THE CORRELATION BETWEEN TRANSCRIPTION AND MEMBRANE-ASSOCIATION OF λ DNA

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Various derivatives of λ were examined for their DNA's ability to associate with bacterial membrane fraction early after infection. The derivatives of λ include λc17, λric, λx13, λdbio c/I nin, all of which are mutants to carry out transcription of specific regions of their genome.

The results indicate that DNA-membrane association is not correlated to transcription of a unique region of the genome. Association occurs whenever there is a transcription stimulated by gene N product from λ in derepressed cells.

The regulatory mechanism of replication is thought to be in close relation to the association of DNA to membrane. Bacteriophage λ provides a unique system to approach this problem by virtue of its wealth of available genetic data. Hallick et al (1) observed that λ DNA associates to bacterial membrane after release of repression in the presence of gene N product. The N product is known to stimulate transcription of the right- and leftward operons located on each side of the immunity region (Fig. 1). Sakakibara & Tomizawa (2) have claimed that it is the occurrence of rightward transcription which is directly correlated to the association.

To investigate this problem in more detail we decided to examine the specificity of N product and the correlation between membrane association and the ability of different λ mutants to transcribe various regions of the genome.

MATERIALS AND METHODS

Bacteriophage. λcI857, λcI857 N_7N_{53}, λcIsus34 ri^c5b, λc17c90, λc17c90 N_7N_{53}, λc17 O_8, λimm^{434}cI6T, λ$imm^{434}O_8P_3$, λ$imm^{434}cN_7$, λimm^{434}c O_8 are reviewed in references 3 and 4; λdbio c/I nin5 (5,6) and λcI857 N_7N_{53}x13 (7) were obtained

from Court and Gottesman, respectively. 434c, 82c, λimm^{21}c, ϕ80c are the clear mutants of the respective phage (8). ^{3}H-labeled phages were prepared as described previously (10). Care was taken to avoid using labeled phage stocks kept over two weeks.

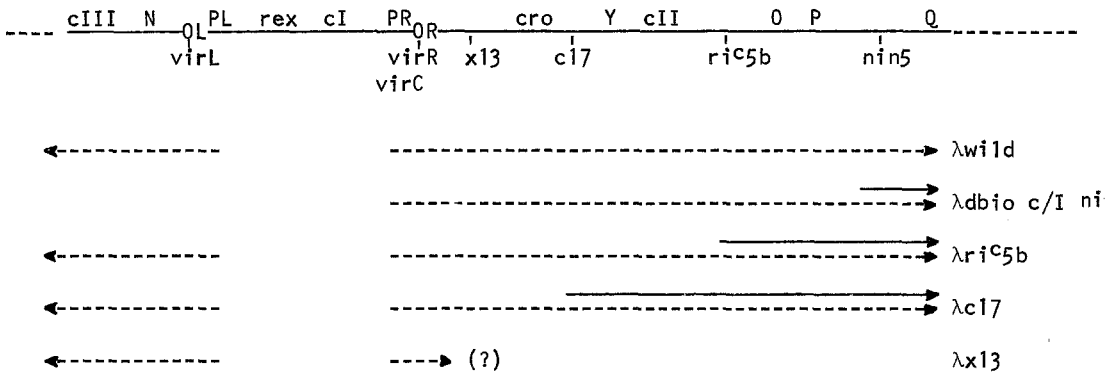

Fig. 1. Genetic map and transcription of λ genome near the immunity region. Genes cIII and cII code for products that control the decision between lysogeny and lytic growth after infection; gene N product plays a general stimulatory role in transcriptions of the λ genome; gene rex product restricts the development of coli phage T4rII; gene cI codes for λ repressor; gene cro product reduces the mRNA synthesis in the cI region and the leftward transcription (11,18); gene O and P products are essential for autonomous λ DNA replication; gene Q product is essential for initiation of late mRNA synthesis. For details of these genes and their products, see (3,4). Arrows indicate the direction and origin of transcription in the presence (----➤) and absence (⟶) of N gene product. In the lytic growth, the leftward transcription starts at the promoter PL, and the rightward transcription at the promoter PR. Promoter mutations c17, ric and nin5 allow new rightward, N-independent transcription from these sites (3,6,13,14),whereas mutation x13 abolishes the rightward transcription from this site (7,15,16). The repressor acts at the operators OL and OR. Mutations virL, virC and virR render the OL and OR, respectively insensitive to repressor (17).

Bacteria. W3102 is an su^{-} galK2 derivative of Escherichia coli K12. C600 is a derivative with a genotype of su^{+}, thr, leu, thi, tonB and lac. The former strain was used as a non-permissive and the latter as permissive host for the sus derivatives of λ (9). Lysogenic derivatives were constructed by infection.

Assays for membrane-association of parental λ DNA were performed by the method of Hallick et al (1) with minor modifications: W3102 lysogenic for a desired phage was grown to 1 x 10^{8}/ml in maltose-casamino medium (1) at 30°, harvested, washed and resuspended in 1/10 volume of λ-adsorption buffer (1). ^{3}H-labeled phage were added at a multiplicity of infection (moi) of 2 - 3 and the

mixture was incubated at 30° for 15 min. Helper phage were added as described in text at a moi of 5 along with the ^{3}H-phage. The infected complexes containing about 2 - 3 x 10^{8} cells were diluted 13 times with maltose-casamino medium and incubated at 30° for 15 min, followed by heating at 42.5° for 5 min to destroy repressor ("repressor-" in tables). Another sample was saved at 30° to serve as a repressed control ("repressor+" in tables). The cells were then harvested, washed with 0.01 M Tris-HCl pH8.1 and resuspended at 0° in 0.3 ml of 0.01 M Tris-HCl pH8.1 containing 12.5 % sucrose. To this were added 5 µl of 0.084 M EDTA and 50 µl of 5.6 mg/ml lysozyme. The mixture was kept at 0° for 20 min, followed by additions of 20 µl of 0.12 M $MgSO_4$ and 100 µl of 3.2 % Brij 58. After standing at 0° for an additional 10 min, a 0.2 ml aliquot of the lysate was layered on top of a 4 ml 5 - 30 % (W/W) sucrose density gradient containing 0.5 ml underlayer of 40 % sucrose solution saturated with CsCl. The sucrose solution was made up in 0.05 M Tris-HCl pH7.4, containing EDTA and NaCl at concentrations of 0.005 M and 0.15 M, respectively. Centrifugation was for 60 min at 35000 rpm in Spinco L using SW39 rotor at 4°. At the end of the run, fractions were collected from the bottom of the tube and the cold trichloroacetic acid-insoluble radioactivity was measured.

The radioactivity was distributed in two peaks; The fast-sedimenting component at the CsCl "shelf" represents the λ DNA associated to membrane. The slow-sedimenting component in the upper half of the sucrose gradient represents the free λ DNA. Radioactivity in each peak was summed up and divided by the total radioactivity to express the fraction of associated and free λ DNA, respectively.

RESULTS AND DISCUSSION

1. Specificity of the N gene product. Hallick et al (1) observed that the membrane-association of parental phage DNA requires i) derepression and ii) synthesis of N gene product. This was confirmed in our system as shown in Table 1, lines 1 - 5. The same table (lines 16 - 17) also shows that chloramphenicol inhibited the association process. The process, therefore, involves some protein synthesis.

Table 1. Effect of Heteroimmune helpers on the membrane-association of λcI857 derivatives.

Sample No.	Host	^{3}H-phage	Helper	Repressor	Radioactive DNA	
					associated to Membrane	in free Form
1	W3102(λcI857)	λcI857	-	+	23	77
2	"	"	-	-	100	0
3	W3102(λcI857NN)	λcI857NN	-	+	27	73
4	"	"	-	-	26	74
5	"	"	λcI857	-	100	0
6	"	"	λimm^{434}c	+	38	62
7	"	"	"	-	86	14
8	"	"	434c	+	27	73
9	"	"	"	-	61	39
10	"	"	λimm^{21}c	+	27	73
11	"	"	"	-	38	62
12	"	"	82c	+	25	75
13	"	"	"	-	40	60
14	"	"	ø80c	+	24	76
15	"	"	"	-	38	62
16	W3102(λcI857)	λcI857	-	- CAM*	51	49
17	"	"	-	- CAM*	34	66

Experiments were carried out as described in Methods. *To samples No. 16 and 17 was added chloramphenicol (CAM) 30 μg/ml and 150 μg/ml, respectively at the time of phage addition. DNA "associated" with membrane in the presence of chloramphenicol is taken as to represent non-specific association.

In the absence of repressor, association of N mutant λ DNA to membrane was efficiently helped by λ wild type or by λimm^{434} (both are N^{+}; Table 1, lines 3-7). Since the radioactive phage lacks the N gene product, this demonstrates that the N product can be provided by a superinfecting particle.

To examine the specificity of the N requirement a number of heteroimmune

phages were tested in the helper system. As shown in Table 1, lines 8 - 15, only phage 434 showed a helper effect, while λimm^{21}, 82 and ø80 failed to do so. Therefore the N requirement is highly specific. This is in good agreement with the known specificity of N action on left- and rightward transcriptions (11,12).

2. Membrane-association in the presence of repressor. Sakakibara & Tomizawa (2) studied a number of operator and promoter mutants and concluded that rightward transcription in the O-P region was the only requirement for membrane association. However, their experiments employed prolonged incubation, and nevertheless, association of the promoter mutants to membrane in an immune cell was never as extensive as the association in derepressed cells.

λc17c90, one of the promoter mutants which carry out constitutive rightward transcription in the O-P region in an immune cell, was found to associate with the membrane in the presence of repressor but only after prolonged incubation and then only to a limited extent (Table 2, lines 6-8). It is possible that such promoter-constitutive mutants have a different association process in immune cells occurring late after infection. However, this problem has not been pursued further as there is evidence which suggests that rightward transcription by these mutants occur early upon infection (13,14) and we have limited ourselves to studying early events.

Table 2 indicates that mutants constitutive for rightward transcription in the O-P region, λc17 and λri^{c}, are unable to associate with the membrane in the presence of repressor even in the presence of N gene product from a helper λimm^{434}c O_8*(lines 3,5). The same results were obtained when the prophage was replaced by those which produce repressors denaturable at lower temperatures. On the other hand, λc17 and λri^{c} readily associate with membrane when derepressed in the presence of gene N product (Table 2, lines 2,6,12).

λvirL has a mutation in left operator OL, and carries out constitutive, N-dependent leftward transcription in an immune cell while the x-O-P operon is repressed (17). The DNA of this phage was observed not to associate with the membrane in the presence of repressor (2).

* The mutation O_8 does not affect membrane-association (1).

Table 2. Membrane-association of λri^c and $\lambda c17$.

Sample No.	Host	^{3}H-phage	Helper	Repressor	Radioactive DNA	
					associated to Membrane	in free Form
1	W3102(λcI857)	λri^c	-	+	32	68
2	"	"	-	-	100	0
3	"	"	λimm^{434} O_8P_3	+	29	71
4	"	$\lambda c17$ O_8	-	+	40	60
5	"	"	$\lambda imm^{434}c$ O_8	+	46	54
6	"	$\lambda c17c90$	-	-	100	0
7	"	"	-	+	52	48
8	"	"	-	+ longer incub.*	75	25
9	"	"	-	+ CAM**	35	65
10	W3102(λcI857NN)	$\lambda c17c90$ NN	-	+	36	64
11	"	"	-	-	54	46
12	"	"	$\lambda imm^{434}c$	-	100	0

Experiments were carried out as described in Method except that phage adsorption was done at 0° instead of 30° for 30 min. λri^c stands for λcIsus34 ric5b.
* For sample No. 8, the incubation period in maltose-casamino medium at 30° was extended to 30 min, instead of 15 min.
** Chloramphenicol was added to sample No. 9 at the time of phage addition.

Thus the λ repressor inhibits the association of all the mutants tested, even in the presence of N gene product: Occurrence of transcription is not necessarily correlated to the association.

3. Membrane-association in the absence of right- or leftward transcriptions.

In order to examine if there is any correlation between the membrane-association and the direction or region of transcription in the absence of repressor, we tested a mutant λcI857 NNx13 which carries out normal leftward transcription, but not rightward transcription beyond the mutation x13 in the right operator region (7,15,16). Table 3 lines 1-3 show that this phage exihibits N-dependent

membrane-association, under derepressed conditions, indicating that high levels of rightward transcription are not required for the association process.

Table 3. Membrane-association of λcI857 NNx13 and λdbio c/I nin5

Sample No.	Host	^{3}H-phage	Helper	Repressor	Radioactive DNA	
					associated to Membrane	in free Form
1	W3102(λcI857NN)	λcI857NNx13	λcI857	+	34	66
2	"	"	"	-	78	22
3	"	"	λcNN	-	49	51
4	"	λdbio c/I nin5	-	+	19	81
5	"	"	-	-	43	57
6	"	"	λimm^{434}c	-	91	9

Experiments were carried out as described in Methods.
λcI857NNx13 was prepared and purified according to Roberts (7).

λdbio c/I nin5 is a mutant devoid of the leftward transcription because it carries a large deletion which covers the genes cI, PL and gene N, along with those genes related to recombination, integration and excision (Fig. 1) (5). The phage can multiply thanks to the nin mutation that bypasses the dependency on N gene product (6). This phage, again carries out N-dependent membrane-association in derepressed state as shown in Table 3, lines 4 - 6. Thus there is no evidence that a unique region of the genome has to be transcribed to allow membrane-association.

Taking these observations together, the requirements for the membrane-association process may be stated as follows: i) absence of repressor (derepressed) ii) the presence of the gene N product from the homologous phage. It seems that membrane-association can occur whenever N stimulated transcription is allowed to occur in derepressed cells.

We have assumed that the mutation x13 completely abolishes rightward transcription. This is based on observations that the site of action of the

N gene product is located downstream from the x13 mutation and therefore would not be functional, and that λcI857x13 phage fails to synthesize recognizable amount of mRNA molecules hybridizable to the x-O-P region (7,15,16). However, one could argue that the x13 mutant carries out either leaky transcription beyond the mutation site or that there are "attempts" at rightward transcription from the normal promoter site located closely to but on the upstream side of the x13 site (mRNA synthesized under these conditions may not be detectable by the current techniques). Thus we cannot rule out the possibility that the observed membrane-association by the λcI857 NNx13 is due to a small amount of rightward transcription.

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