THE EXTRACELLULAR STAGES OF RNA BACTERIOPHAGE INFECTION

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The small bacteriophages such as f2 pose a special problem since they do not possess visible tails for attachment and transport of their nucleic acid into the cell. We have recently presented evidence which suggests that the F-pilus of male cells may serve the role as "tail" for these viruses (Valentine et al., 1965). Continued investigation of this problem indicates that several separate stages are required for RNA injection. We refer to these steps here as the extracellular stages of infection. The hypothetical scheme shown in Fig. 1 shows that the extracellular stages begin with adsorption and terminate with penetration of the phage RNA into the cell. The scheme of Fig. 1 has served as a working hypothesis for the present work and evidence supporting the different steps will be discussed.

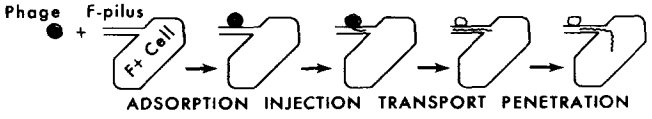

Fig. 1. Hypothetical scheme for extracellular stages of RNA bacteriophage infection. See text for details.

Results and Experimental

Adsorption Step. In an earlier communication (Valentine and Strand, 1965), we reported that RNA phage f2 readily adsorbed to free or fragmented segments of F-pili forming F-pili-phage complexes. The complexes were formed with partially purified F-pili even at 0°C showing that cellular metabolism (energy) was probably not required for simple adsorption. Disruption of F-pili-phage complexes by blending resulted in release of viable phages from the complex, indicating that their RNA was not injected into the core of the F-pilus. Treatment of F-pili-phage complexes with RN'ase neither destroyed the viability of the phage nor disrupted the complex. A similar adsorption step has been observed "in vivo" (Valentine et al., In Press). Taking into account the above experiments, we conclude that the first step in f2 infection is the simple adsorption of phage particles to F-pili.

Injection or RNase Sensitive Step. A second type of experiment to probe the mechanism of phage injection involved the use of ribonuclease (RN'ase). It was found earlier by Loeb (1961) that a culture did not become infected if small quantities of RN'ase were added to the medium prior to infection. In a further study of the mechanism of RN'ase action we have found that the infecting RNA strand (P^{32}-labeled) was degraded if RN'ase was present during the early stages of infection. A likely interpretation was that phage RNA had become exposed to RN'ase during the RNA injection stage. Fig. 2A shows the effect of RN'ase on RNA injection. In this experiment a culture of E. coli K-12 (F^+) grown on Tryptone-Yeast Extract-Calcium Broth (Loeb and Zinder, 1961) to a concentration of 4×10^8 cells/ml was chilled to 0°C and infected with radioactive phage. After 10 min for adsorption of phage the culture was divided into two portions. RN'ase (40 µg/ml) was added to one sample and the other sample without RN'ase served as control; the cultures were next incubated at 37°C to start the injection process. Samples of 0.4 ml were removed at different

times and assayed for adsorbed phage by the membrane filtration method (Ippen and Valentine, In press). As seen in Fig. 2A, when phage-cell complexes formed at 0°C were warmed to 37°C the RN'ase sensitive stage set in rapidly beginning within 1.5 min after raising the temperature to 37°C. Within 5 min more than 60 per cent of the phage-cell complexes have been disrupted. We have named this RN'ase sensitive step the Injection stage of infection. Viable phage particles alone were not degraded by RN'ase. In another experiment (Fig. 2B) RN'ase and radioactive phage were added simultaneously to a culture incubated at 37°C without a period for pre-adsorption of phage. Two stages were again evident; at first the number of adsorbed phage increased rapidly (adsorption phase); an optimum level was reached after 3 min and then a

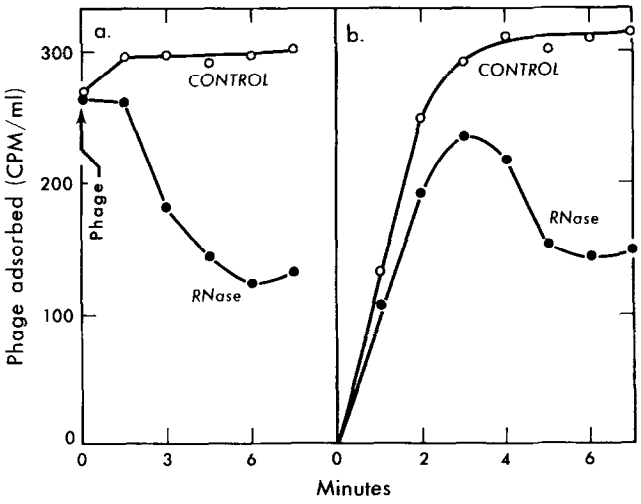

Fig. 2A. The RN'ase sensitive stage of RNA-phage infection. Assay conditions as in text.

Fig. 2B. Conditions similar to Fig. 2A except radioactive phage and RN'ase were added simultaneously and incubation was at 37°C. In the RN'ase treated sample (adsorption) note the initial increase of adsorbed phage followed by the decline in the number of adsorbed particles (injection or RN'ase sensitive stage).

decline occurred (injection phase) as RN'ase began to actively digest the complexes. The adsorbed radioactive phage particles which appeared to escape RN'ase (see Fig. 2) degradation (usually 30-60 per cent depending on the experiment) were probably particles attached to free F-pili fragments or exceptionally long or otherwise inert F-pili. An experiment supporting this idea is summarized in Table I.

TABLE I

THE EFFECT OF RN'ASE ON PENETRATION OF PHAGE RNA

Phage-cell complexes were preformed at 0°C, warmed to 37°C, treated with RN'ase (40 μg/ml) at the times indicated and incubated for a total of 15 min. The samples of 1 ml were diluted into 20 ml water at 0°C to stop further RNA penetration and blended for 2 min to remove extraneously adsorbed phage. The blended cells containing radioactive RNA were collected by centrifugation, precipitated with 5 per cent cold trichloroacetic acid (TCA) and counted after collection of the cells by filtration using glass filter pads. A control without RN'ase was incubated for 15 min at 37°C and blended as above.

Time of Addition of RN'ase	CPM Remaining with Cells after Blending
0	4
2	7
3	26
4.5	70
10	145
Control without RN'ase	165

Phage-cell complexes were incubated at 37°C with RN'ase for 15 min or at different times to allow any RNA to penetrate the cell and finally blended to remove noninfecting particles such as those adsorbed to free F-pili. The penetration of RNA into the cell is described below. As shown by comparison of the first and last lines of Table I blending greatly lowered the observed background value of adsorbed particles

which escaped RN'ase digestion (also see Fig. 2A for unblended values). These findings suggested that free F-pili-phage complexes which were inert to RN'ase digestion were removed from the cells during blending and centrifugation. Addition of RN'ase at later times did not degrade RNA which had already penetrated the cell. RN'ase, then, appears to be a powerful inhibitor totally preventing penetration of phage RNA into the cell.

RNA Transport Step. No direct experimental evidence is available concerning this important step. It can be seen however from electron micrographs that RNA phages adsorb randomly along the surface of an F-pilus, sometimes as far as 5 microns from the cell. It can be argued that RNA from these infecting particles may have to travel the several microns down the core of the pilus before penetrating the cell. It is this stage which we have tentatively designated the Transport step.

Penetration. Entry or penetration of phage RNA into the cell is easily followed by measuring the amount of radioactivity remaining with the infected cells after blending. Phage-cell complexes formed at 0° are completely disrupted by blending whereas after incubation of phage-cell complexes at $37^{\circ}C$ for 10 min a high per cent of the radioactivity of the adsorbed phage remains associated with the bacteria after blending and is assumed to have penetrated inside the cell. This RNA is no longer degraded by RN'ase.

Fig. 3 summarizes an experiment in which phage-cell complexes were blended after different periods of incubation at $37^{\circ}C$. The complexes were formed at $0^{\circ}C$ by incubation of the radioactive phage with the cells. The phage-cell complexes were next incubated at $37^{\circ}C$ and samples of 1 ml were removed and transferred to 20 ml of water at $0^{\circ}C$ to stop further RNA penetration. The samples were next blended for 2 min to remove phages which had not injected their RNA; the cells were collected by centrifugation, washed, precipitated with cold 5 per

cent TCA, and the radioactivity remaining with the cells counted after collecting on glass filters.

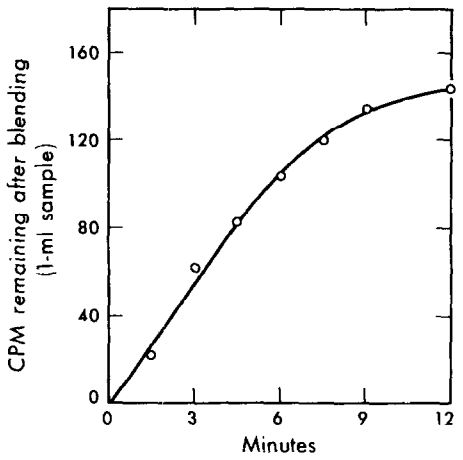

Fig. 3. The RNA penetration stage of infection. Conditions as in text.

The entry of phage RNA into the cell as measured by the above assay marks the final extracellular stage of infection and is readily distinguishable from the first act of simple adsorption. It has not yet been possible however, to separate in time the RN'ase sensitive or transport step from the penetration stage.

Discussion

We have speculated (Fig. 1) that bacteriophage RNA is able to penetrate the male cell in a series of reactions including: (1) simple adsorption of the phage to the F-pilus, (2) injection of phage RNA into the core of the F-pilus, (3) transport of the RNA down the core of the pilus, and (4) final penetration of the RNA into the cell. The RNA transport and penetration stages are particularly interesting because of their possible similarity with DNA transport steps during conjugation where F-pili are thought to play a role. An energy requirement

for transport and penetration may be suggested by the finding that these stages took place only in warm broth unlike the adsorption step which occurs even at $0^{\circ}C$. An integral relationship between cell and F-pilus was suggested by the find that free F-pili fragments carried out only simple adsorption but not subsequent stages such as injection. An additional tool for probing the extracellular stages of RNA-phage injection was recently provided by Paranchych (In Press) who was able to block RNA penetration in Mg^{++} deficient broth cultures without affecting earlier extracellular stages such as the RN'ase sensitive step. Using Fig. 1 as a guide it might also be suggested that the "Mg^{++} block" may have conceivably occurred at the transport stage. The interesting finding of Paranchych (In Press) should shed further light on the extracellular stages of RNA-phage injection.

References

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