ROLE OF HOST RNA POLYMERASE FOR λ PHAGE DEVELOPMENT

Y. Takeda, Y. Oyama, K. Nakajima and T. Yura
Institute for Virus Research
Kyoto University, Kyoto, Japan

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SUMMARY

Rifampicin, a specific inhibitor of RNA synthesis in bacteria, markedly inhibits growth of λ phage on wild-type Escherichia coli. Synthesis of both early and late λ messenger RNA was inhibited by rifampicin even when the drug was added after phage messenger RNA synthesis already started. In contrast, normal phage growth was observed on any of the rifampicin-resistant mutants tested producing RNA polymerase that are themselves resistant to the drug. These results suggest that host RNA polymerase (at least components relating to rifampicin sensitivity) is involved in expression of λ genes throughout the phage development.

It has recently been reported that streptovaricins and rifamycins (including rifampicin) specifically inhibit RNA synthesis in bacteria (Mizuno et. al., 1968; Hartmann et. al., 1967). Mutants of E. coli producing altered RNA polymerase were isolated by selection of those resistant to these antibiotics (Yura and Igarashi, 1968). These studies provide an opportunity to investigate the role of host RNA polymerase in the growth of bacteriophages, including temperate phage λ, the subject of this communication. Evidence presented here suggests that host RNA polymerase (at least components relating to rifampicin sensitivity) is involved in expression of λ genes throughout the phage development.

RESULTS

Streptovaricin-resistant mutants were isolated from E. coli K12, strain KY4185 (a λ-sensitive derivative of PA678) treated with nitrosoguanidine as described

TABLE 1.

Growth of λ phage on different types of streptovaricin-resistant mutants

Bacteria	Sensitivity to		Phage growth		No. of strains tested
	STV	RFP	-RFP	+RFP	
Wild type	S	S	+	-	1
Mutant type 1	R	R	+	+	22
Mutant type 2	R	RS	+	±	2
Mutant type 3	R	S	+	-	14

Sensitivity to streptovaricins(STV) or rifampicin(RFP) was tested in broth containing 500 μg/ml or 25 μg/ml, respectively, of each drug. R, RS and S, respectively, indicate heavy, slight and no growth after overnight incubation in the presence of the drug at 37°C. A log-phase culture (ca. 3×10^8 cells/ml) was harvested, starved in adsorption buffer for 60 minutes, then infected with phage λvir at a multiplicity of infection (moi) of 5. Phage growth was determined by one-step growth experiments in the presence (25 μg/ml) or absence of rifampicin. +, ± and -, respectively, denote normal, some (20-50% of normal) and no (<10% of normal) growth.

previously (Yura and Igarashi, 1968). These mutants can be classified into three types with respect to their cross resistance to rifampicin (See Table 1). The growth of phage λ on these mutant bacteria was examined in the presence or absence of rifampicin. It was found that rifampicin severely inhibits phage growth on sensitive bacteria but not on resistant bacteria. In the latter case, cell lysis occurred after a usual lag, with normal phage yields. When cells of type 2 mutants exhibiting intermediate resistance to rifampicin were infected, however, lysis was delayed somewhat and the phage yield was reduced several fold. Thus, the growth of phage λ in the presence of rifampicin coincided well with the rifampicin sensitivity of the host bacteria used.

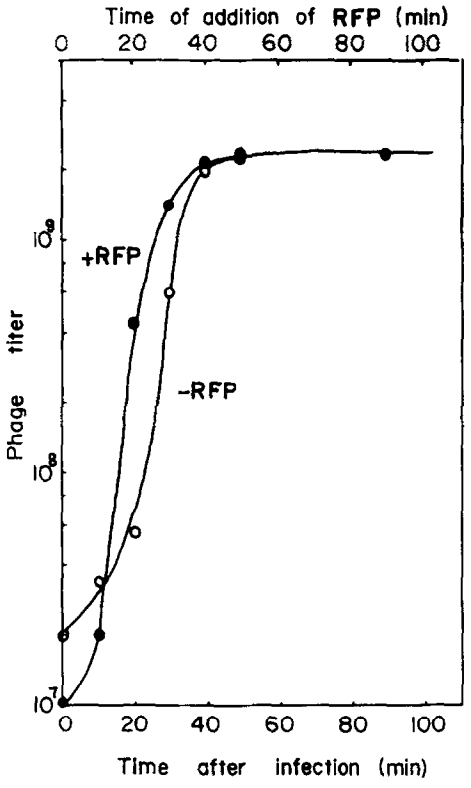

Fig. 1. Effect of rifampicin on λ phage growth. A log-phase culture of E. coli KY4185 (wild type) that had been starved for 60 minutes was infected with phage λvir (moi=5) and treated with anti-λ serum. Then it was diluted and incubated in peptone-glucose medium at 37°C. Rifampicin (50 μg/ml) was added at the times indicated and cultures were incubated till 90 minutes. Aliquots of a control culture taken at intervals (○), as well as rifampicin-treated cultures at the end of incubation (●) were chloroformed and assayed for phage titer.

We then investigated rifampicin-sensitive step(s) of λ phage development in wild-type bacteria. Fig. 1 shows that infectious λ particles are produced at 30 to 40 minutes after infection in the absence of rifampicin. When the drug was added at various stages of infection, subsequent growth of phage was markedly inhibited. A lag of about 5 minutes observed in the inhibition of phage growth presumably reflects a similar time lag in inhibition of RNA synthesis under these conditions.

That this is probably the case is shown by experiments illustrated in Fig. 2. Incorporation of H^3-uridine into RNA was dramatically inhibited even when rifampicin

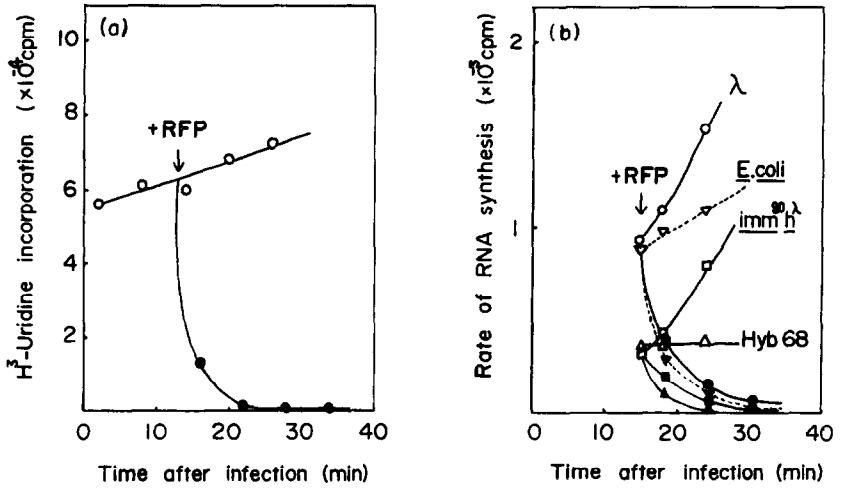

Fig. 2. Effect of rifampicin on RNA synthesis in λ-infected bacteria. Cells of KY4185 were infected with λvir as in Fig. 1 except that the serum treatment was omitted and undiluted cultures were incubated in a minimal-casamino acids medium at 37°C. (a) Aliquots were withdrawn at intervals and pulse-labeled for 1 minute with 1 μc/ml H^3-uridine (specific activity 6.8c/mmole). Labeling was stopped by cold 5%-trichloroacetic acid. Radioactivity in the acid-insoluble fraction was determined in a liquid scintillation counter. ○, control; ●, rifampicin (50 μg/ml) added at the time indicated. (b) Procedures, including pulse-labeling, were as in (a) but H^3-RNA was extracted by the hot phenol method, and DNA-RNA hybridization was carried out as described previously (Takeda and Yura, 1968) using 50 μg E. coli DNA or 30 μg phage DNA. DNAs prepared from a pair of λ-φ80 hybrid phages (Hyb68 and imm $^{80}_{h}$ λ) were used to detect early and late λ mRNA, respectively (Takeda, 1969; Takeda and Yura, 1968). Open symbols represent control cultures, and filled symbols represent cultures to which rifampicin (50 μg/ml) was added at 15 minutes. Rate of RNA synthesis was calculated from total incorporation determined as in (a), multiplied by per cent of RNA hybridized with each DNA used.

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was added at a late phase of the λ growth cycle. Pulse-labeled RNA prepared at various times after addition of rifampicin was then assayed for "early" and "late" phage messenger RNAs, as well as for total phage and bacterial RNAs, using DNA-RNA hybridization. It is clear from Fig. 2b that the synthesis of not only early but also late λ messenger RNA is markedly inhibited by rifampicin and that the kinetics of inhibition paralleled those for host RNA synthesis.

DISCUSSION

When bacteria are infected with phage such as λ, various phage genes are expressed sequentially; early genes are transcribed immediately after infection but late genes only after some lag (Skalka, 1966; Naono and Gros, 1966; Taylor, Hradecna and Szybalski, 1967; Takeda and Yura, 1968). RNA polymerase prepared from uninfected E. coli cells can transcribe only some of the early genes of both the T4 and λ phages (Geiduschek et. al., 1966; Naono and Gros, 1966; Cohen, Maitra and Hurwitz, 1966). Thus, it was conceivable that host RNA polymerase is involved only in transcription of early genes and that a phage-specific RNA polymerase is synthesized for transcription of late genes. If so, then, phage-directed RNA polymerase must be resistant to rifampicin, since rifampicin did not interfere with λ phage growth in the several rifampicin-resistant mutants tested.

However, rifampicin did inhibit λ phage growth in sensitive bacteria when added at any stage of infection. Moreover, the transcription of both early and late genes was inhibited even when the drug was added after late gene transcription already started. Thus, the present results suggest that if a new type of RNA polymerase were made during phage development, it would not be totally directed by the phage genome but must utilize at least some components (relating to rifampicin sensitivity) of host RNA polymerase. Presumably, sequential expression of the phage genome can be achieved by appropriate modifications of host RNA polymerase, allowing transcription of the operons (or genes) with different initiation specificities (or promotors).

A similar conclusion was recently reached for SPO1 and T4 phage development by Geiduschek and Sklar (1969), and by Haselkorn, Vogel, and Brown (1969), respectively.

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REFERENCES

Cohen, S., Maitra, M., and Hurwitz, J., Cold Spr. Harb. Symp. Quant. Biol., 31, 113 (1966).

Geiduschek, E. P., and Sklar, J., Nature, 221, 883 (1969).

Geiduschek, E. P., Snyder, L., Colvill, A. J. E., and Sarnat, M., J. Mol. Biol., 19, 541 (1966).

Hartmann, G., Honikel, K. O., Knüsel, F., and Nüesch, J., Biochem. Biophys. Acta, 145, 843 (1967).

Haselkorn, R., Vogel, M., and Brown, R. D., Nature, 221, 886 (1969).

Mizuno, S., Yamazaki, H., Nitta, K., and Umezawa, H., Biochem. Biophys. Acta, 157, 322 (1968).

Naono, S., and Gros, F., Cold Spr. Harb. Symp. Quant. Biol., 31, 363 (1966).

Skalka, A., Proc. Nat. Acad. Sci. U. S., 55, 1190 (1966).

Takeda, Y., in preparation (1969).

Takeda, Y., and Yura, T., Virology, 36, 55 (1968).

Taylor, K., Hradecna, Z., and Szybalski, W., Proc. Nat. Acad. Sci. U. S., 57, 1618 (1967).

Yura, T., and Igarashi, K., Proc. Nat. Acad. Sci. U. S., 61, 1313 (1968).