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Studies on Polylysogens Containing λ*N*⁻*c*I⁻ Prophages

I. Control of Synthesis and Maintenance of a Large Number of Integrated λ Genomes

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Study of the control of λ DNA synthesis in polylysogens containing 20–25 copies of integrated λ*N*⁻*c*I⁻ prophages per bacterial genome shows that in absence of the functional products of *O* and *P* genes, only 7–8 copies per host genome are maintained. Introduction of constitutive mutations *v1v3* in the right operator increases the prophage genome copies to 52. In a mixed polylysogen containing both λ*N*⁻*c*I⁻ and λ*imm434N*⁻ *c*I⁻ prophages, the total number of phage genomes is also increased to 50. The levels of *cro* activity in all the polylysogens of λ vary almost proportionately with the number of prophages. All these results indicate that the maintenance of 20–25 copies of integrated phage genome in λ*N*⁻*c*I⁻ polylysogens is the result of both passive and *O* and *P* protein-mediated synthesis, and that the latter may be regulated by the *cro* gene product.

INTRODUCTION

Lysogens formed by λ*N*⁻*c*I⁻ phages after infection of a nonpermissive host at high multiplicities grow slowly, produce filamented cells, form colonies having irregular surface (Lieb, 1971), and contain about 20–25 copies of the phage genome per host genome (Lieb, 1972a). These lysogens are called converted lysogens (Lieb, 1972a). The large number of λ genomes are present in these lysogens in covalent association with the host DNA (Lieb, 1971; Mandal *et al.*, 1974). Detailed studies have also shown that the envelope-associated properties (Mandal and Barik, 1979; Barik, 1981) and the division-associated properties of the bacterial cells (Barik, 1981) are significantly altered in these lysogens.

The replication of λ DNA in its two modes of life style follows two different mechanisms. In the lytic mode, it is autonomous and requires the products of *O* and *P* genes which control both initiation from the homologous origin as well as elongation during the process; in the lysogenic mode, the above active synthesis is inhibited by the *c*I product, but the integrated prophage DNA undergoes passive replication controlled by the host-specific DNA initiator proteins which initiate from the host origin of the replicon (for a review, see Herskowitz and Hagen, 1981).

The functional *c*I product is absent in λ*N*⁻*c*I⁻ polylysogens, and hence the negative regulation of the expression of *O* and *P* genes by this gene product should not operate. In the absence of the functional *N* gene product, the expression of the two λ replication genes takes place to a limited extent (Ogawa and Tomizawa, 1968). It is not known whether in the λ polylysogens, the *O* and *P* genes of the prophages are functioning. In other words, it may be asked whether the synthesis of integrated λ DNA in the above polylysogens is only passive or it is passive as well as *O* and *P* mediated. If the *O* and *P*-mediated synthesis of λ DNA occurs simultaneously with the passive one, then the question arises as to how the maintenance of λ DNA copies in λ*N*⁻*c*I⁻ polylysogens is regulated at 20–25 per host genome? In this communication, attempts have been made to answer these questions.

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MATERIALS AND METHODS

Materials

Bacteria and bacteriophage strains. Bacteria and bacteriophage strains are listed in Table 1.

Media and solutions. Tryptone broth (TB): Bacto-tryptone, 1%; NaCl, 0.5%. *Tryptone broth with maltose* (TBM): Bacto-tryptone, 1%; NaCl, 0.5%; maltose, 0.2%; $MgCl_2$, 0.2%. *Phage dilution medium*: Bactotryptone, 0.1%; NaCl, 0.85%; $MgCl_2$, 0.2%. *SSC*: NaCl, 0.15 *M*; sodium citrate, 0.015 *M*. *Normal saline*: NaCl, 0.85%.

Methods

Growth of bacteria. Unless otherwise stated, the bacterial cultures were routinely grown at 32–34°, and the growth was followed by measuring the OD of the culture at 590 nm.

Preparation of converted lysogens. E. coli 594 was grown in TBM to about 4×10^8 cells/ml. The desired phage, at required multiplicities (usually 50–200, depending on the phage strain used), was then mixed with an aliquot of this culture and allowed to adsorb without shaking at 30–34°. After 20 min, the infected bacteria were diluted, and about 200–400 colony formers were plated and incubated at 31°. After 18–20 hr, the prospective lysogens were scored as very slow growing colonies, the cells of which appeared filamented under the microscope. These were then purified.

Determination of efficiency of plating of bacteriophages. The bacteria to be tested were grown in TBM to about 0.6 OD. Ef-

TABLE 1

BACTERIA AND PHAGE STRAINS

Strains	Alternate designation	Reference/Source
Bacterial		
C-600 (*su*$^+$)		Cross and Lieb (1970)
594 (*su*$^-$)		Cross and Lieb (1970)
594 (λ*Nsus7sus53*cI60)	594 (λ*N*$^-$cI$^-$), converted lysogen	Lieb (1972a)
594 (λ*Nsus7sus53*cI60*Osus29*)	594 (λ*N*$^-$cI$^-$*O*$^-$)	This work
594 (λ*Nsus7sus53*cI60*Psus3*)	594 (λ*N*$^-$cI$^-$*P*$^-$)	This work
594 (λ*Nsus7sus53v1v3*)	594 (λ*N*$^-$*v1v3*)	This work
594 (λ*Nsus7sus53*cI*ts2*)	594 (λ*N*$^-$cI*ts2*), convertible lysogen	Lieb (1972b)
594 (λ*Nsus7sus53*cI60, λ*imm434Nsus7sus53*cI$^-$)	594 (λ*N*$^-$cI$^-$, λ*imm434N*$^-$cI$^-$), mixed polylysogen	This work
Phage		
λcI857		Sussman and Jacob (1962)
λ*c47*	λcI$^-$	M. Lieb
λ*imm434*cI$^-$		M. Lieb
λ*imm21*cI$^+$	λ*imm21*	M. Lieb
λ*Nsus7sus53*cI60	λ*N*$^-$cI$^-$	M. Lieb
λcI*ts2Osus29*	λcI*ts2O*$^-$	M. Lieb
λcI*ts2Psus3*	λcI*ts2P*$^-$	M. Lieb
λ*Nsus7sus53*cI60*Osus29*	λ*N*$^-$cI$^-$*O*$^-$	*a*
λ*Nsus7sus53*cI60*Psus3*	λ*N*$^-$cI$^-$*P*$^-$	*a*
λ*Nsus7sus53v1v3*	λ*N*$^-$*v1v3*	*a*
λ*imm434Nsus7sus53*cI$^-$	λ*imm434N*$^-$cI$^-$	*a*
λ*imm21Osus29*	λ*imm21O*$^-$	*a*
λ*imm21Psus3*	λ*imm21P*$^-$	*a*

[a] All these phage strains were constructed by appropriate phage cross during this work.

ficiency of plating (e.o.p.) was calculated as the ratio of plaques formed on experimental bacteria to those formed on control bacteria (either 594 or C-600).

Preparation of labeled cultures. Bacteria were grown in TB to about 0.8 OD and stored at 4°. The next day, the culture was diluted to about 0.05 OD and grown to 0.2 OD. To this culture, were then added 250 μg/ml of deoxyadenosine and [^{3}H]-thymidine at the desired concentrations, and the culture was grown for 2 hr, followed by chilling in ice. The cells were then harvested and washed twice with normal saline in cold. The pelleted cells were then kept at −20° for the subsequent isolation of DNA.

Isolation of DNA. The frozen cells were thawed and suspended in Tris-HCl, pH 8.0, containing 1 m*M* EDTA. Then lysozyme was added to a final concentration of 100 μg/ml, and the mixture was incubated at 30° for 10 min. Sodium dodecyl sulfate (SDS) was then added to a final concentration of 2%, and the mixture was kept at 30° for 10 min when the cells were lysed. The DNA was then isolated from the lysate by extraction with an equal volume of water-saturated phenol. After three such extractions, the aqueous phase containing DNA was dialyzed against a large volume of 0.01 SSC containing 0.1 m*M* EDTA.

DNA-DNA hybridization. To remove the RNA present, if any, in the isolated [^{3}H]DNA solution, the latter was made 0.3 *M* with respect to NaOH and kept at room temperature for 16 hr. Then the alkali was neutralized with a calculated volume of 0.3 *M* HCl, and the solution was dialyzed against a large volume of 0.01 SSC containing 1 m*M* EDTA in cold with two more changes over a period of 36 hr. The hybridization was then done according to the method of Denhardt (1966). In each determination, less than 0.25 μg of labeled DNA was hybridized to 10 μg λ DNA fixed to a membrane filter (Scheicher and Schuell, type B-6). The control hybridization of ^{3}H-λ DNA to λ DNA on filters was used to correct experimental data in each experiment. Hybridizations in controls varied from 60 to 70%.

Calculation of λ genome copies per host genome. The λ DNA has about 1% of the length of *E. coli* DNA. If *D* is the corrected value of the percentage of total cellular DNA which hybridizes to λ DNA, then the number of λ genomes per host genome in that particular sample of DNA is given by $D/(100 - D) \times 100$.

Determination of cro activity in polylysogens. Different polylysogens were grown in TB to mid-log phase. Cells were then harvested, washed with normal saline in cold, and kept frozen at −20°. All the subsequent operations were done at 0–4°, unless otherwise stated.

Cell extracts were prepared by sonication and partially purified through the DEAE-cellulose step according to the procedure of Murotsu *et al.* (1977). The DEAE-purified proteins were concentrated by ammonium sulfate precipitation (70% saturation) and dialyzed against a buffer containing 10 m*M* Tris-HCl (pH 7.4), 10 m*M* $Mg(CH_3COO)_2$, 20 m*M* KCl, 0.1 m*M* EDTA, and 14 m*M* mercaptoethanol. Dialyzed protein solutions were then centrifuged to remove insoluble materials. *Cro* activities in the supernatants were assayed by the DNA-binding procedure of Folkmanis *et al.* (1976) using ^{3}H-λ DNA.

Determination of radioactive counts. All the radioactive counts were determined in 5 ml Omnifluor-toluene scintillation fluid in a Beckman LS-100 scintillation counter.

RESULTS

Expression of Replication Genes of λ Prophages in Converted Lysogens

Results of the experiment determining the e.o.p. of λ and other related phages on 594 (λ^+) and 594 ($\lambda N^- cI^-$) are shown in Table 2. These results verify the earlier observation of Lieb (1972a) that both λ and λ*imm*434 phages plate poorly on the latter lysogen on which λ*imm*21 can plate with 45% efficiency. The *N* function of λ*imm*21 does not complement λN^- mutants and vice versa (Herskowitz and Signer, 1970; Friedman *et al.*, 1973). So, to know if the replication genes, *O* and *P*, of the prophages are expressed at their functional levels in $\lambda N^- cI^-$ lysogens, the growth of

TABLE 2

EFFICIENCY OF PLATING OF DIFFERENT LAMBDA AND LAMBDOID PHAGES ON 594 (λ^+) AND 594 (λN^-cI^-) LYSOGENS

Phage	e.o.p.[a] on 594 (λ^+)	e.o.p.[a] on 594 (λN^-cI^-)
λcI^+ or λcI^-	—	0.0275[b]
$\lambda imm434cI^+$	1	0.03[c]
$\lambda imm21cI^+$	1	0.45[d]
$\lambda imm21cI^+P^-$	0.005[e]	0.42[d]
$\lambda imm21cI^+O^-$	0.002[e]	0.002[e]

[a] Control bacteria used were 594 for wild type, and C-600 for O^- and P^- phages. Results represent the averges of three determinations.

[b] Plaques were small, diffuse, and apparently clear.

[c] Plaques were small, diffuse, and turbid.

[d] Plaques were turbid and of heterogeneous sizes.

[e] Most of the phages in these plaques were found to be wild recombinants of $\lambda imm21$. Other details are given under Methods.

superinfecting O^- and P^- mutants of $\lambda imm21$ by complementation was studied. The results in Table 2 show that on 594 (λN^-cI^-), while the e.o.p. of $\lambda imm21P^-$ and $\lambda imm21P^+$ are equal, that of $\lambda imm21O^-$ is very negligible. Also, the burst size of $\lambda imm21P^-$ in the converted lysogens was 50 per infective center while that of $\lambda imm21O^-$ was only 0.3 (results not shown). These indicate that in the λN^-cI^- polylysogens, only the *P* product and not the *O* product of λ is present at a functional level.

Products of O and P Genes of the Prophages Are Necessary for the Synthesis and Maintenance of 20–25 Copies of Integrated λ Genome in the Converted Lysogens

Lusky and Hobom (1979) described a mini replication system for λdv DNA which does not require the *O* gene product. So, the failure to detect *O* gene function when *P* function could be detected by complementation in 594 (λN^-cI^-) raises a pertinent question as to whether the *O* or both the *O* and *P* gene products of λ are nonessential for the maintenance of 20–25 copies of integrated prophages. This led us to prepare lysogens by infecting 594 separately with $\lambda N^-cI^-O^-$ and $\lambda N^-cI^-P^-$ phages using very high multiplicities and to study their properties. The e.o.p. of different mutants of λ and $\lambda imm21$ on these lysogens as well as the number of λ genomes per host genome in them were determined. The results are presented in Table 3. It appears that λcI857 plates much better on both 594 ($\lambda N^-cI^-O^-$) and 594 ($\lambda N^-cI^-P^-$) than on 594 (λN^-cI^-). On 594 ($\lambda N^-cI^-O^-$), the e.o.p. of $\lambda cIts2P^-$ and λcI857 are equal while on 594 ($\lambda N^-cI^-P^-$), the e.o.p. of $\lambda cIts2O^-$ is 50% of the O^+ phage. While the platings of $\lambda imm21$ on both 594 ($\lambda N^-cI^-O^-$) and 594 ($\lambda N^-cI^-P^-$), and of $\lambda imm21P^-$ on the former lysogen are nearly as efficient as those of the same respective phages on 594 (λN^-cI^-), $\lambda imm21O^-$ shows much higher e.o.p. on 594 ($\lambda N^-cI^-P^-$) than on 594 (λN^-cI^-). Both the lysogens, 594 ($\lambda N^-cI^-O^-$) and 594 ($\lambda N^-cI^-P^-$), contain seven copies of λ genome per host genome.

Number of Copies of Prophage Genome in Converted Lysogens Can Be Increased by Increasing the Expression of O and P Genes of the Prophages

One may reasonably ask why the maintenance level of λ genome copy in the converted lysogens is at 20–25? Eisen *et al.* (1968) showed that $imm\lambda N^+$ phage forms plaques on derepressed lysogens containing λN^-O^- (or P^-). It is also known that the *cro* product of λ having greater affinity for the *prm* promoter, shows the anti-immune property (Eisen *et al.*, 1970; Oppenheim *et al.*, 1970) at relatively low concentrations by interfering with the leftward transcription of *cI* gene from *prm* (Johnson *et al.*, 1978), but at relatively high concentrations, it also inhibits both leftward and rightward transcriptions from the P_L and P_R promoters, respectively, of the λDNA (Takeda *et al.*, 1977; Johnson *et al.*, 1978; Takeda, 1979). The formation of clear plaques by $\lambda cIts$ phages at permissive temperature on $\lambda N^-cI^-O^-$ and $\lambda N^-cI^-P^-$ polylysogens (see footnote to Table 3) indicates the anti-immune state of these ly-

TABLE 3

PLATING CHARACTERS AND λ GENOME CONTENTS OF 594 (λN^-cI^-), 594 ($\lambda N^-CI^-O^-$), AND 594 ($\lambda N^-cI^-P^-$) LYSOGENS

Lysogen	e.o.p.[a]						Copies of λ genome per host genome
	λcI857[b]	λcI*ts*2O^-[b]	λcI*ts*2P^-[b]	λ*imm*21	λ*imm*21O^-	λ*imm*21P^-	
594 (λN^-cI^-)[d]	0.02	0.001	0.03	0.46	0.002	0.42	22
594 ($\lambda N^-cI^-O^-$)[c,d]	0.67	—	0.67	0.41	—	0.57	7[e]
594 ($\lambda N^-cI^-P^-$)[c,d]	0.59	0.30	—	0.50	0.57	—	7[e]

[a] C-600 was used as control bacterium. Results represent the averages of three determinations.

[b] Plaques produced by these phages on all lysogens were of perfectly clear phenotype.

[c] These lysogens were prepared by the procedure described under Methods, using input m.o.i. of 200. Lysogenization frequency was 0.2 to 0.4%. These lysogens grew faster than 594 (λN^-cI^-), producing cells having uniform size of about three times the length of 594 cells.

[d] These lysogens occasionally produce deconverted bacteria which lose simultaneously the prophage copies and the converted morphology. These lysogens need be selected frequently and the selected lysogens show the number of prophages constant around the values shown in the last column as above.

[e] In absence of either *O* or *P* functions, these prophages are maintained only by passive replication indicating that they remain integrated in the bacterial genome. Other details are given under Methods.

sogens. So, a difference in the levels of *cro* product might account for the difference in e.o.p. of λ on lysogens containing λN^- versus λN^-O^- or P^- prophages. The presence of *v*1*v*3 mutations would allow more transcription of *O* and *P* genes from O_RP_R which would increase the copy number of prophages in the converted lysogens. Results presented in Table 4 show that in 594 (λN^-v1v3), the λ genome copies per host genome have increased to 52. Also the e.o.p. of λ on λN^-v1v3 lysogens is much lower than that on λN^-cI^- lysogens. Koga and Horiuchi (1971) showed that $\lambda N^-cI^-O^-$ prophages containing mutations similar to *v*1*v*3 in the right operator also interfere with the plating of $imm\lambda N^+$.

The *cro* protein of *imm*λ does not interact with λ*imm*434 operators (Takeda *et al.*, 1977; Takeda, 1979). If *cro* regulates the copy number of prophages by regulating the expression of *O* and *P* genes, then in mixed polylysogens containing both λN^-cI^- and $\lambda imm434N^-cI^-$ prophages, each *cro* would regulate the expression of *O* and *P* genes from the homologous promoters. This would show some additive effects on the levels of both *O* and *P* proteins and on the prophage copy numbers. Results in Table 4 show that in such polylysogens, the total number of prophage genomes (λ plus λ*imm*434) has increased to 50. However, the e.o.p. of λ on these lysogens is nearly equal to that on λN^-cI^- lysogens.

Cro Level Varies with the Number of Prophages in the Polylysogens

The foregoing results suggest that the levels of *cro* product are different in these different polylysogens. The measurement of *cro* activity in 594 ($\lambda N^-cI^-O^-$), 594 ($\lambda N^-cI^-P^-$), 594 (λN^-cI^-), and 594 (λN^-v1v3) directly supports the above conclusion. It could be seen from the data in Table 5 that the *cro* activity increases

TABLE 4

PROPHAGE GENOME COPIES AND PLATING BEHAVIOR TOWARD LAMBDA OF 594 (λN^-cI^-), 594 (λN^-v1v3)[a], AND 594 (λN^-cI^-, $\lambda imm434N^-cI^-$)[b] LYSOGENS

Lysogen	Total number of prophage genomes per host genome	e.o.p. of λ
594 (λN^-cI^-)	23	0.01
594 (λN^-v1v3)	52[c]	0.0003
594 (λN^-cI^-, $\lambda imm434N^-cI^-$)	50[c]	0.02

[a] This lysogen was prepared by infecting 594 with λN^-v1v3 at an m.o.i. of 100 according to the procedure described under Methods. The frequency of formation of lysogens having converted phenotype was 50%. This lysogen was relatively stable, grew slower than and produced cells with average length greater than 594 (λN^-cI^-).

[b] This lysogen was prepared by using an input m.o.i. of 50 for each of the two phages. Lysogen was phenotypically indistinguishable from 594 (λN^-cI^-) and was found to contain the two prophages in the ratio of 3:2 as was determined by free phage analysis of the purified culture (Lieb, 1967).

[c] Most of these prophage genomes remain in covalent association with the bacterial genome which was shown by the fact that only 1 to 3% of total cellular DNA could be recovered as plasmid by following the procedure of Mandal *et al.* (1974). Other details are given under Methods.

TABLE 5

cro LEVELS IN DIFFERENT LYSOGENS

Lysogens	*cro* levels[a] (units/mg protein)
594 ($\lambda N^- cI^- O^-$)	0.19
594 ($\lambda N^- cI^- P^-$)	0.21
594 ($\lambda N^- cI^-$)	0.53
594 ($\lambda N^- v1 v3$)	1.62

[a] Procedures are described under Methods. Two hundred-fold excess of chicken blood DNA was included in each assay mixture. In blank experiment, extract from nonlysogenic 594 was used, and the counts retained on filter were subtracted from each experimental value. Cold λ*imm*434 DNA did not compete with the ^{3}H-λ DNA for the binding activity under the conditions of assay. Several assays were done with each extract using varying amounts of protein, and the *cro* activity was calculated from the linear region of the DNA-binding activity vs protein concentration curve. One unit of *cro* activity has been defined as the amount of protein which retains 0.1 μg of ^{3}H-λ DNA on filter.

with the number of prophage copies. The gradual decrease in the e.o.p. of λ with the increase of prophage copies (Tables 3 and 4) in these lysogens can, therefore, be correlated with the levels of *cro* activity present in them.

DISCUSSION

The polylysogen, 594 ($\lambda N^- cI^-$), contains 20–25 copies of λ genome in the integrated state per host genome (Mandal *et al.*, 1974), and so these prophage genomes will replicate passively as part of the host replicon. Results presented in this paper show that the synthesis and maintenance of this large number of integrated λ genomes also require active synthesis. We have also presented evidence that the active synthesis is controlled by the *cro* gene product of the prophages.

Though the *O* and *P* gene products are needed for the maintenance of 20–25 copies of λ genome, only the *P* product could be detected at the functional level by complementation study (Table 2). Kleckner (1977) has shown that λN^- phage does not complement *O* function for the growth of a coinfecting $\lambda N^- Osus29$ phage. Whether the noncomplementation of *O* function in 594 ($\lambda N^- cI^-$) for the growth of superinfecting $N^+ Osus29$ phages is due to the fact that the *O* protein has a *cis*-acting character (Kleckner, 1977), and that it has very short half-life (Wyatt and Inokuchi, 1974), is not clear. But, the *O* protein synthesized from seven copies of prophages in 594 ($\lambda N^- cI^- P^-$) complements for the growth of both $\lambda cItsOsus29$ and $\lambda imm21 Osus29$ phages (Table 3) producing homologous and heterologous *N*, respectively. The fact that $\lambda N^- cI^- P^-$ could complement $\lambda imm21 O^-$ in a mixed infection (Chattopadhyay and Mandal, unpublished results) suggested that *O* could act in *trans*. It may be that in $\lambda N^- cI^-$ lysogens in the absence of *N* function and in the presence of higher *cro* level (Table 5), the *O* and *P* genes are expressed with reduced frequency, and these two proteins, immediately after their limited synthesis, form a replicating complex with the integrated λ DNA; such *O* protein, after completing the round of λ DNA synthesis, is inactivated very rapidly (Klinkert and Klein, 1978) and, hence, is not able to complement the superinfecting $\lambda imm21 O^-$ phage. On the other hand, in the presence of a subinhibitory level of *cro* protein, the expression of *O* gene is relatively more frequent in 594 ($\lambda N^- cI^- P^-$) lysogens; in the absence of its own *P* protein, this *O* protein interacts with the *P* protein synthesized from the superinfecting $\lambda imm21 O^-$ or $\lambda cItsO^-$ genome and becomes engaged in a replicating complex either with the resident prophage DNA or with the superinfecting phage DNA, and hence the complementation is observed.

The *O*, *P*, and *cro* functions are indispensable for the maintenance of both λ*dv* (Berg, 1974; Matsubara, 1976) and λN^- (Kleckner and Signer, 1977) plasmids. When the expression of *O* and *P* genes of the integrated $\lambda N^- cIts$ prophages in a convertible lysogen is prevented by the *c*I product at 30° (Mandal *et al.*, 1974), or when the $\lambda N^- cI^-$ prophages also have mutation in either *O* or *P* gene (Table 3), then the prophage copies are maintained by passive replication at 7–8 per host genome; but when the derepressed λN^- pro-

phages are in their O^+ and P^+ states, then these lysogens can maintain 20–25 copies of integrated λ genome. So, the maintenance of this large number of prophage genomes in $\lambda N^- cI^-$ lysogens also requires the active synthesis in addition to the passive one. The *cro* product regulates the λ*dv* copy number by controlling rightward transcription from $O_R P_R$ (Matsubara and Takeda, 1975; Takeda *et al.*, 1975). In the converted lysogens also, the *cro* protein seems to control the number of integrated λ genomes. In these lysogens, and in $\lambda N^- v1v3$ lysogens, the *cro* protein synthesized from 22 and 52 copies of prophage genome, respectively, are possibly just sufficient to regulate the maintenance of these copy numbers. The titration of *cro* protein during passive and active syntheses of integrated phage DNA may temporarily release the P_R promoter from *cro* block and thus maintains the active synthesis in these lysogens. A similar mechanism has been proposed by Murotsu and Matsubara (1980) to explain the control of synthesis and maintenance of λ*dv* DNA copies by *cro* protein. However, in the absence of the *c*I protein, whether *cro* is essential for the maintenance of polylysogeny by $\lambda N^- cI^-$ phages, is not known at this stage.

Initiation of λ DNA synthesis at the phage origin is dependent on two factors: (i) The activation of the origin by rightward transcription from the P_R promoter (Dove *et al.*, 1971) and (ii) the availability of *O* and *P* proteins at the time of initiation (Furth *et al.*, 1978). The copy number of λ*dv* DNA in a carrier bacterium can be increased by increasing the frequency of activation of the origin of this plasmid DNA (Berg and Kellenberger-Gujer, 1975). It is not known whether the synthesis of integrated λ DNA in converted lysogens is similarly controlled at the level of transcriptional activation of the phage origin. The transcriptional condition at $O_R P_R$ in both the λ*dv* DNA and the integrated $\lambda N^- v1v3$ DNA (both contain *v1v3* mutations at this operator-promoter region) is the same. The negative regulation of transcription by the *cro* protein at P_R propmoter of λ is much less effective due to the presence of mutations, *v1v3* (Takeda *et al.*, 1977; Johnson *et al.*, 1978) and so, the initiation of transcription from P_R increases which, in its turn, results in proportionate increase of transcription passing beyond *t*R1 into the *c*II-*O*-*P* region under N^- condition and thus elevates the levels of both *O* and *P* products. Under such conditions, λ copies will have a greater probability of being increased in 594 ($\lambda N^- v1v3$). It may also be noted that due to the constitutive expression of *cro* gene (Kumar and Szybalski, 1970), this protein is maintained in $\lambda N^- v1v3$ lysogens at a level three times higher than that in $\lambda N^- cI^-$ lysogens (Table 5). This higher level of *cro* would reduce the e.o.p. of λ on the former lysogens relative to that on the latter (Table 4).

How 20–25 copies of the λ genome are arranged in the host chromosome is not yet clear. Lysogens morphologically similar to converted lysogens could not be obtained by infecting 594 with $\lambda N^- cI^- O^-$, $\lambda N^- cI^- P^-$, $\lambda N^- v1v3O^-$, or $\lambda N^- v1v3 P^-$ phages at an m.o.i as high as 200 (Chattopadhyay and Mandal, unpublished results). Lysogens of these phages selected as the ones forming colonies larger than the converted lysogens but smaller than the nonlysogenic bacteria, contained seven copies of λ genome per host genome (Table 3 and unpublished results). The convertible lysogen, 594 ($\lambda N^- c$Its), also contain 6–8 copies of integrated prophages (Lieb, 1972b) which rapidly increase to 20–25 after heat induction (Mandal *et al.*, 1974). The fact that the *O* and *P* functions are necessary for the maintenance of 20–25 copies of integrated λ genomes in $\lambda N^- cI^-$ lysogens supports the earlier view (Mandal *et al.*, 1974) about the possible arrangement of λ genomes in the bacterial chromosome. This model may be restated as follows: In both 594 ($\lambda N^- cI^-$) and 594 ($\lambda N^- v1v3$) lysogens, the number of integrated λ genomes being present in linear array in tandem or otherwise in the bacterial DNA is 7–8 copies. Each of these 7–8 prophage genomes undergoes additional replication on an average of one round and a half in the former, and two rounds and a half in the latter by *O* and *P* mediated synthesis per round of passive synthesis

forming local "bubbles" or "puffs"; and such successive rounds of active synthesis, the rate of which being dependent on the transcriptional conditions at O_RP_R, could maintain 20–25 and 50–52 copies of integrated λ genome in the above two respective lysogens. Studies are being made to confirm this proposition and also to determine whether all the integrated λ genomes are present at the same place in tandem sequence, or they are randomly distributed along the whole length of the bacterial chromosome.

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