COMPLEMENTATION EXPERIMENTS ABOUT THE Q GENE OF PHAGE λ

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Received November 12, 1969

Summary: When the Q gene of λi^{434}cIIsusQ was complemented in λsusQ lysogenic, non-permissive E. coli by mix-infected λcII or λcI7, λcI7 but not λcII could grow and support the growth of λi^{434}cIIsusQ. The results will be discussed relating to a temporal control of λ phage development.

Sequential gene expression of λ phage is controlled at least by two regulator genes, N and Q (see reviews, ref. 1 and 2). Mutant in the Q gene permits normal synthesis of λDNA but no or little synthesis of late mRNA and proteins (3,4), thus the Q gene having been indicated as a late inducer gene (4).

By the way, late transcription has been shown to be highly dependent on phage DNA replication (3,5,6,7). In the previous experiments (7), it was shown that necessity of phage DNA replication for late transcription is not due to so called gene dosage effect but that late transcription of λ phage requires continuous replication of phage DNA and, further-more, it seems to be controlled by a subtle coupling of DNA and protein synthesis.

Then, in order to study the characteristics of Q gene expression, I carried out the complementation experiments, the

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thema of this report.

Experimental Principles

Thomas and Bertani (8) observed that when λ lysogenic bacteria were mix-infected with λ and λi^{434} hybrid, hetero-immune λi^{434} grew well but homo-immune λ did not grow at all. In these mix-infections, DNA replication of homo-immune phage was shown to be entirely blocked, despite of the presence of enzymes necessary for phage DNA synthesis (8,9).

Recently, new type of λ mutant, λcI7, was isolated by Pereira da Silva (10) and further characterized as that λcI7, when infected λ lysogen, expresses constitutively its O and P genes and is able to multiple its DNA, but as another functions are still repressed, it cannot make mature phage (10,11,12).

On the basis of these observations, the Q gene of λi^{434}susQ was complemented by mix-infected λcII or λcI7 in λ lysogen to know what conditions are required for the Q gene to be expressed. W3350(λsusQ) was used as an infecting host, for complementation by prophage was observed to take place when wild-type λ lysogenic bacteria were infected with λi^{434}susQ (13). And also all experiments were carried out using clear type phage (all phage except λcI7 contain cII mutation) to exclude disturbance which might be caused with phage lysogenization.

Materials

E. coli K12, W3350 (non-permissive host) and C600 (permissive host) were supplied us from Dr. H. Ozeki and appropriate lysogenic derivatives were prepared in this laboratory. Phage λco$_2$ (14), designated further as λcII, and λcI7 (10) were supplied via Dr. J. Tomizawa. All the clear phage were grown on polypeptone

glucose agar. λi^{434} (15) and $\lambda \underline{sus} Q_{73}$ (16) were supplied us from Dr. H. Ozeki as C600 lysogens and phage were prepared by adding mitomycin C (1 μg/ml) to a logarithmically growing culture. Another phage used were prepared by making these phage to cross in mix-infected cells.

Results and Discussion

Results are illustrated in table 1 and may be summarized as follows:

(1) Control experiments: $\lambda \underline{i}^{434}$cII grew well on W3350 ($\lambda \underline{sus} Q_{73}$) and this phage infection resulted in liberation of about 1 phage per infected cell which has $\underline{i}^{\lambda}$ character, this probably being caused by hetero-immune curing (17). Mix-infection with $\lambda \underline{i}^{434}$cII and λcII did not interefere $\lambda \underline{i}^{434}$cII growth and λcII did not grow in these cells. This confirms the first observation of Thomas and Bertani (8). Single infection of $\lambda \underline{i}^{434}cII\underline{sus} Q_{73}$ liberated 2-3 phage per infected cell (plating efficiency of this phage on W3350 to C600 was 4×10^{-5}). This seems to be caused by leakiness of Q_{73} mutation, for $\lambda \underline{sus} Q_{73}$ infection of sensitive bacteria, W3350, also liberated the similar level of phage. When W3350($\lambda \underline{sus} Q_{73}$) was singly infected with λcII or λc17, λcII liberated only 0,2-0,3 phage per infected cell but λc17 did more phage (2-3 phage per infected cell). This result about λc17 is not consistent with the former results of Packman and Sly (11), for they observed the similar level of repression of phage growth between these two phage in λ lysogen. The reason why our λc17 makes more phage is not certain at present, but our λc17 has its killing activity to λ lysogenic host and also shows clear type phenotype on sensitive indicator as original λc17 does. This phage, of

Table 1. Complementation with the Q gene product.

Phage infected	Phage* assayed on indicator C600	C600(λ)	C600(λi^{434})
λi^{434}cII + λcII	47,5	46,8	2,8
λi^{434}cII$\underline{sus}$Q$_{73}$ + λcII	3,2	2,8	0,6
λi^{434}cII$\underline{sus}$Q$_{73}$ + λcI7	21,6	7,8(0,3)**	14,4
λi^{434}cII	54,3	43,6	1,3
λi^{434}cII$\underline{sus}$Q$_{73}$	2,3	2,1	0,03
λcII	0,2	0,0	0,2
λcI7	2,7	0,0	2,7

E. $\underline{coli}$ K12, W3350(λ$\underline{sus}$Q$_{73}$) were grown to 3 x 10^8 cells/ml, centrifuged and concentrated in adsorption buffer (1 x 10^9 cells/ml). Culture was starved with shaking at 37°C for 60 minutes and then infected with phage as indicated in the table (multiplicity of infection = 3-4). Adsorption was taken place at 37°C for 15 minutes, then anti λ-serum (K=6) was added and incubated for more 5 minutes. Culture was diluted 10^4 fold into prewarmed polypeptone glucose medium and incubated with shaking at 37°C for 120 minutes. Infected cells were assayed on indicator C600, immediately after dilution, and phage produced after 2 hours incubation were assayed after chloroform treatment.

*Phage titer was indicated as average phage yield per infected cell.
**Phage assayed on indicator W3350(λ).

course, does not make any plaque on λ lysogen as indicated in the table.

(2) Experiments: In mix-infection with λi^{434}cII$\underline{sus}$Q$_{73}$ and λcII, both of these phage did not grow above the

background level. But, both λi^{434}cII<u>sus</u>Q$_{73}$ and λc17 grew well when mix-infected. Growth of phage in the latter mix-infection might be argued to come from growth of λi^{434}cII<u>sus</u>Q^{+} which might originate as a result of recombination, but this is unlikely, for when the same lysate was assayed on W3350(λ), only 0,3 phage per infected cell made plaques on the indicator.

λc17, when singly infected λ lysogen, does not make late proteins (11,12) but makes when mix-infected with λi^{434}cII<u>sus</u>Q$_{73}$. This implies that a factor or gene product seems to be necessary for Q gene activation. Synthesis of this factor is repressed in single infection by λc17 but it can be supplied by mix-infected λi^{434}cII<u>sus</u>Q$_{73}$. From the character of λc17 (10,11,12), the factor would be the product of gene which located in the left quarter of λ right arm, probably the N gene. The point was recently visualized clearly by Butler and Echols (18) after isolation of a λ mutant bypassing N control.

Furthermore, another character seems to be necessary for Q gene activation; this requirement can be achieved by λc17 mutation but not by λcII. It is difficult at present to determine exactly what character it is, but the characters of λc17 mutation which are constitutive transcription of OP genes or replication of phage genome appears to be responsible for the activation of Q gene expression.

Thus, it may be concluded that Q gene expression seems to be dually controlled by an early gene product and also by the character relating to phage DNA replication.

Acknowledgements

I am very grateful to Dr. T. Yura for helpful discussion

and encouragement throughout this work. I also thank Drs. H. Ozeki and J. Tomizawa for giving me phage and bacterial strains. This work was supported by a research fund from the Ministry of Education of Japan.

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