DETACHMENT OF LAMBDA PROPHAGE UPON INDUCTION

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Received August 30, 1967

The lambda prophage of a lysogenic bacterial cell is attached to the host chromosome at a specific location. In conjugation it behaves as a chromosomal marker (Jacob & Wollman, 1961). The nature of the attachment of the prophage to the host DNA is not known. Campbell (1962) had suggested that a reciprocal crossing over between the circular lambda DNA and the host chromosome leads to the insertion of the prophage into the bacterial chromosome. After induction the lambda prophage DNA is converted into vegetatively replicating DNA. Ptashne (1965) and Prell (1965) showed that prophage DNA finds its way into mature phage particles.

In this report an experimental system for the assay of prophage detachment is described. It is based on the ability of an induced Hfr cell to allow phage multiplication and at the same time to transfer its chromosome to the F^-. By the use of this experimental approach it was possible to show that protein synthesis during induction is necessary for prophage detachment and that excision regenerates intact chromosomes.

Materials and Methods

Bacterial and phage strains:

Lysogenic derivatives of Hfr 3000(thy^-, B_1^-), Hfr 3000(λ^{++}) and Hfr 3000(λ_{CI857}), were used as donors. These strains donate their genes during conjugation in the order O-leu-gal-tryp. F^- strains D1118 (leu^-, $tryp^-$, arg^-,

met$^-$, lac$^-$, gal$^-$, mal$^-$, / λ , Sm^R) lysogenic for either λ^{++} or λ h, and which were made / λ by selection for resistance to λ vir and mal$^-$ (Clark & Margulies, 1965), were used as recipients. The F$^-$ D1118 strain was originally obtained as a tryp$^-$, his$^+$ recombinant from a cross between Hfr AB1311 (tryp$^-$, his$^+$) and JC1557 (Clark, Chamberlin, Boyce & Howard-Flanders, 1966).

Phage λ^{++}, the "wild type" lambda of Kaiser (1957), λ_{CI857} which is a thermoinducible mutant (Sussman & Jacob, 1962), and λ h (Appleyard, McGregor & Baird, 1956) were used to lysogenize the parental cultures.

Mating experiments:

Hfr and F$^-$ cells were diluted from fresh overnight cultures into tryptone broth (1% Difco tryptone, 0.5 NaCl) and grown at 34°. The Hfr culture was supplemented with 10 μg/ml thymine and 1 μg/ml B_1. To initiate mating, the cultures were first concentrated by centrifugation. The F$^-$ cells were kept in the cold while the Hfr was diluted to a density of 1-2x10^8 cells/ml with pre-warmed supplemented tryptone broth and incubated for 15 min, 5 ml in a 100 ml flask, at 42° or at 33° without shaking. Conjugation was carried out at 33° in 5 ml supplemented tryptone broth in a 100 ml Erlenmeyer flask. Mating was stopped by vigorous shaking and samples were spread on selective minimal plates and incubated for 48 hr at 30°. To test for the lambda content of the recombinants, recombinant colonies were purified on appropriate selective plates. Single recombinant colonies were then picked into 0.5 ml tryptone broth and incubated for at least one hour. After transferring to 42° for 15 min, they were spot tested on either JC1557 or CR63 (Appleyard et al., 1956) at 42°. CR63 is sensitive to λ h and resistant to λ^{++}.

Results and Discussion

When an uninduced Hfr(λ_{CI857}) was mated to an F$^-$(λ^{++})/ λ the recombinants harbored either λ^{++} or λ_{CI857} (Table I, A). There were no recombinants that had lost the ability to produce lambda phages. However, when the Hfr

cells were induced by heat treatment before mating, the majority of the recombinants did not contain prophage (Table I,B). They were neither (λ^{++}) nor (λ_{CI857}), as would be expected from a cross of a non lysogenic Hfr to a

Table I. The Effect of CAP on the Detachment of Lambda upon Heat Treatment

Recombinants obtained in the experiments presented in Table II were tested for their lambda content as described in Materials and Methods. $\lambda c = \lambda_{CI857}$; λ^- = non lysogenic for lambda. 1-2% of the spot tested recombinants were not easily classified, and were omitted from the table. They appeared to contain a mixture of λ^{++} and λ_{CI857}.

Exp.	Treatment	λ Content of the Recombinants							
		leu^+	gal^+	Sm^R		leu^+	$tryp^+$	Sm^R	
	Hfr(λ_{CI857}) X F^-(λ^{++})/λ	λ^{++}	λc	λ^-	$\frac{\lambda^-}{\lambda c + \lambda^-}$	λ^{++}	λc	λ^-	$\frac{\lambda^-}{\lambda c + \lambda^-}$
A	Control, no treatment	7	93	0	0.00	23	60	0	0.00
B	Heat treated; without CAP	16	11	64	0.85	14	11	51	0.82
C	Heat treated; CAP present from -15 to 0 min	5	74	1	0.01	27	65	0	0.00
D	Heat treated; CAP added at 0 min	7	10	16	0.61	--	--	--	--
	Hfr(λ^{++}) X F^-(λh)/λ	λh	λ^{++}	λ^-	$\frac{\lambda^-}{\lambda^{++} + \lambda^-}$	λh	λ^{++}	λ^-	$\frac{\lambda^-}{\lambda^{++} + \lambda^-}$
E	Heat treated; without CAP	18	78	0	0.00	26	64	0	0.00

lysogenic F^- (Jacob & Wollman, 1961). We can therefore conclude that after temperature induction of λ_{CI857} the prophage is detached from the host chromosome. A similat conclusion was reached by Jacob & Wollman (1956) with a different experimental system.

To show that detachment following heat treatment of the Hfr is not the result of some indirect effect on the mated cells, a mating was performed between Hfr 3000(λ^{++}) and F^- D1118(λh)/λ . Both these prophages possess the wild type

repressor and are not heat induced. No detachment of the prophage was observed when Hfr(λ^{++}) was heated before mating (Table I, E). Therefore, one can conclude that the detachment of λ_{CI857} (Table I, B) was the result of thermal induction of this prophage.

The frequency of λ^{++} among the recombinants (Table I) reflects the linkage relationships between the selective markers and the lambda attachment site. Therefore, I have chosen to present the results as the ratio of the recombinants with a vacant lambda site (λ^{-}) to the total lambda sites received from the Hfr ($\lambda_{CI857} + \lambda^{-}$). The ratio of 0.82-0.85 observed in these experiments (Table I) approaches the upper limit of 1.0 which is expected upon complete induction. This suggests that lambda detachment takes place in the majority of the Hfr cells. One can also conclude that the detached prophage chromosome cannot be transferred effectively through the conjugation tube and replace the resident prophage.

Since the lambda DNA is attached to the host chromosome, it is interesting to know if its detachment affects the continuity of the bacterial chromosome. Table II(A & B) shows that both the gal marker, which is proximal to the lambda, and the tryp marker, which is distal to the lambda, were transferred, albeit at a lower level, when the Hfr cells had been induced before mating. These results do not suggest a discontinuity in the bacterial chromosome when assayed by the system described here. Table I (A & B) shows that prophage detachment indeed took place in the transferred chromosomes. This problem deserves further investigation.

It was shown by Lieb (1966) that chloramphenicol (CAP) added during heat treatment of E.coli lysogenic for λ_{CI857} prevents induction of the prophage. It was therefore expected that no detachment will take place under this condition. Indeed, inhibition of protein synthesis by CAP during heat treatment prevented lambda detachment(Table I, B & C). Induction, measured by the loss colony-forming ability (Lieb,1966), was also prevented(Table II,B&C). These results

Table II. The Effect of CAP on Induction and Mating

The mating mixtures contained 6.0×10^6 Hfr(λ_{CI857}) per ml and 1.0×10^8 $F^-(\lambda^{++})/\lambda$ per ml, or 1.5×10^7 Hfr(λ^{++}) per ml and 1.0×10^8 $F^-(\lambda h)/\lambda$ per ml. The heat treated Hfr cultures were incubated at 42° from -15 to 0 min. The non treated Hfr cultures were incubated at 33° at the same time. At 0 time mating was initiated by mixing the Hfr and F^- cells. CAP level, when present, was 25 μg/ml. Survivors were measured by plating on glycerol minimal plates containing 10 μg/ml thymine and 1 μg/ml B_1.

Exp.		Recombinants at 60 min			Viable Hfr cells at 3 min*
		leu^+ gal^+ Sm^R	leu^+ $tryp^+$ Sm^R		
	Hfr(λ_{CI857}) X $F^-(\lambda^{++})/\lambda$				
A	Control, no treatment	1.1×10^5	3.5×10^3		145%
B	Heat treated; without CAP	2.7×10^4	1.8×10^3		1.6%
C	Heat treated; CAP present from -15 to 0 min	4.3×10^4	2.5×10^3		55%
D	Heat treated; CAP added	2.7×10^4	2.5×10^2		4%**
	Hfr(λ^{++}) X $F^-(\lambda h)/\lambda$				
E	Heat treated; without CAP	5.2×10^5	2.7×10^4		--

*The number of Hfr cells at -15 min is taken as 100%.

**Viable Hfr cells at 60 min.

suggest that protein synthesis during induction is needed for prophage detachment.

It was of interest to determine whether protein synthesis during the induction period is sufficient to allow detachment. CAP added immediately after the 15 min of heat induction did not prevent prophage detachment (Table I, D). Therefore it seems that the protein(s) needed for detachment are "early protein(s)". One can speculate that detachment is a necessary step for DNA replication. Experiments are now being conducted to determine more precisely the time of synthesis of

these postulated protein(s) and the time of their action following temperature induction.

These postulated "early protein(s)" may be translated from early lambda m-RNA. It has been shown by Naono & Gros (1967) and by Green (1966) that early lambda m-RNA is synthesized in heat induced lysogenic cells even when CAP is present during the heat treatment. The results presented here suggest that the early m-RNA that was observed by Naono & Gros (1967) and by Green (1966) is transcribed from prophage DNA which is still attached to the host chromosome.

Acknowledgement

I wish to thank R. Ben-Gurion, J. Clark, and A. Ronen for the gift of strains, and D. Godinger for her assistance. The technical help of Mr. A. Srouji is also acknowledged. This work was supported in part by a grant from the Joint Research Fund of the Hebrew University-Hadassah Medical School.

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