EFFECT OF RNase PRE-TREATMENT OF CELLS OF ESCHERICHIA COLI K 12 ON PLAQUE YIELDS RESULTING FROM SUBSEQUENT INFECTION WITH THE RNA PHAGE fr^+

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During studies involving the sensitivity of a phenol-pi-protoplast system (Knolle, P., and Kaudewitz, F., 1962) to the action of RNase it was observed that small amounts of RNase suppressed the formation of plaques in the toplayer of control platings containing mixtures of cells and intact phage fr. For example, 1o μg RNase/ml toplayer reduced the plaque yield by 9o %, while concentrations of 5o μg/ml or higher resulted in complete suppression of the formation of plaques in the presence of $1o^8$ cells and up to $1o^8$ plaque-forming units. This effect has since been used for the rapid estimation of the nucleic acid type of newly isolated phages.

Since free fr is not inactivated by RNase (Knolle, P., in preparation) this effect indicated a cellular involvement in the RNase-suppression of plaque yields. Such involvement could be demonstrated by pre-treatment of the cells with RNase before infection with fr.

Throughout the experiments standard phage techniques were used (Adams, M.H., Bacteriophages, 1959), with modifications as described in the text. RNase was obtained from Nutritional Biochemical Corporation. Phage fr was made available by Dr. H. Hoffmann-Berling. The characteristics of this phage have been described (Knolle, P., and Kaudewitz, F.,

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1962; Hoffmann-Berling, H. in press; Kaudewitz, F., and Knolle, P., in press). Cells of Escherichia coli K 12 strains were grown in nutrient broth (NB). Phage fr antiserum was prepared from rabbits, immunized with purified fr.

Experiment A

Table 1 shows the effect of RNase on the plaque yield resulting from a standard one-step growth experiment.

Table 1: EFFECT OF THE PRESENCE OF 25 μg/ml RNase IN THE STANDARD ADSORPTION TUBE DURING A ONE-STEP GROWTH EXPERIMENT WITH fr

Minutes after adsorption	FGT		SGT	
	RNase-treated adsorption mixture	control	RNase-treated adsorption mixture	control
28	5	55	0	0.5
40	7	60	0	1
82	33	-	0	370
122	540	-	50	680
152	-	-	75	-
Latent period	80 min.;	40, <80 min		
Burst-size			1350	1360

Plaque counts are averages of three plates

In the adsorption tube were mixed 0.9 ml nutrient broth containing 25 μg RNase and 5 x 10^7 cells/ml with 0.1 ml of a fr suspension of the titer 5 x 10^7. A second adsorption tube was identical except for the omission of RNase. After 10 minutes at 37°C the adsorption mixtures were diluted 1/10 into fr antiserum and incubated for another 10 min. A 1/10 dilution+ was made, followed by the standard dilutions into the first growth tube (FGT) and the second growth tube (SGT). Platings

+ This deviation from the standard dilution (Adams, M.H., 1959) was necessary, since during the 10 min. of adsorption, more than 90 % of the free phage was eliminated from the supernatant fluid of a centrifuged adsorption suspension. However, only about 20 % of the input phage established lytic centers on platings from the FGT.

were done during the latent period (which is about 4o minutes), and also later for determinations of the burst size. As can be seen, the plaque yield of fr with RNase in the adsorption tube (as measured in the FGT) was markedly reduced, while its burst size was of the same order of magnitude as in the control. The infection of E.coli K or E.coli K 12 by T2 was not affected by the RNase treatment of the cells under the conditions of this experiment (Knolle, P., in preparation)

Experiment B

In order to obtain larger plaque yields in the FGT, the single-step growth experiment was further modified. The cell titer in the adsorption tube and the titer of the phage suspension added were both 2 x 10^8. Cells were pre-treated with RNase (final concentration 5o μg/ml) at 37° for 1 and for 3o minutes. They were then infected with fr and run through the single-step growth experiment as before. Figure 1 shows the effect of pre-treatment of cells with RNase: with increasing time of pre-incubation the plaque-yield in the FGT was reduced, while the

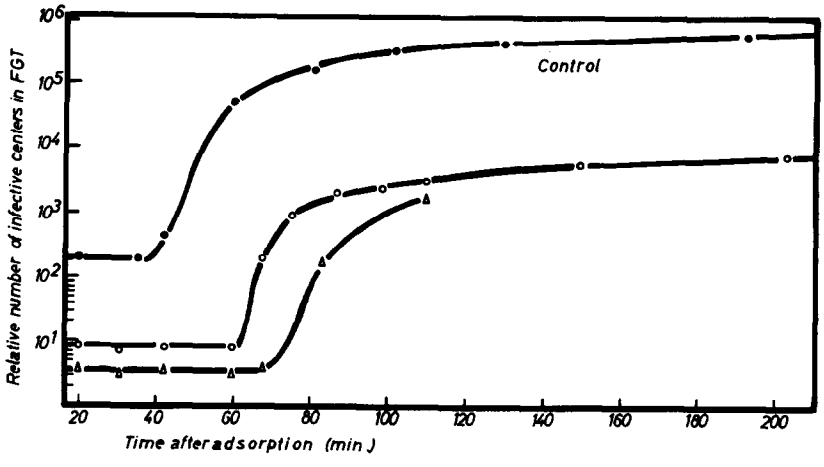

Fig. 1: EFFECT OF RNase-PRE-TREATMENT OF CELLS ON ONE-STEP-GROWTH CURVE
Black circles: No RNase-pre-treatment
White circles: 1 min. RNase-pre-treatment
White triangles: 3o min. RNase-pre-treatment

latent period was elongated. The plaque yield was of the same order of magnitude as in the untreated control. Since the rise period is rather long, a third growth tube (1/1o dilution from SGT) was aerated for the determinations of the burst sizes, in order to reduce the chance for re-infection after the begin of the burst-period.

Experiment C

It was of interest to see if the suppression of the plaque-yield in the FGT could be overcome by incubation of the cells in NB after RNase treatment. Cells were pre-treated with RNase for 3o minutes in o.o5 M pH 7 phosphate buffer. They were then centrifuged, resuspended in 1/2 the original volume of phosphate buffer and diluted 1/5o in NB respectively phosphate buffer. The cells were then infected with fr after being kept in the two media for 3o min at 37°C. Unadsorbed phage was removed by dilution of the adsorption mixture into fr antiserum. Platings were done from further dilutions in NB. The results (table 2) show that the RNase-defect could be restored if RNase-pretreated cells were incubated in NB before infection by fr.

Table 2: RESTAURATION OF RNase EFFECT BY INCUBATION IN NB

Cells pre-treated with RNase, resuspended in		Untreated cells, resuspended in	
NB	PO_4	NB	PO_4
178o+	329	363o	258o

+ see footnote of table 1

While this study was in progress a paper appeard (Brock, T.D., 1962), in which mention is made of an observation by N. Zinder regarding a RNase effect in his f2 system. According to this, RNase interfers speci—

fically with the phage after adsorption and attacks f2 RNA directly. It could be shown (P. Knolle, in preparation), that, in contrast to experiment A, RNase added after successful infection does not reduce the plaque yield, hence RNase may affect indeed fr during early stages of infection. The RNase effect, reported in this paper, suggests a cellular involvement in the susceptibility of the fr-infection to the action of RNase.

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