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THE SEPARATION OF PHAGE PROMOTER FROM BACTERIAL lac PROMOTER FOR β-GALACTOSIDASE EXPRESSION IN TRANSDUCING PHAGE λplac5

Ka-cheung Luk and Kai-keung Mark

Department of Biology
The Chinese University of Hong Kong
Shatin, N.T.
Hong Kong

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Summary

The replication defective transducing phage λplac5029P3 carries a portion of the E. coli lac operon in the b2 region of the lambda phage. This lac operon segment contains the lac promoter, the lac operator, and the β-galactosidase z gene, but does not contain the lac repressor i gene. The z gene can be expressed from both the inserted lac promoter and the phage promoter. When E. coli strain 594 (z^-, i^+) or JC6256 (Δlac) is infected by λplac5029P3 in the absence of additional cyclic AMP, β-galactosidase synthesis is shown to be expressed from the phage promoter. When 594 (λ^+) or JC6256 (λ^+) is infected by λplac5029P3 in the presence of additional cyclic AMP and IPTG, β-galactosidase synthesis is shown to be expressed from the inserted lac promoter.

The ability to separate the phage promoter from the inserted lac promoter for β-galactosidase expression will simplify the interpretation whenever λplac5 is used.

A lac transducing phage, λplac5, was constructed (1), and has wide application for the study of the lac operon and for other purpose (2-7). The λplac5 carries a segment of the E. coli lac operon in the b2 region of the lambda phage. This lac operon segment contains the lac promoter, the lac operator, and the z gene, structural gene for β-galactosidase, but does not carry functional i gene, y gene, and a gene (1,2).

The inserted β-galactosidase z gene can be expressed from both the lac promoter and the phage promoter (6-8). This casts a lot of uncertainty for the interpretation whenever the β-galactosidase is used as a marker.

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This communication describes conditions which allow one to separate the expression of the z gene from either the bacterial lac promoter or from the phage promoter.

Materials and Methods

Escherichia coli strain 594 (genotype su^- gal^- str^r lac^-), 594(λ) (9), JC6256 (genotype F^- trp^- ΔlacX74 su^- str^r) (1) were used, and JC6256(λ^t) was constructed. Bacteriophage lambda mutants λplac5cI857(λplac5) (1), λsus029, λsusP3, λcI857sus029susP3, λcI857susN7susN53 (9), λcI47 (10), λimm434 (10) and λpapa (11) were used, and λplac5cI857sus029susP3 (λplac5029P3) was constructed.

Tryptone broth (15), M9 glyceral medium (16), M63 minimal medium (12), and M63-XG plates (5) were used. The procedures for preparation and purification of bacteriophage has also been described (15).

An overnight culture of E. coli in M9 glycerol medium was diluted 20-folds, and grown in the same medium with aeration at 37^o to a concentration of 2×10^8 cells/ml. The culture was chilled, centrifuges at 5,000 x G for 10 min, and resuspended to 2×10^9 cells/ml with cold M9 glycerol medium supplemented with 10 mM $MgSO_4$. The bacterial culture was then infected with bacteriophages at indicated multiplicity of infection (m.o.i.). 20 min at 0^o were allowed for adsorption. At time zero, fresh prewarmed (42^o) M9 glycerol medium was added to obtain a titre of 2×10^8 cells/ml and the infected cells were incubated with aeration at 39^o. At various times thereafter 1 ml samples were removed, chilled in ice and immediately centrifuged. The cells were resuspended in 1 ml assay mixture without ONPG and disrupted by addition of 0.1 ml toluene. The toluenized samples were incubated at 37^o in a shaker for 30 min and used for assay of β-galactosidase activity (12,13,14).

Results and Discussion

Conditions for β-galactosidase Synthesis Initiated from Phage Promoter

Escherichia coli strain 594 is shown to contain active lac repressor (i gene product), but to be defective in β-galactosidase synthesis (z gene product) (Fig. 1 and 2).

When E. coli 594 cells are infected with λplac5O29P3 (defective in phage DNA replication) in the absence of additional cyclic AMP, β-galactosidase synthesis is shown to begin after a long lag of 10-20 min, and then proceeds for another 40 min (see Fig. 1). The expression of β-galactosidase under these conditions is expected to derive only from the phage promoter because the inserted lac promoter is inhibited both by the presence of the lac repressor as well as by the depressed cyclic AMP level after phage infection (3,17-20).

To further verify this point, 594(λ^+) cells are infected by λplac5O29P3 in the absence of additional cyclic AMP and/or IPTG. We find no β-galactosidase synthesis under these conditions (Figs. 1 and 2). These results confirm that β-galactosidase synthesized in λplac5O29P3-infected 594 cells is initiated only from phage promoter.

E. coli JC6256 is a strain where the whole lac operon including the lac repressor (i) gene has been deleted (1). When JC6256 cells are infected with λplac5O29P3 (or λplac5, data not shown) in the absence of additional cyclic AMP, β-galactosidase synthesis is again shown to appear after a long lag of 10-20 min, and continued for another 40 min (see Fig. 1). However, when JC6256(λ^+) cells are infected with λplac5O29P3 (or λplac5, data not shown) in the absence of additional cyclic AMP, no β-galactosidase is produced (Figs. 1 and 2). These results show clearly that in the absence of additional cyclic AMP, β-galactosidase is expressed only from the phage promotor.

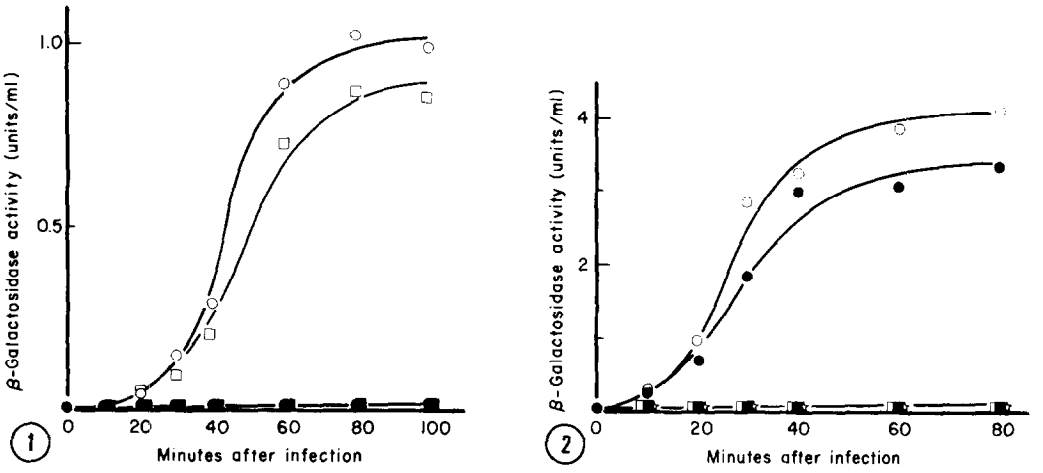

Fig. 1. Synthesis of β-galactosidase from phage promoter. E. coli cell grown in M9 glycerol medium at 39° was infected with λplac5029P3 at m.o.i. of 5. Samples were taken at the indicated times. The infected cells were broken by toluene, and the β-galactosidase activity was assayed (See Materials and Methods).

—○—○— strain 594 infected with λplac5029P3;

—●—●— strain 594(λ^+) infected with λplac5029P3;

—□—□— strain JC6256 infected with λplac5029P3;

—■—■— strain JC6256(λ^+) infected with λplac5029P3.

Fig. 2. Synthesis of β-galactosidase from inserted lac promoter. E. coli cell grown in M9 glycerol medium at 39° was infected with λplac5029P3 at m.o.i. of 2. Samples were taken at the indicated times. The infected cells were broken by toluene, and the β-galactosidase activity was assayed (See Materials and Methods).

—○—○— strain 594(λ^+) was infected with λplac5029P3 in the presence of IPTG (1 mM final concentration) and cyclic AMP (2 mM final concentration) added at time zero;

—●—●— strain JC6256(λ^+) was infected with λplac5029P3 in the presence of cyclic AMP (2 mM final concentration) added at time zero;

—□—□— strain 594(λ^+) was infected with λplac5029P3 in the presence of IPTG (1 mM final concentration) and in the absence of additional cyclic AMP;

—■—■— strain JC6256(λ^+) was infected with λplac5029P3 in the absence of additional cyclic AMP;

—☆—☆— strain 594(λ^+) was infected with λplac5029P3 in the presence of cyclic AMP (2 mM final concentration) and in the absence of IPTG.

Apparently, the lac region is not expressed from the infecting phage, even in the absence of repressor, because of the decreased cyclic AMP level (19,20).

Concerning which phage promoter is responsible for β-galactosidase expression in the absence of cyclic AMP, some authors have assumed that the leftward phage promoter, P_L, in λplac5 is the phage promoter responsible for β-galactosidase expression (6,21). However, our data do not support this proposition. For example, the extensively delayed (10-20 min after infection) appearance of β-galactosidase initiating from phage promoter is difficult to account for if P_L promoter is responsible. We believe that the phage promoter responsible for β-galactosidase expression is a promoter in the b_2 region. The experimental evidence to support this suggestion will be presented separately (7).

Conditions for β-galactosidase Synthesis Initiated from the Inserted lac Promoter

When E. coli strain 594 (λ^+) cells are infected with λplac5O29P3 in the presence of additional cyclic AMP and lac inducer IPTG, β-galactosidase synthesis appears 10 min after induction just as observed in normal induction. β-galactosidase level continues to increase until about 60 min after infection (see Fig. 2). The synthesis of β-galactosidase can only be expressed from the inserted lac promoter since phage promoters are suppressed by the lambda repressor present in the lysogen (22). However, there is a limit beyond which the effective suppression by the lambda repressor could not hold. At multiplicity of infection of 20, effective suppression by existing lambda repressor still holds, but at multiplicity of infection of 40, the lambda repressors are titrated out (23,24) and some degree of β-galactosidase expression from phage promoter is observed (data not shown).

When E. coli JC6256(λ^+) cells are infected with λplac5029P3 in the presence of cyclic AMP, β-galactosidase synthesis is observed in a manner similar to that observed with 594 (λ^+) described above (see Fig. 2).

The addition of cyclic AMP seems very crucial for the proper expression from the inserted lac promoter. No β-galactosidase was synthesized in the absence of exogenous cyclic AMP in the host which did not contain repressor (strain JC6256(λ^+)) or in the host in which repressor was inactivated by IPTG (strain 594(λ^+)) (see Figs. 1 and 2).

Mercereau-Puijalon and Kourilsky (1976) used a λimm21plac5 double lysogen unable to excise and replicate, and reported that β-galactosidase was synthesized presumably from phage promoter P_L only in the presence of cyclic AMP. Our results are different in that cyclic AMP is not essential when β-galactosidase synthesis is initiated from a phage promoter. Cyclic AMP becomes essential only when β-galactosidase synthesis is initiated from the inserted lac promoter in an infected cell. Since the hin or car function (17, 19) is expressed in this system, and there is some uncertainty as to which promoter is responsible for β-galactosidase expression. These discrepancy can be reduced if the inserted lac promoter is responsible for their observed β-galactosidase synthesis.

Mercereau-Puijalon and Kourilsky (1976) also reported that the rate of escape synthesis of β-galactosidase was only 3-4% of the regular IPTG induction. Our results demonstrate that β-galactosidase synthesis initiated from the inserted lac promoter is higher than that initiated from the phage promoter by 4-8 folds (at the same m.o.i.); besides the level of the β-galactosidase synthesis from the inserted lac promoter in λplac5029P3 agrees neatly with the level of β-galactosidase synthesis in normal E. coli K cells after IPTG induction if the gene dosage is taken into consideration (7).

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