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Effects of the *rex* gene of phage λ on lysogeny

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SUMMARY

Mutations in the *rex* gene of phage λ affect lysogeny. λ*rex*⁻ phages have an increased probability of forming abortive lysogens instead of stable lysogens. In addition, established lysogens produce elevated levels of cured cells during anaerobic but not aerobic growth. It is suggested that the function of the *rex* gene is related to excision or repressor function.

1. INTRODUCTION

Bacteriophage λ is a temperate viral parasite of *Escherichia coli*. Upon infection, the phage can either initiate a lytic cycle or establish a lysogenic association. The decision to lysogenize is a complex process involving at least four phage genes *c*I, *c*II, *c*III, and *cro* (Kaiser, 1957; Eisen *et al.* 1970; Herskowitz, 1974). The *c*I gene codes for the λ repressor which establishes and maintains the lysogenic state. Genes *c*II and *c*III enhance the establishment of lysogeny but do not affect the stability of the lysogen. The *cro* gene product acts as an anti-repressor to favour the lytic cycle.

Still other genes may be involved such as the *rex* gene which is recognized by its preventing phage T4*r*II from growing on a λ lysogen (Howard, 1967). The *rex* gene of phage λ is co-transcribed with the adjacent *c*I gene and hence is continuously expressed in the established lysogen as well as during the period when the lysogenic state is being established (Szybalski *et al.* 1969). Moreover, it has no known function, despite a diversity of unexplained effects (see Rolfe & Campbell, 1977). Phage λ*rex*⁻ mutants have been isolated and studied but do not display any obvious alterations in lysogeny (Gussin & Peterson, 1972). We report now, however, that λ*rex*⁻ phage mutants do show subtle defects in both the establishment and maintenance of lysogeny.

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2. MATERIALS AND METHODS

Strains. Escherichia coli K12 strains 233, a *trpE*⁻ 10220 derivative of W3110 from C. Yanofsky, and W3350 *lac*⁻ *gal*⁻ *Su*⁻ from A. Campbell were used in this study as wild type host cells. Strain 483, a colicin-tolerant mutant of W3110 (Nomura & Witten, 1967) that strongly biases infecting λ phage towards lysogeny (Rolfe *et al.* 1973) was obtained from M. Nomura.

Phages λ*c*I857, λ*c*I857*rex*5a (a missense *rex* mutant), λ*c*I857*rex*Q (a nonsense *rex* mutant), λ*c*I857*rex*209 & λ*c*I⁺*rex*30a were all kindly provided by Dr G. Gussin (Gussin & Peterson, 1972). These phages (except *rex*30a) also carry the *ind*⁻ mutation. Phage λ*c*I857*xis*1 and λ*c*I857*xis*1 *nin*5 were from Dr D. Court (Court & Campbell, 1972); λ*b*2*c*I from Dr A. Campbell; λ*c*I857h^{80} from Avitabile *et al.* 1972; and λ⁺, λ*c*I, λ*c*II, λ*c*III, λ*vir* from Rolfe *et al.* (1973). Lysogens were prepared as described elsewhere (Rolfe & Campbell 1977). Maltose negative (*mal*⁻) lambda-resistant (λ^R) derivatives of lysogens were selected using λ*vir* phage (Schwartz, 1966). The *mal*⁻λ^R lysogens of strain W3350 are those described elsewhere (Dykhuizen, Campbell & Rolfe, 1978).

Culture conditions. Phage assays were carried out on LB-maltose agar plates at 30 °C (Rolfe & Campbell, 1974). Anaerobic growth conditions for phage plating were provided by passing nitrogen gas (99 % N_2 plus 1 % CO_2) through large nylon bags (Hill, 1973). Other media and microbial procedures, including the detection of biotin auxotrophy, have been described (Rolfe, 1970; Dykhuizen, 1973; Campbell & Rolfe, 1975).

Determination of Lysogen Frequency. The frequency of formation of lysogens was determined by the method described by Gottesman & Yarmolinsky (1968) using EMBO (dye but no sugar addition) agar plates. Strain W3350 was grown overnight in maltose broth at 30 °C, centrifuged and resuspended in 0·02 M-$MgSO_4$. The cell suspension was divided into two samples, one of which was incubated aerobically and the other anaerobically at 30 °C for 30 min. Phage was added at a multiplicity of infection of 10·0 and the cultures were reincubated for a further 30 min under previous conditions. The phage-infected suspensions were then chilled in an ice bath, centrifuged and the cell pellets resuspended in chilled $MgSO_4$ buffer, diluted and spread onto EMBO agar plates preseeded with about 10^9 λ*b*2*c*I phage particles to kill non-lysogens. The plates were incubated for 24 h at 30 °C under the same conditions (i.e. aerobic or anaerobic) as for phage infection. Strain W3350 has the same colony forming efficiency under aerobic and anaerobic conditions.

The efficiency of phage adsorption was calculated from residual phage in the supernatants and found to be between 95 and 98 % both aerobically and anaerobically. The percentage of cells stably lysogenized was calculated from the ratio of the number of large pink colonies to the number of viable cells before infection. Abortive lysogens in which the prophage is repressed but genetically unstable were scored as purple 'nibbled' colonies.

Determination of frequency of curing. The lysogens used in curing experiments contained the temperature-sensitive lambda repressor (mutation *c*I857), so that cells which grew at 42 °C were considered to have lost the prophage. They were

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also *mal*⁻ to prevent the possibility of reinfection. Each lysogen was streaked out on LB agar and incubated overnight at 30 °C. Five single colonies were suspended in 20 ml of L-broth in separate 250 ml flasks and regrown overnight at 30 °C with vigorous shaking. From each of these overnight cultures an aerobic and an anaerobic culture was prepared, by inoculating 10^6 cells into 20 ml of L-broth. The aerobic cultures were shaken vigorously in flasks at 30 °C. For anaerobic growth 20 ml long glass tubes were completely filled and sealed. After overnight growth at 30 °C both sets of cultures were assayed for viable cells at 30 °C and heat-resistant cells at 42 °C. The aerobic cultures had grown about 15 generations and the anaerobic cultures about 12 generations.

As a control, representative heat-resistant cells obtained were shown still to be killed and lysogenized by phage cI857h^{80}.

3. RESULTS

Plaque morphology. The plaque morphology of λrex^- mutants on wild-type *E. coli* is indistinguishable from that wild-type phage. A difference, however, is discernible on mutant 483. This bacterial mutant strongly biases infecting λ phage towards the establishment of lysogeny (Rolfe *et al*. 1973; Oppenheim, Honigan & Oppenheim, 1974). Wild-type λ phage plates on it at a greatly reduced frequency and with extremely turbid plaques. This alteration of λ plating involves the *c*III gene, as λcIII$^-$ phage mutants produce normal, albeit small, turbid plaques at nearly normal frequencies. Three out of four λrex^- mutants tested behave like *c*III mutants on strain 483 (Table 1). The exceptional mutant, λrexQ is indistinguishable from wild-type phage on this bacterial strain.

Establishment of lysogeny. The role of *rex* in lysogeny was further characterized by infecting wild-type cells with λrex^+ and λrex^- phages and following the fate of the cells (Table 2). In these experiments wild-type λ phage propagates lytically in 85% of cells infected under the conditions used and produces stable lysogens of most of the remaining cells. Unstable lysogens are rarely formed (4%). The λrex5a mutant phage has a greatly reduced ability to form stable lysogens (Table 2). This reduction is due to an increased probability that a cell committed to lysogeny will form an unstable lysogen in which the repressed prophage fails to be inserted into the chromosome and gradually becomes diluted out of a dividing cell population (Ogawa & Tomizawa, 1967). Thus *rex* seems to aid in the proper execution of some physiological process involved in prophage insertion or excision.

Two other *rex* mutants with cI$^+$ alleles, λrex209 and λrex303 also give increased frequencies of unstable lysogens while λrexQ does not differ significantly from λrex^+ (e.g. its ratio of stable to unstable lysogens is 1·7).

We have observed in previous studies that some effects of *rex* gene expression are exaggerated in the absence of oxygen (Rolfe & Campbell, 1977). However, aerobically and anaerobically infected host cells give comparable frequencies of formation of stable and unstable lysogens (Table 2).

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Stability of established lysogens

After aerobic growth, the numbers of heat-resistant (cured) cells in cultures of λ*c*I857*rex*$^{+}$ and λ*c*I857*rex*$^{-}$ lysogens are not significantly different from one another (*ca* 30 per 10^{8} cells) (Table 3). A similar frequency of heat-resistant cells occurs in wild-type lysogens cultured anaerobically. However, the anaerobic cultures of λ*rex*$^{-}$ lysogens have a 200-fold higher frequency of heat-resistant cells. This

Table 1. *Plating of lambda phages on colicin-tolerant mutant 483*

Phage strain	Plating efficiency	Plaque morphology
λ^{+}	10^{-3}	tt
λ*c*I	1·0	c
λ*c*I857	10^{-3}	tt
λ*c*II^{-}	1·0	c
λ*c*III^{-}	0·5	t
λ*c*I857*rex*5a	0·5	t
λ*c*I857*rex*209	0·5	t
λ*c*I^{+}*rex*309	0·5	t
λ*c*I857*rex*Q	10^{-3}	t

c, clear; t, turbid; tt very turbid plaques.

Table 2. *Measurement of lysogenization frequency*

Fate of infected cell	Infecting phage λ	Infecting phage λ*rex*5a
Aerobically grown		
Per cent cells killed	85	82
Per cent surviving cells as		
Unstable lysogens	4	17
Stable lysogens	11	0·8
Ratio $\frac{\text{stable lysogens}}{\text{unstable lysogens}}$	2·7	0·05
Anaerobically grown		
Per cent cells killed	84	79
Per cent surviving cells as		
Unstable lysogens	8	20
Stable lysogens	8·3	0·7
Ratio $\frac{\text{stable lysogens}}{\text{unstable lysogens}}$	1·0	0·04

increase is observed in two different host cell backgrounds and for both λ*rex*$^{-}$ mutants studied, λ*rex*5a and λ*rex*Q. It is not accompanied by elevated titres of free λ phages, indicating that the λ*rex*$^{-}$ lysogens are not spontaneously induced more readily than λ*rex*$^{+}$ lysogens.

A λ*c*I857 lysogen may become heat-resistant either by prophage curing or by a mutational deletion which removes the prophage. Curing requires λ*xis* gene activity and usually entails the exact cutout of the prophage. In contrast, spontaneous deletions have randomly located ends and frequently extend into neigh-

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bouring bacterial genes, for example, those for biotin synthesis. To demonstrate that the conditional instability of λ*rex*⁻ lysogens is due to increased levels of curing we examined the numbers of *bio*⁻ auxotrophs among the heat-resistant cells of our cultures. λcI857*xis*⁻ lysogens can only give rise to heat-resistant cells by mutation

Table 3. *Stability of λ phage lysogens*

Genotype of lysogen	Frequency per 10^8 cells in culture: Heat resistant cells *bio*⁺	Heat resistant cells *bio*⁻	Phage λ
	Aerobic		
233 *mal*⁻ (λcI857)	28	—	< 10
233 *mal*⁻ (λcI857 *rex*5a)	27	—	< 10
233 *mal*⁻ (λcI857 *rex*Q)	44	—	< 10
233 *mal*⁻(λcI857 *xis*-1)	2·1	0·6	—
233 *mal*⁻ (λcI857)	28	0·6	—
W3350 *mal*⁻ (λcI857)	43	1·3	—
W3350 *mal*⁻ (λcI857 *rex*5a)	24	0·5	—
W3350 *mal*⁻ (λcI857 *rex*Q)	25	0·5	—
	Anaerobic		
233 *mal*⁻ (λcI857)	30	—	< 10
233 *mal*⁻ (λcI857 *rex*5a)	6200	—	< 10
233 *mal*⁻ (λcI857 *rex*Q)	5000	—	< 10
233 *mal*⁻ (λcI857 *xis*-1)	0·9	0·3	—
233 *mal*⁻ (λcI857)	33	0·8	—
W3350 *mal*⁻ (λcI857)	21	0·8	—
W3350 *mal*⁻ (λcI857 *rex*5a)	5000	< 0·4	—
W3350 *mal*⁻ (λcI857 *rex*Q)	2400	< 0·4	—

— Not measured.

and, as Table 3 shows, about a quarter of such mutations inactivate *bio* genes. The high incidence of cured cells in anaerobic λ*rex*⁻ lysogenic cultures is not accompanied by increased numbers of *bio*⁻ auxotrophs. Thus the instability is due to an increased rate of curing instead of random mutation. Even in a *rex*⁺ λcI857 lysogen there is sufficient spontaneous *xis* activity to increase the frequency of heat-resistant cells 10–30-fold above the background level from mutation.

4. DISCUSSION

We have shown here that the *rex* gene is yet another gene of phage λ involved in the process of lysogeny. Its role appears to resemble that of the cIII gene in plating behaviour on mutant strain 483, but differs in other respects by affecting both establishment and maintenance of lysogeny. Early studies suggested the *rex* function might be involved in cI repressor control of λ (Ogawa & Tomizawa, 1967; Ptashne, 1971; Mark & Szybalski, 1973). However, Gussin & Peterson (1972), after investigating twenty-five *rex*⁻ mutants of phage λ, concluded that the *rex* and cI genes were distinct and that they acted independently of each other.

For a lysogen to become cured the prophage must be excised from the bacterial

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chromosome but not enter the lytic cycle. Genes for prophage integration and excision, *int* and *xis*, lie on the left arm of the λ chromosome while those for replication are located on the right arm. Transcription of both arms is from promoters under control of the *c*I gene product. One possible mechanism for curing would be for *c*I repression to be temporarily lifted to allow a low level of transcription and then reasserted. Enough left arm messenger might be made to cause prophage excision without enough right arm transcription to kill the cell. According to this explanation *rex* would directly aid in maintenance of *c*I repression.

Alternatively Hayes & Szybalski (1973) have demonstrated a promoter which allows a low level of transcription of the *int-xis* region in established lysogens under *c*I control. If the level of *xis* enzymatic activity from this source were higher during anaerobic growth it might cause an occasional inappropriate prophage excision and the role of *rex* could be to prevent this occurrence.

A regulatory function of *xis* activity would also be consistent with the effect of *rex* on the establishment of stable lysogens. The *rex* gene does not seem to affect the decision between lysogeny versus lytic growth, since cells show similar levels of survivors after λrex^{+} as λrex^{-} phage infection. Instead *rex* seems to help ensure that in the cell committed to lysogeny the prophage ends up in the chromosome instead of becoming repressed in the cytoplasm by 'abortive lysogenization' (Ogawa & Tomizawa, 1967).

One note of caution emerges from these studies. All 'rex^{-}' mutants defined on the basis of T4*r*II exclusion, the classical test for *rex* activity, are not identical in phenotype. We have observed, previously (Rolfe & Campbell, 1977) differences between λ*rex*Q and λ*rex*5a mutants and differences are clearly evident in this study as well. The λ*rex*Q mutation is a nonsense base substitution in the distal portion of the *rex* gene. It seems possible that the chain-terminated *rex*Q peptide may retain some degree of *rex* activity. Alternatively our strains may have some very weak nonsense suppressor activity which is just sufficient for certain *rex*-requiring processes to occur normally and others to be impaired. The limitations of phage T4*r*II growth as a definition of absence of *rex* activity may explain other controversies over the role of the λ*rex* gene (e.g. Dykhuizen *et al.* 1978).

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