qin101: Promoter Mutation Which Allows the Constitutive Expression of the Late Genes

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We describe the isolation and characterization of a mutant (*λqin101*) which renders *λ* growth *Q* independent. We have shown that this mutation creates a new promoter, located between genes *P* and *Q*, which results in the constitutive expression of the entire *Q* late region.

Transcription of the late genes of bacteriophage *λ* is assumed to occur by two pathways. The first is a major pathway which is positively controlled by the *Q* product (8, 15, 20). The *Q* gene product appears to act as an antiterminator, allowing the extension, over the late genes, of a short RNA (6S) initiated at the late promoter (18) (Fig. 1).

The second is a minor pathway which is completely *Q* independent ("leakiness" of the *Q*⁻ mutants) (6). We have shown that this minor transcriptional pathway operates only in the presence of the *N* activator and in the absence of immunity. It consists of the prolongation, over the late genes, of transcription initiated in the immunity region (promoter *p_R* [Fig. 1]). This minor transcription occurs to about 5% of the level of the major pathway and is obscured in the presence of the *Q* product. By itself (that is, in *Q*⁻ mutants), it is too slow to result in plaque formation; however, in liquid medium, *Q*⁻ mutants finally produce normal phage yields.

To elucidate the mechanisms of prolongation and antitermination, we have looked, by different methods, for mutants able to transcribe the late genes at a high rate, even in the absence of the *Q* product. A similar approach has been used by Herskowitz and Signer (14) and Sato and Campbell (19).

MATERIALS AND METHODS

Bacterial strains. All bacterial strains used are listed in Table 1 and are derivatives of *Escherichia coli* K-12. The strains were used as such and, for some of them, as lysogen derivatives.

Bacteriophages. *λc*⁺, *λimm21c*⁺ (16)·*c17* (17) is located in region *y*; it expresses constitutively genes *cII*, *O*, and *P* and replicates even in the presence of immunity.

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Those phages were used as such or labeled with various amber mutations: *Qam21*, *Aam11*, and *Ram5* (4); *Sam7* (13); *Qam203* and *Ram216* (22). Most of the phages used to infect sensitive hosts carry the *cII2002* mutation to diminish lysogenization frequency.

bypE (3) and *byp3* (our laboratory) are located between genes *P* and *Q*. *λQ⁻h⁺80* (*Q⁻* derivative of *λhy6* (21) is a *λ*-*φ80* hybrid with the left arm (*A* → *red*) from *φ80* and the right arm (*red* → *R*) from *λ*. *λ(QSR)₂₁Kam24* (constructed by L. Desmet and F. Salomon) is a *λ*-21 hybrid phage with the *A* → *P* region from *λ* and the *Q* → *R* region from 21. *Kam24* was isolated by Campbell (4) and introduced in the hybrid.

Methods. (i) **Infection experiments.** The infection experiments were described previously by Dambly and Couturier (6). Exponential cultures (lysogens or not) were infected by one or two phages, each at a multiplicity of 3, diluted 100-fold, and incubated at 37°C. At 100 min after infection, the dilutions were treated with CHCl₃, and adequate dilutions were titrated at 37°C.

(ii) **Endolysin assay.** The endolysin assay was as described by Dambly and Couturier (6) except the indicator bacteria were treated with CHCl₃ in buffer (0.05 M Tris-hydrochloride, pH 7.8).

(iii) **Lysogenization experiment.** *λ* Lysogens were selected and lysogenization frequencies were determined as described by Gottesman and Yarmolinsky (12) except that the selection was done on EMBO plates seeded with both *λred₃int₆cIh⁺λ* and *λcIh⁺80*.

RESULTS

Isolation and characterization. The *qin101* mutation was isolated from *λQam203Ram216* as a spontaneous plaque-forming phage on a lawn of the host HB15. The infecting phage carries, in addition to a *Q⁻* mutation, an *R⁻* mutation to be able to further distinguish between the *qin* mutants (*λqinQ⁻R⁻*) and the *Q⁺* revertants (*λQ⁺R⁻*) by transactivation experiments. The HB15 host bacterium was chosen since it carries an ochre suppressor (*supB*) able to restore, to a sufficient level, the *R* function

FIG. 1. Genetic map of λ prophage. Arrows indicate the origin and direction of transcription. The block represents the region of λ -imm21 nonhomology. Markers given are those relative to this study. Symbols: ∇ , transcription termination sites; $\times$, promoter sites.

TABLE 1. Bacterial strains

Bacterial strain	Genotype and relevant properties	Source/reference
H	F^- <i>argH lac am sup</i> ⁺	Ghyssen and Pironio (10)
H15	Isogenic to H but carries the <i>ron</i> mutation (defined by the number 15)	Ghyssen and Pironio (10)
HB15	<i>supB</i> derivative of H15	Ghyssen and Pironio (10)
W3110	F^- <i>sup</i> ⁺	Lederberg via Weigle
594	F^- <i>gal</i> ⁻ <i>strA sup</i> ⁺	Campbell (4)
N100	<i>recA gal</i> ⁻ derivative of W3350	Meselson via Yarmolinsky; Gottesman and Yarmolinsky (12)
RH1413 $\rightarrow$ RH1422	Thermoresistant derivatives of M5020G (Δ NNc1857)	Castellazzi et al. (5)
M5020G	<i>sup</i> ⁺ (<i>lac, pro</i>) <i>strA R</i> ⁺ <i>gly</i>	Provided by J. Davies
CA244.1	Cured derivative of CA244	This laboratory
CA244	HfrH B1 ⁻ <i>lac</i> ⁻ <i>trp</i> ⁻ <i>sup</i> ⁺	Brenner and Beckwith (2)
CA244.1 <i>recA</i>	<i>recA</i> derivative of CA244.1	
C600	<i>sup</i> II, permissive for amber mutations	Appleyard (1)
CA161	<i>sup</i> II, used instead of C600 (which is resistant to ϕ 80) in the experiments involving phages <i>h</i> ⁺ λ and <i>h</i> ⁺ 80	Brenner and Beckwith (2)
CA161/ λ	<i>h</i> ⁺ λ -resistant derivative of CA161	This laboratory

but not the *Q* function and the *ron* mutation which strongly decreases the leakiness of the *Q*⁻ mutations [compare λ Q⁻ productions after infections of H (*ron*⁺) and H15 (*ron*⁻) (Table 2)].

We could isolate only one mutant (λ qin101) able to grow on strain HB15 which plates with the same efficiency on *sup*⁺ hosts and on hosts carrying an amber suppressor. However, the plaques formed on the *sup*⁺ hosts are small, whereas those formed on the hosts carrying an amber suppressor are also normal but clear.

Preliminary characterization of the λ qin101Q⁻R⁻ phage showed that it is not a revertant or pseudorevertant of the λ Q⁻R⁻ phage. Indeed, it failed to transactivate the expression of the *R*⁺ gene of a prophage, showing that it does not synthesize active *Q* product in detectable amounts. Furthermore, when the *qin*101 mutation was combined with other *Qam* mutations, such as *t57*, *Qam*21, *Qam*501, or *Qam*73, the resulting phages behave in the same way as the *qin* derivative of Q203.

Burst sizes of λ qin101Q⁻R⁺ phage on different *sup*⁺ strains are given in Table 2. It is apparent that λ qin101 Q⁻ grows as well on the H15 (*ron*⁻ *sup*⁺) and the H (*ron*⁺ *sup*⁺) strains as on the HB15 (*ron*⁻ *supB*) strain, the yields being about 10⁵ times greater than those of λ Q⁻ plated on H15. The yield of λ qin101Q⁻ on *sup*⁺ strains seems, however, to be impaired by mutations conferring streptomycin resistance, insofar as one can judge from the lower yield on the *strA* strains 594 and N100.

To have a more quantitative measure of the effect of the *qin*101 mutation on late gene expression, we measured directly the expression of the late gene *R* by endolysin titrations. We found that in W3110 (*ron*⁺ *sup*⁺ host bacterium) λ qin101Q⁻ produced two to three times as much endolysin as did λ Q⁻, reaching a value of 10% of that synthesized by λ Q⁺ phage (Table 3). We, therefore, conclude that the *qin*101 mutation permits growth, in Q⁻ conditions, by only increasing the late gene expression by a factor of

TABLE 2. Growth of $\lambda qin101Q^-$ on different bacterial strains^a

Bacterial strain	Yield (phage/bacterium) at 100 min after infection with:			
	λQ^-R^+	$\lambda qin101Q^-R^+$	λQ^+R^+	$\lambda qin101Q^+R^+$
HB15 (<i>ron^- supB</i>)	3	75	52	45
H15 (<i>ron^- sup^+</i>)	5.4×10^{-4}	70.4	58	55
H (<i>ron^+ sup^+</i>)	3.5	56.1	50	52
W3110 (<i>ron^+ sup^+</i>)	2.7	66.7	75	60
CA244.1 (<i>ron^+ sup^+</i>)	3	20.4	69	65
594 (<i>ron^+ sup^+ strA</i>)	2	6.4	33	40
N100 (<i>ron^+ sup^+ strA</i>)	1.75	7.1	35	29

^a The infection experiments were performed as described in the text. Phage productions were measured on the C600 indicator strain. λQ^-R^+ is $\lambda cII_{2002}Qam203$, and $\lambda qin101Q^-R^-$ is $\lambda cII_{2002}qin101Qam203Ram216$; λQ^+ and $\lambda qin101Q^+$ also carry the cII_{2002} mutation.

2 to 3.

Mapping of the *qin101* mutation. Precise localization of the *qin101* mutation has been inferred from three types of crosses. (i) In a cross of $\lambda c^+qin101Q^-R^-$ with $\lambda imm21Q^-R^-$ phages, *imm21qin101Q^-R^-* recombinants [selected on HB15 (λ)] are found with a frequency of 1%, indicating that the *qin101* mutation is localized outside the *imm21* region.

(ii) A more precise mapping was obtained by crossing $\lambda c^+qin101Q^-R^-$ phage with various λ prophage deletion mutants. The deletions used enter the prophage from the left and end at different points in the *P-Q* region. For these crosses, the clear phenotype given by the *qin101* mutation was used as marker, and the recovery of turbid-plaque λ recombinants indicated that the *qin101*⁺ region was present within the deleted prophage. The crosses between $\lambda c^+qin101Q^-R^-$ and different deleted prophages localized *qin101* between the ends of the deletions RH1417 and RH1415 (Table 4).

(iii) We also mapped the *qin101* mutation in respect to two mutations located in the *P-Q* region: *bypE* (1) and *byp3* (previously isolated in this laboratory). These two mutations, although they have the same properties of *N* independence for the relief of the *t₁₂* terminator sites and of clear-plaque phenotype, are distinct on the λ map: *byp3* is within the RH1415 deletion and *bypE* is not. Crosses between $\lambda c^+qin101Q^-R^-$ phage and $\lambda imm21bypE$ (Fig. 2) show that the turbid recombinants ($\nu = 5.10^{-2}$) present almost all (97%) the *imm21* and the Q^-R^- mutations. On the contrary, the same cross made with $\lambda imm21byp3$ (Fig. 2) gives turbid recombinants with a much lower frequency ($\nu = 6.10^{-3}$) which have indifferently *imm* λ or *imm21*, the *am* or *am*⁺ character. This shows that the *qin101* and *byp3* mutations are very close. The presence with equal probability of one or the other outside marker is probably due to high negative interference. From these results, we inferred that the

TABLE 3. Endolysin synthesis by *qin101* phage infecting a *sup*⁺ strain^a

W3110 infected with:	Endolysin activity (units/100 μ l of lysate at 100 min)
λQ^-	36
$\lambda qin101Q^-$	95
λQ^+	595
$\lambda qin101Q^+$	950

^a All the phages carry an additional cII_{2002} mutation. Q^- is *Qam203*. Endolysin was assayed as described by Dambly and Couturier (6).

TABLE 4. Deletion mapping of the *qin101* mutation^a

Host	Clear <i>am</i> ⁺ (%) ^b	Turbid <i>am</i> ⁺ (%) ^b	Ratio of <i>c</i> ⁺ <i>am</i> ⁺ (%) / <i>c</i> <i>am</i> ⁺ (%) ^b
RH1422 (Q^-/R^+)	100	10^{-1}	10^{-3}
RH1415 (P^-/Q^+)	100	10^{-1}	10^{-3}
RH1417 (P^-/Q^+)	51.8	48.2	0.93
RH1416 (P^-/Q^+)	38	62	1.62
RH1413 (P^-/Q^+)	33.65	66.35	1.97

^a The deleted strains derive from M5020G (*sup*⁺ *strA*) (see text). The blocks represent the deleted prophage region. The *am*⁺ recombinants were titrated on 594 (*sup*⁺ *strA*). The percentage of clear *am*⁺ (or turbid *am*⁺) represents the fraction of *am*⁺ recombinants which present a clear (or turbid) phenotype. Q^- is *Qam203*; R^- is *Ram216*.

^b After infection with $\lambda c^+qin101Q^-R^-$.

qin101 mutation is located to the left of *bypE*, very near *byp3*.

Dominance-recessivity test. To test the dominant or recessive, *cis* or *trans* character of the *qin101* mutation, we coinfecting strain H15 with $\lambda qin101Q^-h^+\lambda$ (able to grow on H15) and λQ^-h^+80 (unable to grow on H15) and measured the production of each phage. The h^+80 character was used to test for a *trans* effect of the *qin101* mutation on late gene expression since the presence of the h^+80 late protein results in a host range different from that of λ .

We observed that the mixed infection results in high, comparable yields of both $\lambda qin101Q^-$ and λQ^- phages (Table 5). The difference in $\lambda qin101Q^-$ yields between mixed and single infections (compare lines 3 and 1 of Table 5) may be explained by phenotypic mixing; as will become apparent later, there is no synthesis of late $\phi 80$ proteins, and the coats made by $\lambda qin101Q^-h^+\lambda$ package not only their own DNA but also λQ^-h^+80 DNA. This competition limits the amount of $\lambda qin101Q^-h^+\lambda$ phage packaged.

Indeed, we can see (Table 5) that, among progeny, the number of h^+80 phages which present the host range of $\phi 80$ [h^+80 (h^+80)] remains very low. As expected, this production is again high when the *qin101* derivative is Q^+ (Table 5).

All of these results show that, in mixed infection, $\lambda qin101Q^-$ phage continues to express its own late genes but is unable to induce in *trans* the late genes of the coinfecting phage. We, therefore, conclude that *qin101* is a *cis*-dominant mutation.

Nature of the *qin101* mutation. As already described, the *qin101* mutation gives a clear phenotype in Q^+ conditions (phage $\lambda qin101Q^+$ or $\lambda qin101Q^-$ on a *sup am* host). In Q^- conditions (phage $\lambda qin101Q^-$ on a *sup*⁺ host), plaques are very small, and the phenotype is not as easy to determine. To determine the phenotype, we measured the percentage of lysogenization after infection of a *sup*⁺ host with different derivatives of $\lambda qin101$ and found that $\lambda qin101Q^-$ lysogenizes normally (33%), in contrast to $\lambda qin101Q^+$ (<0.05%; Table 6). The observation that the

FIG. 2. Mapping of *qin101* in respect to *byp3* and *bypE*.

TABLE 5. *Cis*-dominant effect of the *qin101* mutation^a

Phage	Phage production (phage/bacterium) at 90 min			
	Total	$\lambda qin101$ ($h^+\lambda$) or (h^+80)	λQ^- ($h^+\lambda$) or (h^+80)	λQ^-h^+80 (h^+80)
$\lambda qin101Q^-h^+\lambda$	35	35		
λQ^-h^+80	2×10^{-1}		2×10^{-1}	2×10^{-1}
$\lambda qin101Q^-h^+\lambda + \lambda Q^-h^+80$	24	13	11	2×10^{-1}
$\lambda qin101Q^+h^+\lambda$	28			
$\lambda qin101Q^+h^+\lambda + \lambda Q^-h^+80$	42	25	17	12

^a Strain H15 (a sensitive host to both $h^+\lambda$ and h^+80 derivatives) was infected by each phage at a multiplicity of 3. This mixed infection gave (4) classes of phages following their genotype ($h^+\lambda$ or h^+80) and their phenotype [$(h^+\lambda)$ or (h^+80)]: the two parental types, $h^+\lambda$ ($h^+\lambda$) and h^+80 (h^+80), and two types due to the phenotypic mixing, $h^+\lambda$ (h^+80) and h^+80 ($h^+\lambda$). Among the four progeny types, only the phages which are h^+80 (h^+80) should be able to form a plaque on a strain resistant to λ and thus may be titrated. λQ^-h^+80 is a Q^- derivative of $\lambda hy6$ (see text). Total phage production was titrated on CA161, $\lambda qin101Q^-$ were titrated on H15, $\lambda qin101Q^+$ were titrated on 594, and λQ^- were titrated by the difference between the total and $\lambda qin101$ yields. λQ^-h^+80 (h^+80) production was titrated on CA161/ λ . All the phages carry the *cII*₂₀₀₂ mutation.

frequency of lysogenization by $\lambda qinQ^+$ is improved (5%) in the presence of mutations S^- and R^- (Table 6) and almost reaches the normal level (26%) with an $S^-R^-A^-$ triple mutant (Table 6) shows that these functions are expressed by $\lambda qin101Q^+$ under immunity and thus indicates that the $qin101$ mutation constitutes a promoter able to initiate, even under immunity, the constitutive expression of gene Q (in Q^+ conditions) and of all the late genes. In Q^- conditions, this additional transcription does not provoke the killing of the lysogens but suffices to allow phage growth.

To obtain more direct evidence supporting this conclusion, we measured the expression of two late genes (R and K) by a $\lambda qin101$ phage infecting an immune host. In the R experiment, the prophage only provides the immunity, and we tested whether, despite this immunity, the superinfecting $\lambda qin101Q^+$ phage is able to synthesize endolysin. Results are shown in Table 7, part 1. We observed a small but significant production of endolysin when the homoimmune phage carries the $qin101$ mutation, production which disappears entirely if the $\lambda qin101$ phage is Q^- (Table 7). This production of endolysin is low (11 units), as would be expected in view of the low dosage of gene R^+ present in the cell. Indeed, the presence of the $c17$ promoter mutation, which enables the phage to replicate even in the presence of immunity, greatly improves the endolysin production (Table 7). Moreover, the $\lambda c17qin101$ phage gives high phage bursts under immunity and is N independent for its development (data not shown). We also examined the *trans* effect of the Q product synthesized by the homoimmune $\lambda qin101Q^+$ phage on an R^+ gene located on the prophage. In this experiment, the strain is lysogenic for λR^+ prophage, and the $\lambda qin101$ infecting phage carries an R^- mutation (Table 7, part 2). It was found that $\lambda qin101 Q^+R^-$ phage completely fails to elicit endolysin expression in *trans*. However, the positive result obtained with the $c17$ derivative shows that this *trans* expression is completely dependent on the number of $\lambda qin101Q^+$

copies present. The fact that this transactivation is not seen with the product of a single Q^+ gene shows that Q acts preferentially in *cis*, in agreement with previous observations (7, 9).

In the K experiment, the prophage constitutively provides the K gene product, and we tested the growth characteristics of a heteroimmune superinfecting phage which was neither able to synthesize the K product itself (being K^-) nor able to transactivate the K^+ gene of the λ prophage (since its Q product has a different specificity: $Q21$). Thus, we infected the $\lambda imm21c^+qin101Q^+S^-R^-A^-K^+$ lysogen with a hybrid phage $\lambda(QSR)_{21}K^-$ and looked for complementation of the K deficiency by the K^+ gene of the prophage, in λK^- progeny. This complementation by a unique K^+ gene is easily detectable in view of the catalytic character of the K function. The yield of the superinfecting $\lambda(Q)^+_{21}K^-$ phage was greatly improved when the prophage carried the $qin101$ mutation (Table 8)

TABLE 7. *R* experiment^a

Strain	Endolysin activity (units/100 μ g of lysate) at 100 min
Part 1: CA244.1 <i>recA</i> (λc^+R^-) infected with:	
λc^+	<2
$\lambda c^+qin101Q^+R^+$	11
$\lambda c^+qin101Q^-$	<2
$\lambda c17qin101Q^+R^+$	570
$\lambda c17$	<2
Part 2: CA244.1 <i>recA</i> (λc^+R^+) infected with:	
$\lambda qin101Q^+R^-$	<2
$\lambda c17qin101Q^+R^-$	8

^a Infection experiments were performed as described in the text. Q^- is *Qam203*, and R^- is *Ram216*.

TABLE 8. *K* experiment^a

Bacterial strain	Yield (phage/bacterium) after infection with $\lambda(QSR)_{21}K^-$ phage		
	Super-infecting type	K^+ recombinants or revertants	
CA244.1	0.1	0.02	
CA244.1 ($\lambda imm21$)	0.5	0.3	
CA244.1 (21c ⁺)	7	0.3	
CA244.1 ($\lambda imm21qin101S^-R^-A^-$)	9	0.28	
	<i>A</i>	<i>K^-</i>	<i>J</i>
	<i>imm</i> λ	<i>Q</i>	<i>S</i>
			<i>R</i>

^a Superinfecting phage production was titrated on C600 (21c⁺), and λK^+ production was titrated on 594 (21c⁺). Heterologous substitutions of the superinfecting phage DNA with λ DNA are given by the dark area.

TABLE 6. Lysogenization by $\lambda qin101$ derivatives^a

CA244.1 infected with:	Lysogeny (%)
λc^+ or λc^+Q^-	35
$\lambda c^+qin101Q^-$	33
$\lambda c^+qin101Q^+$	<0.05
$\lambda c^+qin101S^-R^-$	5
$\lambda c^+qin101S^-R^-A^-$	26

^a The procedure used is described in the text. The percentage of lysogeny represents the number of lysogens divided by the number of infected cells. Q^- is *Qam203*, S^- is *Sam7*, R^- is *Ram5*, and A^- is *Aam11*.

and even reached the optimal values obtained after K^+ transactivation (Table 8).

The results of these experiments show that the late functions tested are expressed under immunity in the presence of the *qin101* mutation, confirming the promoter character of this mutation.

DISCUSSION

qin101 is a promoter mutation located in the *P-Q* region which allows expression of *Q* and of the late genes. The levels of endolysin synthesized by λ *qin101Q*⁻ phage suggest that late gene expression, in the presence of this promoter, results in a two- to threefold increase in the level of expression over that observed with *Q*⁻ phage (residual levels of endolysin are synthesized by *Q*⁻ phage as a result of the leakiness of the *Q*⁻ mutation). Although the increase of late transcription brought about by the *qin101* mutation is relatively small, it is nevertheless sufficient to make *qin101Q*⁻ phage plaque formers, in contrast to *Q*⁻ phage. Moreover, the fact that the λ *qin101Q*⁻ phage continues to make plaques in a *ron* host, which completely eliminates the leakiness of the *Q*⁻ mutation, shows that the level of late transcription, resulting from the presence of the *qin101* promoter, is sufficient to allow the growth of a *Q*⁻ derivative. This constitutive late expression does not alter the viability of *qin101Q*⁻ lysogens. Late expression, which is normally amplified by active replication in the absence of immunity, is not amplified in the λ *qin101Q*⁻ lysogen as a result of the presence of immunity.

Endolysin titrations with λ *qin101Q*⁺ phage showed that, after superinfection of normal lysogens (and transactivation experiments), this phage produced significant amounts of *Q* product. It is therefore not surprising that *qin101Q*⁺ lysogens are nonviable. However, *qin101Q*⁺ lysogens with mutationally inactivated *S*, *R*, and *A* genes are viable. We have also shown that the *qin101Q*⁺ phage under immunity constitutively expresses the late gene *K*.

How can we explain the molecular mechanism by which the *qin101* mutation gives rise to late gene expression? We propose that the *qin101* mutation, by promoting a reinitiation of transcription before gene *Q*, increases *Q* mRNA synthesis to a level which results in the production of a small amount of *Q* product despite the presence of the *Qam* mutation. These few *Q* molecules would then allow the partial relief of the late terminator, resulting in late gene transcription.

This explanation is consistent with the absence of growth of *qin101Q*⁻ phage on *strA*

hosts. Indeed, since the *strA* mutation greatly decreases the degree of misreading of codons in general and of amber codons in particular (11), one would expect λ *qin101Qam* phage to synthesize less *Q* product in the *strA* host and consequently fewer late proteins. Furthermore, our explanation is consistent with the absence of growth of λ *qin101* phage containing two amber mutations in gene *Q* (data not shown). The presence of such a double mutation in gene *Q* should reduce the amount of *Q* proteins synthesized.

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