ON THE MECHANISM OF DEREPRESSION OF HOST GALACTOSE OPERON FOLLOWING INDUCTION OF BACTERIOPHAGE λ IN ESCHERICHIA COLI STRAIN K12*

Yasuo Imae and Toshio Fukasawa

Institute for Protein Research, Osaka University, Osaka, Japan

Received May 23, 1967

When the cells of Escherichia coli strain K12 lysogenic for bacteriophage λ are treated with inducing agents, such as ultraviolet light (UV) or mitomycin C, one observes, besides the vegetative development of the virus, an increase (3 to 4 folds uninduced control) of the levels of a group of inducible enzymes concerning galactose metabolism (Buttin et al., 1960; Yarmolinsky and Wiesmeyer, 1960). These enzymes are synthesized under the direction of a bacterial operon, namely galactose operon which maps closely to the prophage site in the host chromosome. The observed increase of the enzyme levels can be accounted for by assuming that more than 30% of the induced cell populations are synthesizing these enzymes constitutively (see Buttin, 1963). The derepressed synthesis of the enzymes therefore, cannot readily be ascribed to the production of λdg, which is a rare event. Despite their extensive studies of the effect of the prophage induction on the host function (Buttin, 1963; Yarmolinsky, 1963), which is referred to as BY-effect in this paper, its molecular mechanism has remained obscure. We have been engaged in reinvestigation of this problem with the hope that its elucidation could shed light on the mechanism of prophage induction from a new point of view. In this communication, we report our recent experiments, revealing the following features: 1) Some viral cistrons known to control viral DNA replication must function in order for galactose operon to be derepressed. 2)

* A part of these results have been presented at 14th Annual Meeting of Japanese Virologists in 1966.

Replication of viral genome, however, is not required. 3) Instead, is required DNA synthesis of host bacteria.

EXPERIMENTAL RESULTS AND DISCUSSION

(I) Requirement for λ functions for BY-effect:

In the first place we attempted to study what functions of λ are essential for the occurrence of BY-effect. A non-permissive host, strain U155 was lysogenized with various λ sus mutants (Campbell, 1961). Cultures of the respective lysogens were treated with mitomycin C in the logarithmic phase of growth and the activity of UDPG 4-epimerase (epimerase), one of the galactose metabolizing enzymes was measured in each culture.

TABLE I

Derepression of UDPG 4-epimerase synthesis following induction of various prophage λ by mitomycin C

Strains	UDPG 4-epimerase activity[1] (a) non-induced	(b) induced	(b)/(a)	Phage yield[2]
U155[3]	4.2	4.9	1.2	---
U155 (λ)	5.1	12.9	2.5	2.3×10^9
U155 (λsus N_7)	6.7	21.2	3.2	$<10^3$
U155 (λsus N_{53})	6.8	17.3	2.5	1.4×10^5
U155 (λsus O_8)	3.7	5.6	1.5	2.2×10^6
	6.8	8.9	1.3	---
U155 (λsus P_3)	4.6	6.6	1.4	2.2×10^7
U155 (λsus P_{72})	8.8	11.8	1.3	1.9×10^7
U155 (λsus Q_{21})	5.8	58.7	10.1	4.9×10^7
U155 (λsus R_5)	6.0	23.8	4.0	---

1) The activity is expressed as mμmoles UDPG formed at 25°C per min per ml culture. 2) Plaque forming units assayed on C600 per ml culture. 3) A derivative of E. coli K12 requiring histidine and tryptophan.

Cells were grown at 37°C with aeration in a glycerol-salts medium, M63 (Buttin, 1963) supplemented with 5 μg/ml thiamine and the required aminoacids (100 μg/ml each). When the optical density reached 0.2 at 650 mμ in a spectrophotometer (Beckman DU), the culture was separated into two equal portions. To one of the two flasks was added mitomycin C (0 min) to a final concentration of 1 μg/ml (induced), while the other received none (non-induced). At 120 min aliquots were withdrawn and poured into ice-cold tubes containing chloramphenicol (50 μg/ml). Cells were then treated by toluene at 37°C for 5 min in the presence of 0.02M mercaptoethanol and 0.002M EDTA. UDPG 4-epimerase was assayed in the toluenized cells by the two step method described previously (Imae et al., 1964).

As shown in Table I, the derepressed synthesis of epimerase took place following induction of all the mutant prophages that have defects in cistrons N, Q, and R as well as in the wild type control. Such enzyme increase was not observed in the mutants belonging to cistrons O and P as in non-lysogenic control. On the basis of the above findings, we may be led to the following conclusions: 1) In order for BY-effect to be seen, cistrons O and P known to control DNA replication (Joyner et al., 1966; Eisen et al., 1966) must function. 2) N gene function and the replication of viral genome are not required: The mutants of N cistron are also known to be unable to initiate DNA replication (Eisen et al., 1966; Joyner et al., 1966). This is in agreement with the previous experiment of Buttin (1963) with a defective lysogen for λd22 which has recently been classified in cistron N (Eisen et al., 1966). 3) It is unnecessary for cistron Q and its controlling late functions to be expressed, which is impaired by the mutation in cistron Q (Dove, 1966; Protass and Korn, 1966).

Essentially the similar results were obtained if the prophage induction was achieved with heat treatment by the use of the sus mutants carrying an additional mutation (tsI) in C_I region (Horiuchi and Inokuchi, 1966). Thus, following the heat induction, the defectives in cistrons N and Q showed BY-effect normally in the non-permissive host, U155 as well as in the permissive host, C600 (Table II). Note in the table that the defective in cistron O failed to cause this effect even in C600. This finding, while puzzling, could be interpreted as implying that the O gene function is restored by suppression in the host cells such that it is sufficient to initiate the viral DNA replication but not to cause the derepression of epimerase. Regardless of the mechanism, this behavior of the mutant of O cistron in the permissive host can be taken as evidence indicating that the replication of viral genome by itself is not sufficient condition for the initiation of BY-effect.

TABLE II

Derepression of UDPG 4-epimerase synthesis following induction of various heat-inducible prophage λ by heating

Strains	UDPG 4-epimerase activity[1] (a) non-induced	(b) induced	(b)/(a)	Phage yield
C600[2]	7.7	7.4	1.0	---
C600 (λtsI)	7.0	19.3	2.8	1.8×10^8
C600 (λsus N_7-tsI)	9.6	54.8	5.7	3.8×10^8
C600 (λsus O_8-tsI)	8.0	9.8	1.2	8.7×10^9
	9.6	11.2	1.2	1.6×10^7
C600 (λsus Q_{21}-tsI)	5.9	20.3	3.4	8.0×10^7
U155	3.7	3.1	0.8	---
U155 (λtsI)	3.0	13.0	4.3	2.2×10^9
U155 (λsus N_7tsI)	8.6	22.0	2.6	$< 10^3$
U155 (λsus O_8-tsI)	4.3	4.8	1.1	9.4×10^6
U155 (λsus Q_{21}-tsI)	3.8	9.1	2.4	1.6×10^6

1) The activity is expressed as in Table I.
2) A derivative of E. coli K12, requiring threonine, leucine and thiamine.
Culture conditions were as described in the legend for Table I except for the growth temperature (33°C in this experiment). The heat induction was carried out by keeping a culture flask for 15 min in a water bath at 48°C with occasional shaking (induced). The flask of the non-induced control was allowed to stand at 33°C for 15 min also with occasional shaking. The flasks were then transferred to the water bath at 33°C (0-time), in which shaking was continued. For other procedures, see the legend for Table I.

(II) Requirement for host DNA synthesis for BY-effect:

A requirement for DNA synthesis, either viral or bacterial, was suggested by Yarmolinsky (1963) from the fact that 5-fluorodeoxyuridine (FUDR) arrested the increase of epimerase level following UV-induction of λ lysogens. However, he also reported that FUDR caused only a delay of the enzyme increase if the prophage induction was achieved by heat treatment using a heat-inducible mutant phage, λC_{I857}. The ambiguity of the effect of FUDR promoted us to conduct the following experiment in which DNA synthesis was blocked by thymine starvation. Thus, a thymine-requiring strain W3110 T^- was lysogenized with λC_{I857} which is inducible by heating (Sussman and Jacob, 1962) but not by UV-irradiation nor by thymine-starvation (Sicard and Devoret, 1962). The lysogen was subjected to heat treatment and the

synthesis of epimerase was followed in the presence or absence of thymine.

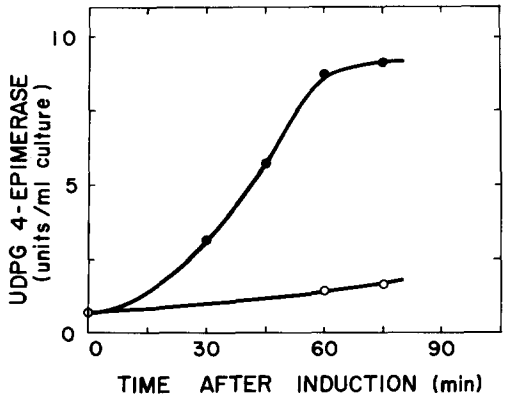

Fig. 1. Strain W3110 T^- (λC_{I857}) was grown in M63 supplemented with 20 μg/ml of thymine at 33°C. At the logarithmic phase of growth, cells were harvested and starved of thymine for 60 min at 33°C. The cell suspension was heated at 45°C for 15 min and then divided into equal halves. One received 20 μg/ml of thymine at 0 time while the other none. Cultivation was continued at 33°C with shaking. At intervals aliquots were withdrawn as described in the legend for Table I. One unit of epimerase is defined as 1 mμmole UDPG formed per min at 25°C. (●); with thymine, (○); without thymine.

As shown in Figure 1, the epimerase derepression was completely blocked in the absence of thymine. This experiment, confirming that of FUDR, indicates that a thymine-requiring step is involved in the derepression of galactose operon after the prophage enters upon the vegetative phase. Taking account of the findings with the N mutants in the preceding section, we suggest the thymine-requiring step to represent host DNA synthesis that participates in BY-effect.

From these considerations we may propose a tentative model for the aberrant derepression of galactose operon: An 'anomalous' replication of host chromosome is initiated, irrespectively of that of λ genome, at the site of the prophage detachment through action of the enzymic systems provided by the viral cistrons (i.e., O and P). This replication causes the host operon to be derepressed either by increasing copies of the corresponding chromosomal segment or by other unknown mechanisms.

ADDENDUM: After these experiments have been accomplished, we have noted that Echols et al. (1967) have conducted a similar work in which they have shown λ sus mutants, N_7, O_{29}, and P_3 fail to cause the escape synthesis of epimerase whereas Q_{21} and R_{54} initiate the enzyme synthesis. The only discrepancy with respect to the N mutant between their results and ours has remained to be clarified.

ACKNOWLEDGMENT: We are indebted to Drs. Allan M. Campbell for supplying the λ sus mutants, Tadao Horiuchi for the heat-inducible λ mutants, and Nozomu Otsuji for the thymine-requiring bacteria. This work was supported in part by a research grant (Tokutei) from the Ministry of Education of Japan, United States Public Health Service Research Grants AM-04600 to Dr. K. Kurahashi, and GM-14350-01. Our thanks are also due to Dr. Kiyoshi Kurahashi for helpful discussions.

REFERENCES

Buttin, G., F. Jacob, and J. Monod, C. R. Acad. Sci. Paris, 250 2471 (1960).
Buttin, G., J. Mol. Biol. 7 206 (1963).
Campbell, A., Virology 14 22 (1961).
Dove, W. F., J. Mol. Biol. 19 187 (1966).
Echols, H., B. Butler, A. Joyner, M. Willard, and L. Pilarski, Edmonton Symposium on the Molecular Biology of Viruses, p.125 (1967) Academic Press.
Eisen, H. A., C. R. Fuerst, L. Siminovitch, R. Thomas, L. Lambert, L. Pereira Da Silva, and F. Jacob, Virology 30 224 (1966).
Horiuchi, T., and H. Inokuchi, J. Mol. Biol. 15 674 (1966).
Imae, Y., N. Morikawa, and K. Kurahashi, J. Biochem. 56 138 (1964).
Joyner, A., L. N. Isaacs, H. Echols, and W. S. Sly, J. Mol. Biol. 19 174 (1966).
Protass, J. J., and D. Korn, Proc. Natl. Acad. Sci., Wash. 55 1089 (1966).
Sicard, N., and R. Devoret, C. R. Acad. Sci. 255 1417 (1962).
Sussman, R., and F. Jacob, C. R. Acad. Sci. 254 1517 (1962).
Yarmolinsky, M. B., and H. Wiesmeyer, Proc. Natl. Acad. Sci., Wash. 46 1626 (1960).
Yarmolinsky, M. B., Viruses, Nucleic Acids, and Cancer, p.151 (1963), The Williams and Wilkins Co., Baltimore.