentially the same as those reported, and no evidence for a strong "immunity" was obtained. In addition, the rapid effect of f2 on f1 penetration makes it appear unlikely that phage immunity could have been generated during this short-time period.

Penetration of Radioactive f2 RNA--Blocking by f1. In an experiment similar to those above, nonradioactive f1 was found to effectively block f2 RNA penetration (Fig. 3). Competition was not as strong as in the reverse case above, and f1 appeared to be most competitive when added a short time before f2--a time which presumably allowed f1 to adsorb and initiate its own injection process. This is in agreement with the fact that f1 possesses only a few adsorption sites on the end of the F-pilus whereas f2 may adsorb and presumably penetrate at numerous sites along the F-pilus.

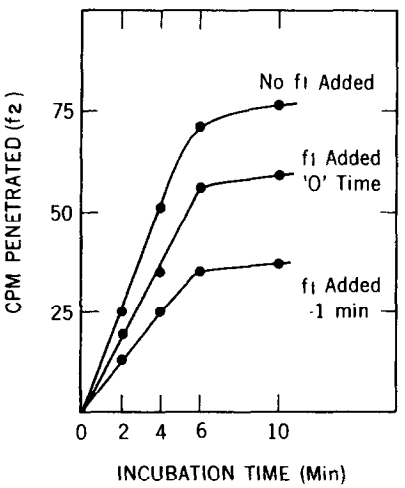

Fig. 3. Rod-shaped phage f1 blocked penetration of radioactive f2 RNA. Penetration of radioactive f2 RNA was measured as described in the text for f1. Cold f1 (5×10^{10} per ml cell culture) was added simultaneously with (0 time), and 1 minute before infection with f2 (-1 min), as indicated on the curves. Samples of 2 ml were removed; f2 concentration was 2×10^{9}/ml (~1000 cpm).

Discussion: The Nature of the Pump. The experiments reported here support the F-pili model for active transport of nucleic acids across the membrane of the male cell. In this model, originally proposed by Brinton et al. (1965), the nucleic acids of the male-specific viruses are somehow injected into the hollow core of the F-pilus (sex hair) and then transported into the cell. The critical experiment is, of course, to prove that the viral nucleic acid is actually injected directly into the F-pilus. As far as we are aware this experiment has not yet been reported. We feel that the experiments reported above, although still indirect, strongly support such a model. First of all, it was interesting that two morphologically distinct viruses attached to the same sex hair or F-pili--though at different sites. The competition experiments strongly suggest that a common penetration mechanism operates for both forms of nucleic acid and perhaps even for nucleic acid transport during mating.

The common feature of all these systems is obviously the F-pilus

itself. Thus, once again, attention is focused on this unique bacterial filament and its function as part of an active nucleic acid pump of the male bacterium. It is interesting to speculate that other cellular metabolites might have similar "pumps." The existence of other types of pili (Brinton, 1965) suggests the possibility that these hairlike appendages may be produced by the cell to function in the active uptake or transport of various compounds. The possible relationship of the cellular permeases to this type of pump system is an interesting question.

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