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Studies on Polylysogens Containing $\lambda N^{-}cI^{-}$ Prophages

II. Role of High Multiplicities in Lysogen Formation by $\lambda N^{-}cI^{-}$ Phage

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Results of the experiments presented in this paper show that $\lambda N^{-}cI^{-}$ phage can lysogenize a nonpermissive host *Escherichia coli* when it infects at very high multiplicities (around 100), and $\lambda N^{-}cI^{-}cII^{-}$ and $\lambda cIII^{-}N^{-}cI^{-}$ lysogenize poorly at similar high multiplicities. The latter two phages lysogenize with appreciable frequency when either $\lambda N^{-}cI^{-}$ or λ*int*-c$N^{-}cI^{-}cII^{-}$ is used as helper. The phages, $\lambda N^{-}cI^{-}$, $\lambda N^{-}cI^{-}cII^{-}$, and $\lambda cIII^{-}N^{-}cI^{-}$ can lysogenize also at relatively low m.o.i. of 20 in presence of the above λ*int*-c helper, and the λ*int*-c$N^{-}cI^{-}cII^{-}$ phage alone forms converted lysogens at an m.o.i. as low as 12. All these results suggest that the establishment of prophage integration by $\lambda N^{-}cI^{-}$ is positively regulated, like $\lambda N^{+}cI^{+}$ phage, by the cII/cIII-promoted expression of the *int* gene of λ, and under the N^{-} condition, high multiplicities are needed to provide optimum levels of cII and cIII products, especially the latter.

INTRODUCTION

Lysogens of $\lambda N^{-}cI^{-}$ phage in a nonpermissive host *Escherichia coli* are called converted lysogens (Lieb, 1971), and they contain 20-25 copies of the prophage genome per host genome (Lieb, 1972). The facts that (1) the formation of converted lysogens by the above phage requires phage *int* function (Lieb, 1971), (2) about 90% of the prophage genomes could not be separated from the host DNA (Mandal *et al.*, 1974), and (3) $\lambda N^{-}cI^{-}$ phage having a mutation in either the *O* or *P* gene can form polylysogens which could maintain 7-8 copies of prophage genome by passive replication (Chattopadhyay and Mandal, 1982) support the view that most, if not all, of the 20-25 copies of $\lambda N^{-}cI^{-}$ genome remain in covalent association with the host genome in the converted lysogens.

The lysogenization by $\lambda N^{-}cI^{-}$ is dependent on high multiplicities of infection (m.o.i.) of the phage (Lieb, 1971). For the establishment of lysogeny by wild-type λ, expressions of two genes, cI and *int*, are essential, and these expressions are positively and coordinately controlled at *pre* and *pI* promoters respectively by the cII gene product (see Herskowitz and Hagen, 1980, for a review). The maintenance of the prophages in $\lambda N^{-}cI^{-}$ polylysogens in absence of the cI product is controlled by the *cro* product (Chattopadhyay and Mandal, 1982). It is not known whether the establishment of integration of phage genomes leading to polylysogeny by $\lambda N^{-}cI^{-}$ in the absence of cI and *N* functions is effected by the above control of *int* expression by the cII gene product. If this happens, the question may be asked whether the need for very high m.o.i. of $\lambda N^{-}cI^{-}$ phage for the polylysogen formation has any relation with the cII/cIII mediated control of *int* expression. In this paper, an attempt has been made to answer these questions.

MATERIALS AND METHODS

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

Media and solutions. Compositions of tryptone broth with maltose (TBM), phage dilution medium (DIL), and tryptone agar

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TABLE 1

BACTERIA AND PHAGE STRAINS

Strains	Alternate designation	Reference/Source
Bacterial		
594 (su^-)		Cross and Lieb (1970)
Phage		
λ*Nsus7sus53*	λN^-	M. Lieb
λcI*sus34*	λcI$^-$	M. Lieb
λ*c*II68	λcII$^-$	H. Echols
λ*c*III67	λcIII$^-$	D. I. Friedman
λ*int*-cNsus7*sus*53cI857	λ*int*-cN^-cI857	D. K. Chattoraj
λ*v*1*v*3	—	M. Lieb
λ*c*17	—	H. Echols
λ*Nsus7sus*53cI*sus*34	λN^-cI$^-$	[a]
λ*Nsus7sus*53cI*sus*34cII68	λN^-cI$^-$cII$^-$	[a]
λcIII67*Nsus7sus*53cI*sus*34	λcIII$^-$$N^-cI^-$	[a]
λ*int*-c*Nsus7sus*53cI*sus*34	λ*int*-cN^-cI$^-$	[a]
λ*int*-c*Nsus7sus*53cI*sus*34cII68	λ*int*-cN^-cI$^-$cII$^-$	[a]
λ*Nsus7sus*53cI60*v*1*v*3	λN^-cI$^-$*v*1*v*3	[a]
λ*Nsus7sus*53cI*sus*34c17	λN^-cI$^-$c17	[a]
λ*Nsus7sus*53cI857	λN^-cI857	[a]

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

(TA) are given in Chattopadhyay and Mandal (1982).

Methods. Bacteria were routinely grown in TBM at 32° to 0.6 OD (measured at 590 nm) and were infected by phage at desired m.o.i. After allowing 20 min for adsorption, about 300-400 colony formers were plated on TA plates and incubated at 30°. After 18-20 hr, the prospective lysogens were scored as very slow growing colonies, the cells of which appeared filamented under microscope.

RESULTS

Polylysogen Formation by λN^-cI$^-$ Phages is Dependent on Very High Multiplicities of Infection and on the Presence of Functional cII and cIII Genes

Wild-type λ lysogenizes with appreciable efficiency at m.o.i. as low as 1, but this is increased when an m.o.i. greater than 1 is used (Kourilsky, 1973; Knoll, 1979). The results presented in Table 2 (lines 1-4) show that with λN^-cI$^-$ phage, polylysogens are not formed at the m.o.i. of 25 or less, and at the m.o.i. of 50, the frequency of polylysogen formation is 2% which becomes 13% at the m.o.i. of 100. With λN^-cI$^-$cII$^-$ and λcIII$^-$$N^-cI^-$, the frequencies are 0.08 and 1%, respectively, both at the m.o.i. of 100 (lines 5 and 6, Table 2). This suggests that both *c*II and *c*III gene functions are essential for the polylysogen formation by λN^-cI$^-$ phage. Since the lysogenization frequency with λN^-cI$^-$cII$^-$ is much lower than that with λcIII$^-$$N^-cI^-$, it may be assumed that the cII gene product probably plays a more direct role than the *c*III gene product in the above lysogenization process. Furthermore, when λN^-cI$^-$ contains either *c*17 or *v*1*v*3 mutations both of which cause constitutive expression of *c*II gene (Herskowitz and Hagen, 1980), then the lysogenization frequency is markedly increased (lines 7-9, Table 2). The fact that the polylysogens formed by λN^-cI$^-$cII$^-$ as well as by λcIII$^-$$N^-cI^-$ are stable (see foot note to Table 2) suggests that the λN^-cI$^-$ polylysogens, once formed, do not require *c*II and *c*III products for maintenance.

TABLE 2

EFFECT OF MULTIPLICITIES AND OF HAVING MUTATION IN cII AND cIII GENES ON THE FREQUENCY OF LYSOGEN FORMATION BY λN^-cI^- PHAGE

Infecting phage	m.o.i.	Survival fraction	Frequency[a]
λN^-cI^-	25 or less	1.00	nil
λN^-cI^-	50	1.00	2
λN^-cI^-	75	1.00	6
λN^-cI^-	100	1.00	13
$\lambda N^-cI^-cII^-$	100	1.00	0.08[b]
$\lambda cIII^-N^-cI^-$	100	1.00	1[b]
λN^-cI^-v1v3	50	1.00	20[b]
λN^-cI^-c17	50	0.60	9[c]
λN^-cI^-c17	100	0.30	27[c]

[a] The host bacterium used was *E. coli* 594. Lysogenization frequency was calculated as percentage of total cells infected that were scored as converted lysogens. The lysogenization frequencies of λN^-cI^+ phage (these were determined using λN^-cI857 at 30°) at multiplicities of 50, 75, and 100 were respectively 0.4, 1, and 2% under identical conditions. Other details are given under Materials and Methods.

[b] These lysogens were identical with those of λN^-cI^- with respect to converted morphology, stability, and viability.

[c] These lysogens were identical with λN^-cI^- lysogens with respect to converted morphology, but they were relatively less stable than the latter.

$\lambda N^-cI^-cII^-$ Phage Can Form Polylysogens in Presence of Low Multiplicities of λN^-cI^- Helper

To distinguish between the roles of cII and cIII genes, the effect of a low m.o.i. of λN^-cI^- helper on polylysogen formation by $\lambda N^-cI^-cII^-$ and $\lambda cIII^-N^-cI^-$ phages was studied. In both the cases, the total m.o.i. was kept at or around 100. The results presented in Table 3 show that the mixed infection by $\lambda N^-cI^-cII^-$ and $\lambda N^-cI^-cII^+$ phages in the m.o.i. ratio of 99:1 results in increase of the lysogenization frequency to 6%, which increases further to the value obtained with λN^-cI^- phage alone (Table 2) on increasing the m.o.i. of the helper to 8. Under identical conditions, the same helper phage does not show such helper action towards the $\lambda cIII^-N^-cI^-$ phage (lines 7-10, Table 3). These results suggest that the low level of cII activity provided by 1-8 copies of λN^-cI^- helper can promote polylysogen formation efficiently by $\lambda N^-cI^-cII^-$ in presence of a high level of cIII activity synthesized from 99 to 92 copies of the latter genome; but under identical conditions of the experiment, the low level of cIII activity supplied by 1-8 copies of λN^-cI^- helper genome cannot effect polylysogen formation so efficiently by $\lambda cIII^-N^-cI^-$ in presence of a high level of cII protein synthesized from 99 to 92 copies of the latter genome.

Polylysogen Formation by $\lambda N^-cI^-cII^-$ Phage Also Takes Place Efficiently in the Presence of λint-c$N^-cI^-cII^-$ Helper

The foregoing results indicate that both the cII and cIII gene products are indispensable for polylysogen formation by λN^-cI^- phage but not for the maintenance of polylysogeny. Then, the question arises as to whether the ultimate role of these two genes is to promote *int* gene expression from the *p*I promoter. It is known that the transcription of the *int* gene becomes cII independent when a constitutive mutation is present in the *p*I promoter region as in λ*int*-c phage (Shimada and Campbell, 1974). So, to clarify the above point, the effect of λint-c$N^-cI^-cII^-$ helper on polylysogen formation by $\lambda N^-cI^-cII^-$ phage was studied. The data presented in Table 4 clearly show that when *int* function is provided by a low m.o.i. of the helper, the lysogenization frequency with $\lambda N^-cI^-cII^-$

TABLE 3

EFFECT OF USING $\lambda N^{-}cI^{-}$ HELPER ON THE FREQUENCY OF FORMATION OF LYSOGEN BY $\lambda N^{-}cI^{-}cII^{-}$ AND $\lambda cIII^{-}N^{-}cI^{-}$ PHAGES

m.o.i.			
Phage 1		Phage 2 (helper)	
$\lambda N^{-}cI^{-}cII^{-}$	$\lambda cIII^{-}N^{-}cI^{-}$	$\lambda N^{-}cI^{-}$	Frequency[a]
100	—	—	0.08
99	—	1	6
98	—	2	8
96	—	4	10
92	—	8	15
84	—	16	15
—	100	—	1
—	98	2	1
—	96	4	1
—	92	8	1
—	84	16	4
—	—	16	Nil

[a] The host bacterium used was 594. Frequency was calculated as percentage of total cells infected that were scored as converted lysogens. In all the cases, survival was 100%. All these lysogens were identical with $\lambda N^{-}cI^{-}$ lysogens with respect to converted morphology, stability, and viability, and were found to contain 23–25 copies of prophage genome per host genome. For other details, see Materials and Methods.

becomes as high as 56% (line 2, Table 4). Also, it is interesting to note that when *int* function is supplied by the *int*-constitutive helper in absence of both *c*II and *N* functions, lysogenization occurs also with high frequency of 45–47% even at a total m.o.i. of 25. It was also observed that both $\lambda N^{-}cI^{-}$ and $\lambda cIII^{-}N^{-}cI^{-}$, at the m.o.i. of 25, could form polylysogens with appreciable frequency in presence of low m.o.i. of either $\lambda int\text{-}cN^{-}cI^{-}$ or $\lambda int\text{-}cN^{-}cI^{-}cII^{-}$ helper (data not shown). Furthermore, the infection by $\lambda int\text{-}cN^{-}cI^{-}cII^{-}$ alone at a relatively low m.o.i. of 12 results in polylysogen formation to the extent of 5% which increases further with m.o.i. (lines 6–9, Table 4).

DISCUSSION

The fact that $\lambda int^{-}N^{-}cI^{-}$ phage lysogenizes poorly (Lieb, 1971) suggests that like wild-type λ, the *int* function is essential for the lysogenization by this mutant phage. How the expression of *int* gene is regulated in absence of *N* function was not clear. The results presented in this paper show that the presence of a mutation in *c*II or *c*III gene in $\lambda N^{-}cI^{-}$ phage drastically affects the lysogenization process (Table 2) and that both *c*II and *c*III genes could be spared if *int* function is supplied by an *int*-constitutive helper phage (Table 4). These results suggest that like λN^{+} phage (Herskowitz and Hagen, 1980), the expression of *int* gene is positively regulated by the *c*II and *c*III gene products of λ under N^{-} and cI^{-} conditions.

The present status of our knowledge about the regulation of *int* gene expression via the *c*II/*c*III circuit is that the action of *c*II product is more direct and that of *c*III gene product is indirect in the process

TABLE 4

EFFECT OF USING $\lambda int\text{-}cN^{-}cI^{-}cII^{-}$ HELPER ON THE FREQUENCY OF FORMATION OF LYSOGEN BY $\lambda N^{-}cI^{-}cII^{-}$ PHAGE

m.o.i.			
$\lambda N^{-}cI^{-}cII^{-}$	$\lambda int\text{-}cN^{-}cI^{-}cII^{-}$	Survival fraction	Frequency[a]
100	—	1.00	0.1[b]
98	5	1.00	56.0[b]
30	5	0.80	45.0[b]
23	2	0.90	47.0[b]
20	5	0.80	45.0[b]
—	12	0.70	5.0[b]
—	25	0.62	8.0[c]
—	62	0.50	10.0[c]
—	130	0.47	26.6[c]

[a] The host bacterium used was *E. coli* 594. Frequency was calculated as percentage of total cell infected that were scored as converted lysogens. For other details, see Materials and Methods.

[b] These lysogens were identical with $\lambda N^{-}cI^{-}$ lysogens with respect to converted morphology, stability, and viability.

[c] These lysogens were identical with those of $\lambda N^{-}cI^{-}$ with respect to converted morphology only producing very tiny colonies which were barely visible. When they were transferred to TA plates or to TB, they did not grow anymore; this indicated that they became nonviable after a few cycles of growth.

(Herskowitz and Hagen, 1980). The *c*II protein activates the *p*I promoter thereby facilitating leftward transcription of *int* gene by RNA polymerase from this promoter, while the *c*III protein antagonizes a host protein defined by the *hfl* mutation (Belfort and Wulff, 1974) which otherwise inhibits the above transcription by inactivating the *c*II protein. Since the *c*III protein has been assumed to be unstable (Reichardt, 1975), it seems likely that the *c*II-promoted transcription of *int* gene necessitates an optimum level of *c*III protein which can antagonize completely the host *hfl* function, and thereby stabilizing the *c*II protein. Then the latter, even at relatively low concentrations can promote *int* expression from the *p*I promoter. Alternatively, in absence of an optimum level of *c*III protein, a relatively high level of *c*II protein provided by the constitutive expression of the latter may also promote *int* expression from the *p*I promoter. This view is supported by the following facts: (*i*) the $\lambda N^- cI^- cII^-$ phage at the m.o.i. of 100 forms polylysogens with much improved frequency in presence of an m.o.i. of 1 of $\lambda N^- cI^- cII^+$ helper, but the $\lambda cIII^- N^- cI^-$ phage is unable to do so under identical conditions (Table 3); (*ii*) the lysogenization frequency of wild-type λ is much better with multiple infections (Kourilsky, 1973; Knoll, 1979); (*iii*) constitutive synthesis of *c*II product by $\lambda N^- cI^- c17$ and $\lambda N^- cI^- v1v3$ results in the formation of polylysogens with relatively high frequency at relatively low m.o.i. of these phages (Table 2); and (*iv*) when *int* function is provided by λint-$cN^- cI^- cII^-$ helper, the $\lambda N^- cI^- cII^-$ can form polylysogens not only at high m.o.i. but also at low m.o.i. of 25, and also λint-$cN^- cI^- cII^-$ alone forms polylysogens with a frequency of 5 at relatively low m.o.i. of 12 (Table 4). Taken together, it may be concluded that for lysogenization by $\lambda N^- cI^-$ phage, high m.o.i. is needed for providing an optimum level of *c*III product that is sufficient to inactivate the host *hfl* protein, thereby stabilizing the *c*II protein, and the latter under this condition, even at low concentrations, can promote *int* expression from the *p*I promoter. The possible role of such super-high m.o.i. of $\lambda N^- cI^-$ phage in providing sufficiently high level of *cro* repressor for the establishment of *cro*-mediated repression may be ruled out by the fact that the converted lysogeny is established when the host is infected either by λint-$cN^- cI^- cIII^-$ at m.o.i. of 12 or by a mixture of $\lambda N^- cI^-$ and λint-$cN^- cI^- cII^-$ at a total m.o.i. of 25 and that the constitutive expression of *c*II gene from $\lambda N^- cI^- c17$ genome helps this phage to form polylysogens with a relatively high frequency at relatively low m.o.i. (Table 2). Lysogenization by $\lambda N^- cI^+$ also needs very high m.o.i. (see footnote to Table 2 and Lieb, 1971).

In absence of *N* function, about 90% of the transcriptions initiated from *o*L*p*L and 50% of those initiated from *o*R*p*R are terminated at *t*L1 and *t*R1, respectively (Rosenberg *et al.*, 1978; Salstrom and Szybalski, 1978; Salstrom *et al.*, 1979). In wild-type λ infections, increasing the m.o.i. upto 10 increases the lysogenization frequency (Kourilsky, 1973; Knoll, 1979). As *p*L is about 10 times more efficient than *p*R in transcription initiation (Johnson *et al.*, 1978), then assuming that the efficiency of translation of both *c*II and *c*III messages is the same, the amounts of *c*III product will be expected to be about 10 times higher than that of the *c*II product in a λN^+-infected bacterium. The lysogenization efficiency is maximum with about 10 m.o.i. of $\lambda N^+ cI^+$ phage (Kourilsky, 1973), and hence the *c*III product synthesized from those 10 λN^+ phages may be considered optimum for the above purpose. If one considers the above-mentioned differential effect of transcription termination at *t*L1 and *t*R1 in absence of *N* function, one can calculate the amounts of *c*III product synthesized from 100 infecting $\lambda N^- cI^-$ phages to be nearly equal to that synthesized from 10 $\lambda N^+ cI^+$ phages. So, to provide the optimum amounts of *c*III product, a bacterial culture needs be infected by $\lambda N^- cI^-$ phage at m.o.i. of 100 or more (Table 2). The fact that the "super *c*III" mutants of λ lysogenize with high frequency at an m.o.i. of 1 or less (Knoll, 1979; 1980) and that λ *can* mutants lysogenize even in absence of *c*III (Jones and Herskowitz, 1978) suggests that the above anticipated high amounts of *c*III

product may not be needed when the stability of either *c*III or *c*II protein is increased by the above respective mutations.

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