A Genetic Analysis of Defective Plasmid Formation by *N*-deficient Phage λ Chromosomes

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Plasmid formation by *N*⁻ derivatives of lambdoid phages has been reinvestigated with transducing phages carrying the *trp*, *lac* and *gal* genes of *Escherichia coli*. Transduction by $\lambda N^{-}cI$ derivatives was inefficient and short-lived in each case, under both selective and non-selective conditions. Mutant operators were introduced to relieve possible auto-repression by the *cro* gene product. Such *N*-defective phage genomes were able to propagate continually as plasmids, although without selection they were gradually lost from the carrier cells. Plasmid formation remained inefficient, however. The entire chromosome of *N*⁻ phages can be expressed by transcription that leaks through the serially arranged Rho-dependent terminators. Some functions so expressed are deleterious to the plasmid state and cause the instability of λN^{-} plasmids.

INTRODUCTION

Mutants of bacteriophage λ defective in *N* gene function fail to grow lytically, and the *Escherichia coli* cells they infect survive and continue to grow (Campbell, 1961). Additionally, the lysogenic pathway is blocked so that the formation of lysogens with *N*⁻ phages is extremely rare (Lieb, 1971). This mutant phenotype results because the *N* gene product, synthesized immediately upon infection, acts as an anti-terminator for transcription and is required for further development along either of the two alternative pathways of lysis and lysogeny (reviewed by Herskowitz, 1973; Herskowitz & Hagen, 1980).

Although λN^{-} phage genomes are blocked for normal development, they are not inert within the cytoplasm of infected cells. Such molecules can replicate autonomously, although much more slowly than when the *N* product is present (Joyner *et al.*, 1966; Eisen *et al.*, 1966; Ogawa & Tomizawa, 1968), and so form plasmids within the bacterial cells they infect (Signer, 1969). Plasmid formation is dependent on functional *O*, *P* and *cro* genes and is antagonized by the λ repressor (Kleckner & Signer, 1977).

Plasmids formed by *N*-defective lambdoid phages are unstable (Signer, 1969). Since there are advantages in being able to use λ either as a plasmid or as a phage, we have investigated the instability of λN^{-} plasmids and determined its causes. This has enabled us to derive phages that successfully adopt the plasmid mode of growth upon infection of an *E. coli* host cell. Plasmid growth has different genetic requirements to those governing lytic or lysogenic growth, and therefore provides the possibility of an alternative approach to the analysis and manipulation of the λ genome.

METHODS

Bacterial and phage strains. These are described in Tables 1 and 2 respectively.

Media. (a) *Tryptophan-free medium*: 1 $\times$ Vogel-Bonner salts (Vogel & Bonner, 1956), 0.2% (w/v) glucose, 0.2% (w/v) Casamino acids, 10 μ g biotin ml⁻¹ (+ 1.5% w/v Difco agar for plates). (b) *Galactose selection medium*: 1 $\times$

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Table 1. *E. coli* strains

Strain	Genotype	Reference or construction
C600	<i>supE tonA thr leu thi</i>	Appleyard (1954)
W3350	<i>sup^o gal</i>	Campbell & Balbinder (1958)
AA129	<i>sup^o lac13 lacZ^ΔM15 (gal-att⁻-bio-uerB)^Δ trp^ΔLD102 recA56</i>	Anilionis (1977)
Ymel	<i>supF</i>	Dennert & Henning (1968); Goldberg & Howe (1969)
JC5088	Hfr (KL16) <i>recA56 thr ilr Spc^R Sm^R</i>	Clark (1967)
QR47	<i>supE</i>	Weil & Signer (1968)
QR48	<i>supE recA1</i>	Weil & Signer (1968)
WJB869	W3110 <i>sup^o trpBE^Δ9(λimm²¹)</i>	W.J.B., unpublished
WJB890	C-1a <i>sup^o trp Cam1024,am9870 recA56</i>	W.J.B., unpublished
WJB885	C-1a <i>sup^o trp Cam1024,am9870 thyA</i>	W.J.B., unpublished
	W3110 <i>sup^o trpR cysB</i>	Yanofsky & Ito (1966)
CH18	<i>supE tonA thr leu thi (λ)</i>	Lysogen of C600
CH20	<i>sup^o gal (λ)</i>	Lysogen of W3350
CH32	<i>sup^o gal λ^R</i>	λr2,r1,r3 selection of W3350
CH43	<i>supF (λimm⁴³⁴)</i>	Lysogen of Ymel
CH49	<i>supF (λimm⁴³⁴) λ^R</i>	λr2,r1,r3 selection of CH43
CH54	<i>supF λ^R</i>	λr2,r1,r3 selection of Ymel
CH59	<i>trp(ABCDE)^Δ8 rha lac^{am} Sm^R polA1</i>	C.H., unpublished
CH73	<i>sup^o gal (λimm⁴³⁴)</i>	Lysogen of W3350
CH85	<i>supE (λimm²¹) λ^R</i>	λr2,r1,r3 selection of CH102
CH102	<i>supE (λimm²¹)</i>	Lysogen of QR47
CH110	<i>supE λ^R</i>	λr2,r1,r3 selection of QR47
CH131	<i>supE recA1 tonA</i>	T5 selection of QR48
CH156	C-1a <i>sup^o trpCam1024,am9870 recA tonA</i>	T5 selection of WJB890
CH161	C-1a <i>sup^o thyA cysB</i>	P1 (W3110 <i>cysB</i>) × WJB885 to <i>trp⁺ cysB</i>
CH167	<i>sup^o thyA trp(ABCDE)^Δ8</i>	P1 (CH59) × CH161 to <i>trp cysB⁺</i>
CH175	<i>sup^o gal (λimm⁴³⁴) λ^R</i>	λr2,r1,r3 selection of CH73
CH207	<i>sup^o trp(ABCDE)^Δ8 recA56</i>	Mating of JC5088 × CH167 to <i>thy⁺ recA</i>
CH233	<i>sup^o gal (λimm⁴³⁴) λ^R (P2)</i>	P2 lysogen of CH175
CH259	<i>supE recA1 tonA (λNam7,am53 cIts857)</i>	Lysogen of CH131 at 32 °C
CH266	<i>supF λ^R (λh⁸⁰ att⁺ imm²¹ ninR5)</i>	Lysogen of CH54

Vogel Bonner salts, 0.2% (w/v) galactose, 0.2% (w/v) Casamino acids, 10 µg biotin ml⁻¹, 40 µg L-tryptophan ml⁻¹ (+1.5% w/v, Difco agar for plates). (c) *MacConkey indicator agar*: 4% (w/v) Difco MacConkey agar base, 1% (w/v) lactose or galactose. (d) *L broth*: after Lennox (1955); for plates, glucose omitted and Difco agar added to 1.5% (w/v). This was the rich medium that was generally used for bacterial and phage growth. (e) *BBL agar* (Parkinson, 1968): this was used for plaque assays. (f) *Xg indicator plates*: L-agar plates containing 40 µg 5-bromo-4-chloro-3-indolyl-β-D-galactoside ml⁻¹ (Sigma). (g) *Bacterial buffer*: 0.3% (w/v) KH₂PO₄, 0.7% (w/v) Na₂HPO₄ (anhydrous), 0.4% (w/v) NaCl, 0.02% (w/v) MgSO₄·7H₂O. (h) *λ buffer*: 0.3% (w/v) KH₂PO₄, 0.7% (w/v) Na₂HPO₄ (anhydrous), 0.5% (w/v) NaCl, 1 mM-CaCl₂, 10⁻³% (w/v) gelatin, 1 mM-MgSO₄.

Bacterial strain construction. (a) *Phage-resistant strains*. λ-resistant derivatives were selected by infecting 10⁷ cells (in the presence of 10⁻² M-MgSO₄) with 10⁶ λvir and plating on L agar with 3 ml BBL top-layer. For h⁸⁰-resistant mutants, the cells (in 10⁻² M-CaCl₂) were infected with 10⁶ T5, to select *tonA* mutants. (b) *Lysogens*. A 0.2 ml sample of exponential phase or fresh overnight cells was mixed with an equal volume of 0.1 M-MgSO₄ (for λ) or CaCl₂ (for P2), left for 5 min at room temperature and then plated on a BBL plate with 3 ml BBL top-layer containing 10⁻² M-MgSO₄ or CaCl₂ respectively. A drop of phage suspension (10⁸ p.f.u. ml⁻¹) was spotted onto the lawn and allowed to dry, and the plate was incubated overnight at 37 °C. A small area of top-layer agar from the resulting turbid spot was picked into L broth and the cells were grown to a thick culture. A loopful of this was streaked for single colonies. Usually 50–100% of these colonies were lysogens. λ lysogens were identified by their failure to permit the growth of homoimmune phages, while allowing the growth of heteroimmune or virulent phages. P2 lysogens were identified by their interference with λ growth (Signer, 1971; Zissler *et al.*, 1971).

λ phage growth and manipulation. (a) *Host cells*. These were prepared as described by Murray *et al.* (1973). (b) *Preparation of phage stocks*. The methods of plaque purification, preparation of high-titre stocks by plate lysates and assay of phage titres have been described by Davis *et al.* (1980). (c) *Phage crosses*. These were essentially as described by Murray *et al.* (1973). The host cells used were sensitive to both φ80 and h⁺, *rec⁺* and carried an amber suppressor (QR47 or Ymel).

Table 2. *Phage λ strains*

λ stock no. (CH #)	Genotype	Reference or cross
1	λ^+	—
2	$\lambda r2, r1, r3$	Jacob & Wollman (1954); Hopkins & Ptashne (1971)
5	λimm^{+34}	Kaiser & Jacob (1957)
6	λimm^{+1}	Liedke-Kulke & Kaiser (1967)
15	$\lambda h^{800} att^+ c1$	Franklin (1967)
16	$\lambda plac5 (srI2-att)^+ Nam7, am53 c1$	Anilionis (1977)
21	$\lambda h^{800} trpBG2 imm^{+34}$	Murray & Brammar (1973)
30	$\lambda Nam7, am53 c1s857$	Signer (1969)
34	$\lambda h^{800} trp51 imm^{+1}$	Brammar <i>et al.</i> (1974)
37	$\lambda h^{800} att^+ Nam7, am53 imm^{+34} c1$	W.J.B., unpublished
41	$\lambda h^{800} att^+ Nam7, am53 imm^{+34} ninR5$	W.J.B., unpublished
44	$\lambda b2 Nam7, am53 c1 ninR5$	W.J.B., unpublished
58	$\lambda h^{800} trp51 Nam7, am53$	Brammar <i>et al.</i> (1974)
59	$\lambda pgal8 ninR5$	Feiss <i>et al.</i> (1972)
85	$\lambda srI(1-2)^+ trpABC^{10}, (att-xis)^+ gam imm^{+1} ninR5 shn6^a$	Hopkins <i>et al.</i> (1976)
112	$\lambda h^{800} att^+ Nam7, am53 r2, r1, r3 ninR5$	$\lambda 2 \times \lambda 41$: selected on CH233; purified on CH32
113	$\lambda Nam7, am53 r2, r1, r3 ninR5$	$\lambda 112 \times \lambda 6$: clear plaques on C600
114	$\lambda h^{800} trp51 Nam7, am53 c1$	$\lambda 58 \times \lambda 44$: clear plaques on CH110 that failed on CH32
115	$\lambda pgal8 c1$	$\lambda 15 \times \lambda 59$: clear plaques on C600
116	$\lambda Nam7, am53 c1^+ KH54$	$\lambda 126 \times \lambda 30$: clear plaques on C600 at 32 °C
118	$\lambda srI(1-2)^+ trpA^+ imm^{+1} ninR5$	Hopkins <i>et al.</i> (1976)
125	$\lambda c1^+ KH54$	Blattner <i>et al.</i> (1974)
126	$\lambda h^{800} trpBG2 c1^+ KH54$	$\lambda 21 \times \lambda 125$: selected on CH49; purified on CH32
127	$\lambda h^{800} att^+ Nam7, am53 c1^+ KH54$	$\lambda 226 \times \lambda 116$: selected on CH85; purified on CH110
135	$\lambda h^{800} att^+ imm^{+1} ninR5$	$\lambda 118 \times \lambda 37$: turbid plaques on CH110
136	$\lambda srI(1-2)^+ trpABC^{10}, (att-xis)^+ Nam7, am53 r2, r1, r3$	$\lambda 147 \times \lambda 85$: clear plaques on C600
147	$\lambda h^{800} att^+ Nam7, am53 r2, r1, r3$	$\lambda 112 \times \lambda 6$: clear plaques selected on CH85 that failed on WJB869; purified on CH110
173	$\lambda h^{800} trpBG2 r2, r1, r3$	$\lambda 221$ grown on CH20 to make $Q^+ S^+$ by recombination with prophage; purified on C600
221	$\lambda h^{800} trpBG2 r2, r1, r3 Qam73 Sam7$	Moir & Brammar (1976)
226	$\lambda h^{800} att^+ imm^{+1}$	Burt (1981)
245	$\lambda pgal8 imm^{+1}$	$\lambda 115 \times \lambda 135$: selected on CH18; purified on C600
247	$\lambda pgal8 Nam7, am53 c1^+ KH54$	$\lambda 245 \times \lambda 127$: clear plaques on C600
248	$\lambda plac5 (srI2-att)^+ Nam7, am53 r2, r1, r3$	$\lambda 16 \times \lambda 173$: clear plaques selected on CH259 at 32 °C that failed on CH156; purified on C600
263	$\lambda pgal8 Nam7, am53 r2, r1, r3$	$\lambda 245 \times \lambda 147$: clear plaques on C600
281	$\lambda h^{800} trp51 Nam7, am53 r2, r1, r3$	$\lambda 34 \times \lambda 248$: selected on CH266; purified on CH54

λ plasmid transductions. Cultures of the host cells were grown to an OD_{600} of 0.5, which corresponded approximately to 2×10^8 cells ml⁻¹, as measured by microscopic counting and viable plate counts. The cells were then pelleted (5000 r.p.m., 10 min) and resuspended in half the volume of 10^{-2} M-MgSO₄. For each transduction, the phages were added in a volume of less than 0.5 ml to give the desired m.o.i. and the volume was made up to 1.0 ml with 10^{-2} M-MgSO₄. After mixing, followed by 15 min for adsorption at 37 °C, the cells were diluted in bacterial buffer and 0.1 ml samples of the appropriate dilutions spread on selection or detection plates. All transductions were repeated independently at least three times. For all transductions, host cell numbers were verified by the plating of serial dilutions on non-selective media, to ensure that the multiplicity of phage infection was approximately correct.

RESULTS

Characteristics of plasmid formation by λN^- phages

The efficiency with which λN^- phage chromosomes are able to form plasmids can readily be determined by the frequency of transduction of an appropriate host strain by a transducing phage. Since the behaviour of a host/plasmid combination might be affected by the characteristics of the particular selection imposed, we compared the responses of infecting the same host strain with three different types of transducing phage.

Design of the plasmid transduction system. (a) The phages. We required phages lacking *N* activity but retaining the transcription terminators t_{R1} and t_{R2} in order to minimize late gene expression. Phages carrying two amber mutations, *Nam7* and *Nam53*, were used to provide a 'non-leaky' deficiency in *N*-function, minimize reversion and allow stock preparation on amber-suppressing host strains.

The transducing phages used carried bacterial genes in the non-essential region of their genomes, on either side of *att*. In each case the bacterial genes within the phage genome carry their own promoters to liberate their expression from phage control systems and thereby allow the transducing genes to provide a dependable and direct means of monitoring the success of plasmid formation.

Phages carrying three different sets of bacterial genes were used. These were derivatives of λ pgal8, which carries the entire *E. coli gal* operon, including the operator and promoter (Feiss *et al.*, 1972), λ trp51, which carries the *trpD* and *E* genes, the attenuator, leader sequence, operator and promoter (Brammar *et al.*, 1974) and λ plac5, which carries the *lacZ* gene, operator and promoter (Ippen *et al.*, 1971; Malamy *et al.*, 1972). The structures of λ Nam7,am53 *cl*⁻ phages carrying these insertions are shown in Fig. 1. All three sets of transducing genes are transcribed in the leftward orientation.

(b) The bacterial host. The same host strain was used for all of the transductions, to avoid strain variations. The host used, AA129, has the genotype *trp(ED)*^Δ (*gal-att*^Δ-*bio*)^Δ *lacZ*^Δ *sup*^o *recA*: it carries deletions of each of the three selectable markers and is suppressor-free. The *att*^Δ deletion eliminates the possibility of transduction via site-specific recombination. The *recA* mutation was considered necessary to prevent transduction by recombinational rescue of the *trp* and *lac* lesions, where the bacterial genes on the transducing phage genome span the deletion in the recipient chromosome. The *recA* mutation also blocks the possibility of secondary transduction through leaky lytic growth of the phage, since in the absence of the *red* and *gam* products and the host *recA* product the phages are unable to render their DNAs suitable for packaging into virions (Enquist & Skalka, 1973). Furthermore, since the λ plasmids cannot form dimers or higher oligomers the number of individual replicons will be maintained at the highest level (see Hobom & Hogness, 1974), thus maximizing the genetic stability of the plasmids.

*Transduction by λ pgal8 Nam7,am53 *cl*^Δ.* The recipient cells (AA129) were infected over a range of multiplicities and transductants either detected on galactose MacConkey agar or selected on galactose minimal agar plates. Different levels and patterns of transductions were found on these two types of plates (Table 3a).

At an m.o.i. of 0.1, when most infected cells are singly infected, the frequency of transduction was 67% per infected cell on galactose minimal plates. This was slightly higher than that obtained for λ pgal8 Nam7,am53 *cl*857 by Signer (1969). On galactose MacConkey plates, the frequency of transduction indicated by the production of red, sectorial colonies, was only 34%. When the m.o.i. was increased to 1, the transduction frequency dropped dramatically on both media. As the m.o.i. was further increased, the frequency of transduction remained low in both cases, with only a few percent of cells infected at high multiplicities giving rise to Gal⁺ transductants.

Whatever the means of detecting transduced cells, only a fraction of singly-infected cells became transduced to the Gal⁺ phenotype. Multiple infection unexpectedly lowered the frequency of transduction rather than enabling all the cells to become transduced.

The transductants produced on the galactose minimal plates were abortive, forming very

Fig. 1. Structures of transducing phage genomes used to monitor λN^- plasmid formation (not to scale). (a) λ wild-type; (b) λ^{pgal8} *Nam7.am53*, carrying *cI Δ* KH54 or *r2, r1, r3*; (c) λ^{h80} *trp51* *Nam7.am53*, carrying *cI Δ* or *r2, r1, r3*; (d) λ^{plac5} (*srI2-att*) Δ *Nam7.am53*, carrying *cI Δ* or *r2, r1, r3*; (e) $\lambda^{srI(1-2)\Delta}$ *trpABC^{1,2}* (*att-xis*) Δ *Nam7.am53* *r2, r1, r3*. The 'replicon region' indicated, which is present in all of the λ phages, contains all the elements required to facilitate the replication of λ DNA within an *E. coli* cell: the promoter *p_R*, the origin of replication and the essential replication genes, *O* and *P*.

small colonies (about 1 mm diameter) that did not increase in size on prolonged incubation. When such colonies, or red transductant colonies from galactose MacConkey plates, were picked into galactose minimal liquid medium no further detectable growth occurred. After subculture without selection in L broth, all the cells were Gal⁻ and appeared to have lost the plasmid.

Transduction by λ^{h80} *trp51* *Nam7.am53* *cI Δ* . The recipient cells were infected over the same range of multiplicities as above and transductants selected on tryptophan-free plates. Transduction frequencies showed a different pattern to that found with λ^{pgal8} *Nam7.am53* *cI Δ* (Table 3b). Cells infected at an m.o.i. of 0.1 produced transductants very rarely, at a frequency of less than 0.01% per infected cell. At an m.o.i. of 1, however, 6.1% of the infected cells were transduced. As the m.o.i. was increased beyond 1 the frequency of transduction progressively declined. The transductant colonies produced on tryptophan-free plates behaved like the Gal⁺ transductants in remaining very small (about 0.5 mm diameter), despite prolonged incubation at 37 °C. Such colonies could not be grown in liquid minimal medium. After subculture in tryptophan-containing L broth, all cells tested were Trp⁻ and appeared to have lost the plasmid.

Transduction by λ^{plac5} *Nam7.am53* *cI Δ* . Since lactose preparations are often contaminated with its breakdown products, galactose and glucose, a minimal lactose plate selection was not used for λ^{plac5} *Nam7.am53* *cI Δ* transduced cells. Instead, transduction was monitored on lactose MacConkey agar indicator plates. Thus, the host cells were infected over the same range of multiplicities as above, diluted and spread on lactose MacConkey plates. None of the resulting colonies showed any distinguishable red colouration. Plasmid-mediated transduction, if it had occurred, was not able to elicit observable lactose fermentation by this test. An alternative means of screening for Lac⁺ transductants was therefore tried, using the chromogenic substrate 5-bromo-4-chloro-3-indolyl- β -D-galactoside (Xg). This screening is much more sensitive to *lac* operon expression than that given by the MacConkey indicator dyes (see

Table 3. Transduction of a *sup^o recA (gal-att⁺-bio)⁺ lacI lacZ⁺* host strain (AA129) by *N⁻ cI⁻* derivatives of lambdoid transducing phages

M.o.i.	Transduction frequency (%)* to:				
	Gal ⁺ (a)		Trp ⁺ (b)	Lac ⁺ (c)	
	Minimal	MacConkey	Minimal	MacConkey	Xg
0.1	67.0	34.1	<10 ⁻²	<10 ⁻²	3.5
1	11.5	1.1	6.1	<10 ⁻²	2.6
2	7.4	2.5	5.2	<10 ⁻²	3.0
6	1.6	3.7	4.3	<10 ⁻²	3.8
8	1.9	3.6	3.6	<10 ⁻²	3.6
10	1.4	5.4	3.3	<10 ⁻²	3.6
20	0.4	3.4	2.5	<10 ⁻²	3.4

* Mean value of at least three independent transductions at each m.o.i. The independent transductions varied on either side of the mean by less than one-tenth of the mean for values over 5% and by less than one-fifth of the mean for values under 5%.

(a) *λpgal8 Nam7,am53 cI⁺* KH54 transductants, either selected on galactose minimal agar or detected on galactose MacConkey agar.

(b) *λh⁸⁰trp51 Nam7,am53 cI⁻* transductants selected on tryptophan-free minimal agar.

(c) *λplac5 Nam7,am53 cI⁻* transductants detected either on lactose MacConkey agar or L-Xg agar.

Beckwith, 1978). Using L-Xg plates, a proportion of the infected cells were observed to produce faint blue colonies. At an m.o.i. of 0.1, 3.5% of the mainly singly-infected cells produced such weakly Lac⁺ colonies. Progressive increases in the m.o.i. failed to raise the transduction frequency significantly (Table 3c). Lac-transduction therefore showed another different response. Most marked was the failure to detect transductants on lactose MacConkey agar plates, since galactose fermentation due to *λpgal8 Nam7,am53 cI⁻* infection had been readily detected on the comparable galactose MacConkey agar plates as a strong response. The pattern of transduction frequencies, as detected on Xg plates, was also quite different, staying close to 3% irrespective of multiplicity. Following growth of transduced cells in non-selecting L broth, no Lac⁺ transductants could be detected by plating on L-Xg plates.

Plasmid formation by phages with *o_R* mutations

The results of the investigations with *λN⁻ cI⁻* transducing phages showed that although these genomes succeeded in transducing a proportion of the *E. coli* host cells, they did so for only a short limited period after infection. After this time, selected transduced colonies failed to grow further, and in subsequent non-selective growth the plasmid-carried genetic marker was rapidly lost. Similarly, transductants detected on non-selecting indicator medium formed sectorial colonies, from which the cells were Gal⁻ on further subculture. Since infection by the *N⁻* phages did not lead to killing of the host cells, this apparently short-lived plasmid formation suggests there is a defect in plasmid replication that becomes progressively more acute. Since multiple infection of host cells resulted in reduced levels of initial plasmid establishment, it would seem that the replication defect occurs more rapidly with greater input plasmid genomes.

Such inhibition of plasmid replication could result from the action of increasing concentrations of the *cro* gene product, causing progressively severe repression at *o_R* and thereby reducing *p_R*-initiated transcription of the replication genes, *O* and *P*, and the accompanying *cis*-activation of the origin. If such *cro* product inhibition was indeed responsible for the failure of the *λN⁻ cI⁻* plasmids to transduce permanently, then a relief of this inhibition would allow the plasmids to continue replicating and thereby maintain the transducing marker. The *cro* protein cannot be removed altogether, however, since it is essential for plasmid formation (Kleckner & Signer, 1977).

The level of *cro* product inhibition of transcription from *p_R* can be reduced by the introduction of mutations at the operator sites that lower the binding affinity of the protein for the DNA.

Table 4. Transduction of $sup^o recA$ host strains by $N^- r2, r1, r3$ derivatives of lambdoid transducing phages

M.o.i.	Transduction frequency (%) [*] to:					
	Gal ⁺ (a)		TrpB ⁺ (b)	Lac ⁺ (c)		TrpED ⁺ (d)
	Minimal	MacConkey	Minimal	MacConkey	Xg	Minimal
0.1	0.2	0.2	0.03	<0.1	<0.1	<10 ⁻⁵
1	1.7	1.3	0.04	0.05	0.1	<10 ⁻⁶
2	8.0	8.4	0.4	0.6	1.2	<10 ⁻⁶
6	9.5	9.0	3.5	1.0	2.3	<10 ⁻⁶
10	4.2	3.5	3.0	2.3	3.3	<10 ⁻⁶
20	1.5	0.5	2.6	2.0	3.0	<10 ⁻⁶

^{*} Mean value of at least three independent transductions at each m.o.i. The independent transductions varied on either side of the mean by less than one-tenth of the mean value.

(a) $\lambda pgal8$ *Nam7,am53 r2,r1,r3* transducing $sup^o recA$ (*gal-att_l-bio*)^Δ host strain (AA129) to Gal⁺, either selected on galactose minimal agar or detected on galactose MacConkey agar (red sector and non-sector colonies).

(b) $\lambda(sr11-2)^Δ trpABC^{1,9}$ (*att-xis*)^Δ *Nam7,am53 r2,r1,r3* transducing $sup^o recA$ (*trpABCDE*)^Δ host strain (CH207) to growth on indole minimal medium.

(c) $\lambda plac5$ *Nam7,am53 r2,r1,r3* transducing $sup^o recA att_{l1}$ *lacI lacZ*^Δ host strain (AA129) to Lac⁺, detected either as blue colonies on L-Xg agar or as red colonies (sector and non-sector) on lactose MacConkey agar.

(d) $\lambda h^{80} trp51$ *Nam7,am53 r2,r1,r3* transducing $sup^o recA att_{l1}$ (*trpED*)^Δ host strain (AA129) to Trp⁺ on minimal medium.

Previous studies of the effects of mutations in *o_R* on the copy number and stability of λdv plasmids have established that genomes containing the doubly mutant *o_{R2}r1, o_{R1}r3* operator gave the greatest increase in plasmid copy number without being so insensitive to *cro*-protein as to be unstable (Matsubara, 1974). This doubly mutant *o_R* was therefore introduced into a series of *N*-defective transducing phages as a means of reducing the inhibitory effect of the *cro* product on plasmid replication.

In these studies, in order to detect possible inconsistencies in the transduction data due to plasmid instability, four different phage-carried sets of *E. coli* genes were utilized and two different bacterial strains employed as hosts. In addition to derivatives of $\lambda trp51$, $\lambda pgal8$ and $\lambda plac5$, phages carrying the *trpA*, *B* and *C* genes were also used. The *trpABC*^{1,9} fragment included in phage $\lambda CH136$, cloned by Hopkins *et al.* (1976), contains the internal *trp* promoter, *pI*, located within the portion of the *trpD* gene present, which provides independent expression of the transducing genes (Fig. 1). The *trpB* gene product catalyses the conversion of indole to tryptophan, so that transductants can be selected on indole minimal plates.

Efficiency of plasmid transduction. Transduction experiments using $\lambda h^{80} trp51$, $\lambda pgal8$ and $\lambda plac5$ derivatives of $\lambda Nam7,am53 r2,r1,r3$ were carried out with the same $sup^o recA lacZ^Δ trpED^Δ$ (*gal-att_l-bio*)^Δ host strain as used previously (AA129). Experiments with $\lambda trpABC^1$ (*att-xis*)^Δ *Nam7,am53 r2,r1,r3* used a different $sup^o recA$ host strain (CH207) that contained a deletion of the entire *trp* operon, but retained *att_l*. In this case the phage genome carries an (*att-int-xis*) deletion, eliminating site-specific recombination. Thus the system for assaying plasmid formation was the same as that previously described. Transductants were either selected on the appropriate minimal agar or detected on indicator plates.

With the exception of $\lambda h^{80} trp51$ *Nam7,am53 r2,r1,r3*, which failed to produce Trp⁺ transductants of AA129, the transduction frequencies obtained were similar for all the other phages, despite the various monitoring systems employed (Table 4). Singly-infected cells gave rise to transductants at low frequencies (<0.5%). Increases in the m.o.i. up to 6–10 also increased the number of transductants, although the maximum frequency was under 10% in each case.

Unlike the transduced colonies obtained with the λN^- plasmid genomes carrying wild-type operators, the cells transduced by these $\lambda N^- r2,r1,r3$ genomes continued to grow and formed

Table 5. *Retention of λ Nam7,am53 r2,r1,r3 plasmids in sup^o recA host cells during non-selecting growth*

About 10^5 cells from a transduced colony, either growing on a minimal selection plate or a non-sectored transductant on an indicator plate, were used to inoculate 5 ml non-selecting liquid medium and grown up for approximately 15 generations to stationary phase. The proportion of cells in the population retaining the transduced phenotype was determined by plating on the same selection or detection medium and is given as the maintenance stability value. Four independent transductants arising at each m.o.i. were assayed. Mean values are given only when the independent stability values varied on either side of the mean by less than one-fifth of the mean value.

M.o.i.	Maintenance stability (%)				
	TrpB ⁺ (a)	Lac ⁺ (b)		Gal ⁺ (c)	
	Minimal	MacConkey	Xg	Minimal	MacConkey
0.1	0.9	<0.2-3.3	<0.5-9.2	0.9	<0.2-2.9
1	0.9	<0.2-4.6	<0.3-4.5	1.0	<0.2-4.9
2	5.3	<0.5-9.1	0.2-10.0	4.2	<0.2-5.2
6	16.8	0.5-8.3	<0.5-2.5	13.1	0.3-9.3
10	14.2	<0.2-6.7	0.7-14.7	11.0	<0.2-12.0
20	12.4	0.7-5.1	1.2-12.6	10.2	0.2-8.7

(a) CH207(λ srI(1-2)⁺ *trpABC*_{1,2} (*att-xis*)⁺ *Nam7,am53 r2,r1,r3*) transductants were grown in L broth supplemented with tryptophan, then diluted and plated on indole minimal agar (TrpB⁺) and non-selecting agar (total cells). Mean values are given.

(b) AA129(λ plac5 *Nam7,am53 r2,r1,r3*) transductants were grown in L broth supplemented with glucose, then diluted and plated on lactose MacConkey agar or L-Xg agar to detect Lac⁺ and Lac⁻ colonies. Maintenance stability values were widely variable: the lowest and highest values obtained are given.

(c) AA129(λ pgal8 *Nam7,am53 r2,r1,r3*) transductants were grown in L broth supplemented with glucose, then diluted and plated either on galactose minimal agar (Gal⁺) and L agar (total cells) or on galactose MacConkey agar to detect Gal⁺ and Gal⁻ cells. Mean values are given for transductants initially grown on selecting medium; lowest and highest values given for transductants initially grown on indicator medium.

progressively larger colonies with prolonged incubation. Transduced cells could be purified on selective plates and grown to high cell densities in selective liquid media.

The continued propagation of these transducing plasmids, and the positive effect of increases in the m.o.i. on the number of transductants formed, both contrast with the short-lived growth of the wild-type operator plasmids and the negative effect of multiple infection on the number of transductants formed by such genomes. Thus, relief from repression mediated by the *cro* product had discernible effects on λ N⁻ plasmid growth, although the frequency of initial plasmid formation was significantly reduced, indicating an increased effect of an underlying genetic instability.

Maintenance of the λ N⁻ r2,r1,r3 plasmids during non-selecting growth. Selected transduced colonies were purified and approximately 10^5 cells used to inoculate 5 ml non-selecting rich liquid medium. These cultures were grown to saturation, about 15 generations of growth, and the proportion of cells that still retained the transducing plasmids was determined. The stabilities of the *trpABC*⁺ and *pgal8* transducing derivatives of *λNam7,am53 r2,r1,r3* monitored in this way showed a clear positive influence on the level of maintenance of the plasmid of the m.o.i. at which the transductants were formed. Maximum stabilities of 13-17% were observed for transductants formed at an m.o.i. of 6 (Table 5).

In the case of transductants detected on MacConkey indicator plates, non-sectored colonies were used as inocula for the stability test. For both *lac* and *gal*, very variable stability values were obtained, independent of the initial m.o.i. However, the higher values were of the same order as obtained when selected colonies were used as inocula. This variability presumably reflects the gradual tendency to lose the λ N⁻ r2,r1,r3 plasmids under non-selecting conditions, which will affect the cells used as inocula for the stability tests.

Lac⁺ transductants detected on L-Xg plates did not give rise to sectorized colonies, and so there was no criterion for choosing colonies as inocula for the stability test. Again, very variable maintenance stability values were obtained, but these were no different from those obtained with the MacConkey-detected transductants.

Evidence for leaky transcription. Although cells transduced to TrpB⁺ and Gal⁺ by the *trpABC*^l and *pgal8* derivatives of λ *Nam7,am53 r2,r1,r3*, respectively, grew to high cell densities in selective media, such cultures always contained the debris of lysed cells. Such lysis could only be due to expression of the λ plasmid's lysis genes, which lie downstream of the *t*_{R2} terminator. For such expression to occur, some *p*_R-initiated transcripts must be able to leak through both of the serially arranged *t*_{R1} and *t*_{R2} terminators, even though the *N* protein is absent. Possible inefficient termination of *p*_R'-initiated transcription by *t*_R, in the absence of the *Q* gene product, could also contribute to leaky late gene expression. However, since AA129 cells multiply infected with λ *pgal8 Nam7,am53 cI⁺* showed no evident cell lysis when subsequently grown in selecting liquid medium, there is no evidence for such a contribution. Host cell lysis evident when the phage genome carried the *r1,r3* mutations would therefore appear to result almost entirely from increased *p*_R-initiated transcription. If expression of the lysis genes occurs, then expression of all the other late genes is also likely, since they are part of the same transcription unit.

When such $\lambda h^+ N^- r2,r1,r3$ plasmid-carrier cells were plated on lawns of *sup*⁺ cells (Ymel or C600), they failed to give rise to centres of lytic infection. The possible production of free phages was also investigated by mixing the supernatants from chloroform-lysed minimal media cultures with *sup*⁺ cells and plating to form lawns on BBL agar. No plaques were detected, again indicating that intact phages were not liberated from the lysed cells.

The AA129 *sup^o recA* cells infected with $\lambda h^{80} trp51 Nam7,am53 r2,r1,r3$ phages were also screened for the liberation of free phages. The infected cells were washed several times to remove unadsorbed phages, and then plated on lawns of *sup*⁺ cells (Ymel). Plaques arose from 1% of the infected cells. For such phage production to occur, leaky transcription of both the leftward and rightward operons of the infecting phage genomes must have occurred, despite the absence of the *N* protein. Thus the presence of the left arm of the $\phi 80$ chromosome, which carries the head, tail and *b* region genes of $\phi 80$, facilitated the occasional lytic development of λ *Nam7,am53 r2,r1,r3*. This phenomenon is possibly associated with the failure of plasmid transduction by $\lambda h^{80} trp51 Nam7,am53 r2,r1,r3$.

In contrast to these findings with λ *Nam7,am53 r2,r1,r3* derivatives, release of viable phages from *sup^o recA* cells infected with the λ *Nam7,am53 cI⁻* transducing phage derivatives could not be detected.

DISCUSSION

Although *N*-defective lambdoid phages carrying a *cI⁻* lesion (to eliminate possible repression by the *cI*-encoded repressor) were able to transduce suitable *E. coli* cells by propagation of the infecting phage genomes within the cytoplasm of their hosts, as reported by Signer (1969) and Lieb (1970), this transduction was found to be essentially abortive. Growth of transductant colonies was temporary and, in the absence of selection, the plasmids were rapidly lost during subsequent propagation of the host cells. This behaviour suggested a gradually increasing inhibition of plasmid DNA replication, the most likely cause of which would be repression of transcription from *p*_R by the plasmid's own *cro* gene product. This possibility is consistent with the observation that reduced transduction frequencies were found at higher multiplicities, where the *cro* gene product would more rapidly reach its threshold concentration (Franklin, 1971).

When the repressive effect of the *cro* product was alleviated, by the introduction of mutant *o*_R operator sites that are bound by *cro* protein with reduced affinity, the λN^- plasmids continued to replicate within the transduced cells and were only slowly lost in non-selective growth conditions. However, plasmid formation by these genomes occurred with low efficiency, suggesting that there must be some interference with the adoption of the plasmid state.

Fig. 2. Leaky transcription of the λ genome in the absence of the N gene product. Transcripts initiated at p_L and p_R are inefficiently terminated at the downstream terminators, so that a proportion of the transcripts is able to pass through. The level of transcription, and hence the amount of leaky gene expression, is thereby reduced at each successive terminator, as illustrated. Transcripts from p_R that leak through t_{R2} lead to expression of the Q gene, whose product further promotes late gene transcription from p_R . Transcripts that leak through terminators t_{R1} and t_{L2} give rise to expression of the cII and $cIII$ genes, respectively, whose products facilitate transcription from pI . In this manner the whole λ genome can be transcribed in N^- conditions. As the infection progresses, the amount of cro gene product synthesized will increase, and, by binding at o_L and o_R , this weak repressor will negatively regulate transcription from p_L and p_R .

For plasmid establishment to occur, the infecting λN^- genome must circularize and then be replicated in the theta-form, as in a wild-type infection, but avoiding the usual development along the lytic pathway. This would require transcription to be initiated from p_R and be limited to the ori - O - P region bounded by t_{R2} . In the absence of the N protein, t_{R1} is bypassed by 30–50% of approaching transcripts (Rosenberg *et al.*, 1978), allowing transcription of the t_{R1} - t_{R2} segment. Terminator t_{L1} has also been shown to be leaky to 10–20% of p_L -initiated transcripts (Salstrom & Szybalski, 1978; Drahos & Szybalski, 1981). Several other terminators, including t_{L2} , t_{L3} , t_1 , t_1' , and t_1'' have been cloned individually and shown to be only about 90% efficient in the absence of N -protein (Drahos & Szybalski, 1981; Luk & Szybalski, 1982, 1983*a, b*; Benedik *et al.*, 1983; Luk *et al.*, 1982). These findings suggest that t_{L2} and t_{R2} may not be effective in stopping all of the transcripts that leak through the first terminators.

Our results with the $\lambda N^- t_{L2} t_{R1} t_{R3}$ plasmids show that the t_{L2} and t_{R2} terminators are inefficient in blocking those transcripts that escape the first terminator, since downstream gene expression was detected. Consequently, it would appear that the entire λ genome can be leakily transcribed in the absence of N -protein, as depicted in Fig. 2. This additional gene expression could significantly influence the viability of the newly formed λ plasmids and be the cause of their defective characteristics.

Further information indicates that λN^- genomes with wild-type operators must also be leakily transcribed, albeit to a lesser extent due to cro protein mediated repression, and be destabilized by these leakily expressed λ gene products. Thus, Lieb (1970) reported that some of the cells in a colony derived from a $\lambda N^- cI^-$ -infected rec^+ cell lyse and release phage particles. For this to occur, t_{R2} or t_R must be bypassed for the late genes to be expressed. Our failure to detect phages released from $recA$ cells infected by $\lambda N^- cI^-$ indicates that transcription through

t_{L2} is insufficient to enable such lytic growth. However, transcription past t_{L2} did occur when *cro* protein mediated repression was reduced due to the $r2$ mutation in ϕ_{L1} . When *cro* protein mediated repression is completely eliminated, as in the case of $\lambda Nam7, am53 cI^u cro^- ninR5$ at 37 °C, there is sufficient escape of leftward transcription for the phage to form plaques on a *sup⁺ recA* host (Court & Campbell, 1972). Because the *cro* protein is a weakly binding repressor, early in an infection by a $\lambda N^- cI^-$ phage (while the level of the *cro* product is low within the cell) transcription will not be fully repressed and will be frequent enough to provide substantial expression of the whole genome.

A $\lambda N^- cI^-$ plasmid will therefore be subject to the effects of any deleterious products expressed from the λ genome. Later in the infection reduction of DNA replication due to repression by *cro*-protein will be superimposed. Presumably, it is the combination of these two factors that leads to the loss of $\lambda N^- cI^-$ plasmids after a few cycles of host cell growth.

The genetic composition of the λ chromosome is sufficiently well understood to allow prediction of at least some of the genes whose products might adversely affect plasmid formation. Thus one potential anti-plasmid function is the phage's terminase, which binds to the *cos* site in λ DNA and cleaves the DNA site-specifically as part of the encapsidation process (reviewed by Feiss & Becker, 1983). The λ endonucleases, encoded by genes *endo* (Roades & Meselson, 1973) and *ben* (Benchimol *et al.*, 1982; Sumner-Smith *et al.*, 1982), could also be capable of inhibiting plasmid formation, since they are able to introduce double-stranded breaks into DNA and are able to cleave both λ and *E. coli* DNA *in vitro*.

Expression of the *gam* gene could also destabilize λ plasmid growth, since the *gam* gene product promotes rolling-circle DNA replication (Greenstein & Skalka, 1975). Conversion to this mode of replication would result in the channelling of DNA replication towards the production of linear concatenated tails rather than the production of more plasmid molecules. Additionally, recombination catalysed by the *red* and *int* gene products could cause the monomeric circular λ chromosomes formed at the start of the infection to form dimers or higher multimeric forms (Enquist & Skalka, 1973; Syvanen, 1974). This would decrease the number of individual replicons within the carrier cells and so reduce the heritability of the λ plasmids during host cell growth.

Although expression of the lysis genes was detected, the leakily expressed lysis proteins may not substantially influence plasmid formation, since the great majority of plasmid-containing cells grew without apparent impairment. Other independent observations indicate that *E. coli* cells are quite tolerant of the expression of the lysis genes (Adhya *et al.*, 1971; Reader & Siminovitch, 1971*a, b*; Garrett *et al.*, 1981).

Although our results show that plasmid formation by λN^- genomes is defective, inactivation by mutation of those genes whose products have a destabilizing activity should make possible the successful adoption of the plasmid mode of growth. We have applied this approach to developing non-defective λ plasmids, as reported in the accompanying paper (Hadfield & Brammar, 1984).

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