THE DEGRADATION OF TWISTED CIRCULAR LAMBDA DNA BY PHAGE LAMBDA MUTANTS

Robert C. Shuster [1] and Arthur Weissbach [2, 3]

National Institute of Arthritis and Metabolic Diseases
National Institutes of Health, Bethesda, Maryland

Received October 4, 1968

Summary: The stability of the twisted circular, covalent form of phage lambda DNA (species I) has been examined after infection of thymine auxotrophs of E. coli with early sus mutants and integration negative mutants of lambda. After infection of the permissive host, E. coli CR34 (thy⁻), all of the lambda mutants tested lost over 90% of their species I DNA after 60 minutes of incubation in a thymine deficient medium. In the nonpermissive host, E. coli W3350 (thy⁻), it was found that the sus P lambda mutants were the least efficient in degrading species I phage DNA. It is suggested that the P cistron may be involved in directing the synthesis of a phage specific endonuclease which attacks the twisted, circular, covalent configuration of phage DNA.

INTRODUCTION

When Escherichia coli K12 are infected with lambda bacteriophage, a significant portion of the entering linear duplex phage DNA is converted to a covalently closed, twisted circular duplex molecule (species I lambda DNA) (1) which contains no single stranded breaks. Once formed in the infected cells, this twisted circular configuration of phage DNA is stable and persists for at least one hour under normal incubation conditions (1, 2). However, if the host bacteria are thymine auxotrophs, the twisted circular form of the phage DNA is rapidly destroyed when bacteria are infected and incubated in the absence of thymine (2). The degradation of species I lambda DNA which occurs under these conditions is apparently caused by a new endonuclease whose synthesis is controlled by the phage genome. Thus, it has been previously shown

---

[1] Present Address - Dept. Biochemistry, Emory University, Atlanta, Georgia

[2] Visiting Scientist from the Roche Institute of Molecular Biology, Nutley, N. J. 07110

[3] Present Address - Institut de Biologie Physico-Chimique 13, Rue Pierre et Marie Curie, Paris V^e, France

that the destruction of species I lambda DNA does not occur either in the presence of chloramphenicol or in a bacterial host carrying a noninducible lambda prophage which prevents the expression of the superinfecting phage genes (2). At the present time it is not known if this new, phage directed endonuclease, makes single or double stranded breaks in the species I lambda DNA.

In the present paper, we have examined various lambda mutants for their ability to cause the disappearance of species I phage DNA during incubation of the infected bacteria in the absence of thymine. The results suggest that the P cistron, which is one of the lambda genes required for phage DNA synthesis, may be partly responsible for directing the formation of a new lambda endonuclease which attacks species I lambda DNA.

MATERIALS AND METHODS

A.) Bacteria:

E. coli K12 strain W3350(S) (non-lysogenic) was the gift of Dr. A. Campbell. A thymine requiring mutant of this strain was isolated by the method of Stacey and Simson (3).

E. coli CR34(S) (non-lysogenic) Leu$^-$ Thr$^-$ Thy$^-$ B_1^- was obtained from Dr. M. Meselson.

Lysogenic E. coli CR34 (λ ind$^-$) was isolated in this laboratory.

B.) Bacteriophages:

All lambda bacteriophages used in these experiments, except lambda C_{II}, contained the mutation C_I857 which leads to the formation of a temperature sensitive phage repressor (4). Incubation of infected cells was carried out at 39° in order to minimize repression of phage functions. The following lambda mutants were the kind gift of Dr. W. Sly:

Lambda C_I857 sus N_{96A}, lambda C_I857 sus O_{29}, lambda C_I857 sus P_{80}, lambda C_I857 sus Q_{73} and lambda C_I857 sus R_5.

The integration negative mutants lambda C_I857 434hy int_6 and lambda C_I857 434hy int_4 sus P_3 were generously donated by Drs. M. Gottesman

and M. Yarmolinsky.

Lambda $C_{II}68$ was obtained from Dr. Vernon Bode.

C.) Infection of bacteria and isolation of phage DNA:

Bacteria were grown to a concentration of 2×10^8 cells/ml in synthetic medium supplemented with 60 mM thymine (5). They were washed, resuspended in thymine deficient medium and infected with ^3H thymine-labeled bacteriophage at a multiplicity of five phage/cell (2). After allowing the phage to absorb for 15 min at 37°, the infected cells were centrifuged, and resuspended in thymine deficient medium. Incubation was continued at 39° for 60 min. The infected bacteria were lysed, treated with pronase and sedimented in alkaline sucrose as previously described (2). Fractions were collected and assayed for radioactivity (2).

RESULTS

Table I summarizes the results obtained when various lambda mutants infect either the permissive su^+ *E. coli* strain CR34 or the nonpermissive su^- strain W3350. In all of the experiments, the initial percentage of twisted, circular lambda DNA formed was rather low and may reflect the conditions of thymine deprivation and host strains used in these experiments. When *E. coli* W3350 is infected with lambda C_I857 and incubated in the absence of thymine, about 80% of the species I phage DNA which is initially formed disappears within 60 minutes. When infection was carried out with a series of lambda C_I857 suppressor sensitive mutants (sus mutants), it was found that phage with mutations in either N, Q, or R were also able to destroy from 70-80% of the species I lambda DNA under these conditions. However, the sus P lambda mutants were not as efficient in causing the destruction of their own species I DNA. Thus, infection of the nonpermissive host, *E. coli* W3350 with lambda C_I857 sus P resulted in a loss of only 56% of species I DNA during incubation in the absence of thymine. When the infecting phage was lambda C_I857 int_4 sus P, the decrease in species I after incubation was also 50%. The lambda C_I857 sus O mutant also seemed to be slightly deficient in its capacity to

Stability of Twisted Circular Lambda DNA in Bacterial Hosts During Thymine Deprivation

Host Cell	Infecting λ Phage Mutant	% Circular DNA[1] Formed at 0'	% Circular DNA[1] Remaining at 60'	% Circular DNA[1] Destroyed
A) E. coli W3350 (thy⁻) (non-permissive)				
	C_I857	27	5.0	81
	" sus N_{96A}	9.0	1.6	82
	" sus O_{29}	11	4.1	63
	" sus P_{80}*	17	7.5	56
	" sus Q_{73}	7.5	2.1	72
	" sus R_5	16	3.0	81
	" 434hy int_6*	5.2	1.6	70
	" 434hy int_4* sus P_3*	13	6.5	50
	C_{II}68	7.5	2.4	68
B) E. coli CR34 (thy-) (permissive)				
	C_I857	20	1.0	95
	" sus O_{29}	22	1.2	95
	" sus P_{80}	24	1.4	94
	" 434hy int_6	14	1.0	93
	" 434hy int_4 sus P_3	16	1.0	94
	C_{II}68	6.3	0.5	92
C) E. coli CR34(λ ind⁻) (thy⁻) (immune)				
	C_I857	31	25	19

[1] Covalent, twisted, circular configuration of lambda DNA (species I DNA).

* Average of two experiments.

destroy species I lambda DNA but the effect was not as pronounced as the sus P mutant and may be equivocal.

The int_6 and C_{II} lambda mutants were found to have an apparently normal ability to destroy species I phage DNA under these experimental conditions. Since neither of these mutations are supressor sensitive, they are expressed both in E. coli CR34 and E. coli W3350. As shown in Table I, both of these lambda mutants destroy over 90% of the species I phage DNA in E. coli CR34 as does the wild type lambda C_I857. The same is true of the sus mutants which are not expressed in E. coli CR34 and therefore behave as the wild type lambda C_I857 in this host strain. The extensive disappearance of species I lambda DNA in thymine deprived host cells after infection by lambda mutants is predominantly due to a phage directed process as shown in part C of Table I when lambda C_I857 infects E. coli CR34 carrying a non-inducible lambda prophage In this host bacteria, the functions of the superinfecting phage are repressed and only 20% of the twisted circular lambda DNA disappears in 60 minutes. This may be compared to the 95% disappearance of species I lambda DNA in the non-lysogenic E. coli CR34 when infected by the same bacteriophage.

DISCUSSION

Of the lambda mutants tested in these experiments, the sus P mutant was the least efficient in causing the destruction of species I phage DNA. However, even with the sus P mutants, up to 50% of the species I lambda DNA was still destroyed. If the P gene is the structural gene for a new lambda endonuclease or controls the synthesis of such an enzyme, it is necessary to explain the aforementioned 50% destruction of species I lambda DNA observed with the sus P mutant. An obvious explanation is the existence of other phage or bacterial nucleases which are synthesized or already present and which attack the twisted, circular configuration of lambda DNA. It is also possible that the sus P mutant in E. coli W3350 is slightly leaky and can therefore synthesize some of the proposed endonuclease.

The importance of an endonuclease which would make single strand

breaks in the lambda DNA molecule to permit either initiation or continuation of phage DNA synthesis has been widely discussed (6). Our results suggest that the function of the P cistron may be to direct the synthesis of such an endonuclease which would permit the replication of circular lambda DNA, since it is known that mutations in the P cistron of lambda prevent the synthesis of the phage DNA (7, 8, 9).

REFERENCES

1. Bode, V.C., and Kaiser, A.D., J. Mol. Biol., 14, 399 (1965).
2. Shuster, R.C., and Weissbach, A., J. Virol., in press.
3. Stacey, K., and Simson, E., J. Bacteriol., 90, 1517 (1962).
4. Sussman, R., and Jacob, R., Compt. Rend., 254, 1517 (1962).
5. Salzman, L.A., and Weissbach, A., Virol. 31, 70 (1966).
6. Ogawa, T., Tomizawa, J. and Fuke, M., Proc. Nat. Acad. Sci. (U.S.) 60, 861, (1968).
7. Brooks, K., Virol. 26, 489 (1965).
8. Eisen, H.A., Fuerst, C.R., SiminoVitch, L., Thomas, R., Lambert, L., da Silva, P. and Jacob, F. Virol. 30, 224 (1966)
9. Joyner, A., Isaacs, L.N., Echols, H. and Sly, W.S., J. Mol. Biol. 19, 174, (1966).