NUCLEASE ACTIVITY IN DEFECTIVE LYSOGENS OF PHAGE λ

Charles M. Radding
Department of Human Genetics
University of Michigan, Ann Arbor, Michigan

Received January 7, 1964

Introduction

In the course of studies on DNA synthesis by induced K12 (λ) (Radding, 1963), we have tested various defective mutants of λ for the λ-associated nuclease (Korn and Weissbach, 1963). Three independently isolated λ*sus* mutants in cistron N (Campbell, 1961) failed to show the increase in nuclease activity shown by wild type λ or other *sus* mutants on treatment with inducing doses of ultraviolet light, streptonigrin (Levine and Borthwick, 1963), or mitomycin C.

Methods

Suppressor sensitive (*sus*) mutants of λ and unpublished information about their genetic properties were generous gifts of Drs. Katherine Brooks and Allan Campbell. The *sus* mutants are suppressible defective mutants; they will not grow in strain W3350 of *E. coli* K12, a non-permissive host, but will grow in strain C600 and other permissive strains. Although the *sus* mutants cannot grow in strain W3350, the latter can be lysogenized by λ*sus*, forming defective lysogens which cannot be induced to yield phage. Other permissive strains of *E. coli*, CR63 and CS^P, were gifts of Dr. M. Dirksen (Dirksen *et al.*, 1963).

Cultures were grown in Tryptone broth, or synthetic media, to a density of about 2×10^8 cells/ml. and treated with UV, streptonigrin, or mitomycin C. Aliquots of the cultures, taken prior to treatment

This research was supported by USPHS GM-09252-02.

and at various times later (usually 60 minutes), were chilled on ice, centrifuged, resuspended, and frozen in .05M glycylglycine, pH 10, .001M glutathione. Extracts of the thawed aliquots were prepared by sonication with four 15 second pulses in the MSE ultrasonic disintegrator. Nuclease assays were done for 30 minutes at 37°C in a 0.3 ml. reaction mixture containing 10 μmoles glycine buffer at pH 10, 1 μmole $MgCl_2$, 50 mμmoles of E. coli DNA labelled with H^3 thymine to have 1-2 x 10^6 cpm/μmole, and 0 to 0.4 units of nuclease activity. 1 unit was defined as 10 mμmoles of nucleotide made acid soluble in 30 minutes. The reaction was stopped by chilling, addition of 0.2 ml. of .25% calf thymus DNA and 0.5 ml. of 3.5% PCA. 0.1 ml. of supernatant was counted by scintillation counting. H^3 thymine labelled DNA was prepared from E. coli 15T⁻. Unlabelled DNA from E. coli B or K12 (λ), induced by streptonigrin, was used to dilute the labelled DNA to desired specific activity. Streptonigrin, a generous gift of Chas. Pfizer and Co., was used at a final concentration of 4 to 8 μg/ml. Mitomycin C was used at 3 μg/ml.

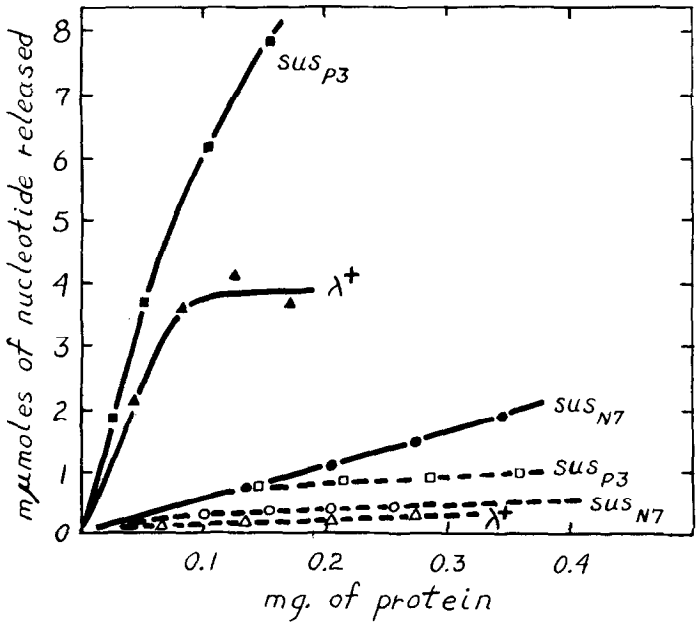

Fig. 1. Nuclease Activity vs. Protein in Crude Extracts of Lysogens of W3350 induced by Streptonigrin.
---------- Before; ———— After "induction".

Results

Proportionality of nuclease activity to the amount of protein used from the crude extracts is shown in Fig. 1. Also shown is the magnitude of the increase in nuclease activity after induction of W3350 (λ^+) and the defective lysogen W3350 ($\underline{sus}_{P3}$), as contrasted with the failure of another defective lysogen, W3350 ($\lambda\underline{sus}_{N7}$), to show a similar increase.

Of the tested derivatives of W3350, only the non-lysogenic parent strain and the defective lysogens made with sus mutants in the N cistron failed to show fourfold or greater increases in nuclease activity (Table I).

Table I. Nuclease Activity of W3350 and Lysogenic Derivatives. Specific Activity (units/mg. protein)

Strain	Inducibility (lysis)	Inducer	Before	After
W3350	-	UV	.73	.78
		SN	.13	.10
W3350 (λ^+)	+	UV	1.1	4.0
		SN	.1	6.5
		MC	.1	5.9
W3350 ($\underline{sus}_{I2}$)	+	SN	.38	2.1
W3350 ($\underline{sus}_{O29}$)	-	UV	.49	4.6
		SN	.45	9.2
W3350 ($\underline{sus}_{P3}$)	-	UV	.63	4.2
		SN	.51	7.0
W3350 ($\underline{sus}_{N7}$)	-	UV	.86	.47
		SN	.47	.60
		MC	.16	.23
W3350 ($\underline{sus}_{N53}$)	-	SN	.85	1.1
W3350 ($\underline{sus}_{N96A}$)	-	SN	.91	1.0

UV = ultraviolet light, SN = streptonigrin, MC = mitomycin C

Included in Table I are: a mutant in cistron I which governs a late λ function, and mutants in cistrons N, O, and P which govern early

functions necessary for multiplication of the phage genome (Brooks and Campbell, 1963). Preliminary studies support the conclusion that N and P mutants are blocked prior to DNA synthesis (Radding, 1963). Defective lysogens made from any of these early mutants do not express, on treatment with inducers, any known λ functions, including lysis, except for the increase in nuclease activity shown by O and P; N mutants are deficient in nuclease activity as well. Failure of W3350 ($\lambda \underline{sus}_{N7}$) to show an increase in nuclease activity is not due to an inhibitor; mixtures of extracts from "induced" W3350 ($\lambda \underline{sus}_{N7}$) and induced W3350 ($\lambda \underline{sus}_{P3}$) showed the sum of the respective activities.

The function of the N cistron is essential for the lytic cycle as well as for induction, since $\lambda \underline{sus}_{N}$ (or any sus mutants) will not grow in W3350 after infection. Nuclease activity, after infection by $\lambda \underline{sus}_{N}$, has not been tested yet.

The suppressor sensitive mutants of λ grow in several permissive hosts where the phenotypic effect of the phage mutation is suppressed. For example, the relative efficiency of plating of $\lambda h \underline{sus}_{N96A}$ on C600, CR63, CS^{P} and W3350 respectively was 1.0, 0.71, 0.76, and 2×10^{-6}. In Table II are shown the results of nuclease assays on extracts from the permissive lysogens of the N mutants. An increase in nuclease activity after induction was restored in the permissive hosts along with the ability of the phage to be induced and grow.

Discussion

A probable interpretation of these results is that the N cistron is the structural gene for the λ-associated nuclease. Favoring that interpretation is the restoration of nuclease activity by the same suppressor strains which produce qualitatively altered hydroxymethylase from T4 am mutants (Dirksen et al., 1963).

Table II. Nuclease Activity of Permissive Lysogens Induced by Streptonigrin. (Units/mg. protein)

Strain	Before	After
C600	.65	.58
C600 (λ^+)	.64	8.5
C600 (λsus_{N7})	.92	4.0
(N53)	.81	4.0
(N96A)	.94	2.1
CR63	.51	.52
CR63 (λsus_{N7})	.91	3.1
(N53)	1.1	5.0
(N96A)	.91	5.1
CS^P	.60	.39
CS^P (λsus_{N7})	.60	3.4
(N53)	.23	2.5
(N96A)	.57	11.

The tentative identification of the function of one of the early cistrons of λ raises many interesting questions about the possible role of the λ nuclease in initiation of λ DNA synthesis or in replication.

Other interpretations are possible, however, and present efforts are directed at attempting (1) to confirm with purified enzyme preparations the results from assays of the crude extracts, and (2) to find a qualitatively altered form of the nuclease made by an N mutant, perhaps in one of the suppressed strains.

References

Brooks, K., and Campbell, A., Bacteriological Proc. p. 137 (1963), and personal communication.
Campbell, A., Virology 14: 22 (1961).
Dirksen, M., Hutson, J.C., and Buchanan, J.M., Proc. Nat. Acad. Sci. 50: 507 (1963).
Levine, M., and Borthwick, M., Bacteriological Proc. p. 153 (1963).
Korn, D., and Weissbach, A., J. Biol. Chem. 238: 3390 (1963).
Radding, C.M., Proc. XI Intern. Congr. Genet. p. 22 (1963); & unpublished observations.