The Time Required for cro Gene Product to Establish Dominance in Coliphage Lambda Lysogens

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SUMMARY

To measure the length of heating required to convert a λ lysogen in the immune (im⁺) phase into the anti-immune (im⁻) phase, rex gene activity was used as an indicator. It was observed that 5 min heating at 41 °C did not shift any lysogenic cells of 594(λN−cI857O−) from the im⁺ phase into the im⁻ phase, and it took 17 min heating at 41 °C followed by long hours of culture at 30 °C to shift half of the lysogenic cells into the im⁻ phase. Such a length of heating is too long to be accounted for by blocking of the expression of lambda repressor by cro gene product. The result is more consistent with accumulation of a certain level of cro gene product during heating so that the synthesis of the repressor is blocked even after a return to low temperature.

The repressor of bacteriophage lambda, encoded by the cI gene, controls the expression of most other genes in the lambda genome by blocking the leftward and rightward promoters PL and PR (Ptashne, 1971). However, the expression of the cl-rex operon is also under complicated control (Furth, 1980; Ptashne et al., 1980). The most important controlling factor is the product of gene cro (Eisen et al., 1970) or tof (Pero, 1970). This cro gene maps in the x region inside the immunity region (Pero, 1971) and its expression is controlled by the rightward promoter PR (Szybalski, 1971). The cro gene product has two negative regulatory functions: turn-off of transcription of the cl-rex operon (Hayes, 1972) and repression of the leftward and rightward transcription controlled by promoters PL and PR (Reichardt, 1975). Therefore, the product of the cro gene and the cl repressor are counteracting each other.

With a heat inducible lambda lysogen such as 594 (λN−cI857O−), carrying mutations in the N and O genes which enable the lysogen to grow at inducible temperature (41 °C) without being killed, it was shown that the lysogen can exist in two distinct physiological states: an immune (im⁺) phase and an anti-immune (im⁻) phase. Before heat induction the repressor is in dominance, and the lysogens have an imm⁺ and rex⁺ phenotype. This is referred to as the im⁺ phase. If the lysogen is grown at the inducible temperature (41 °C), the cro gene product is produced upon denaturation of the thermolabile repressor. Thus, after some period of growth at the inducible temperature (41 °C) the cro gene becomes dominant, and the lysogen cannot recover immunity and rex gene activity (restriction for T4rII growth), even when the lysogen is allowed to grow again at the low temperature (30 °C). Now the lysogen has imm⁻ and rex⁻ phenotype. This state is known as the im⁻ phase (Calef et al., 1971; Mark & Szybalski, 1973). Because of this correlation the rex⁻ phenotype can be used as an indicator for the im⁻ phase.

In this communication, we present experimental results on the time needed for the cro gene product to establish dominance or to reach the im⁻ phase.

Coliphage T4D carrying the mutation rII638 was used for the assay of rex activity. Escherichia coli strain B was used for the plating assay of T4 phage. E. coli strains 594 and 594(λ⁺) were used as controls and 594(λN7cI857Odef3) was the test lysogen. This lysogen was isolated by Dr H. Inokuchi, and can grow at 41 °C without being killed (Mark & Szybalski, 1973). The stock culture was purified, grown up at 30 °C and stored in the cold to

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make sure that all lysogenic cells had the rex + phenotype before the experiments took place. Tryptone broth and T4 antiserum and the infective centre method used for the assay of rex gene activity have been described previously (Mark & Szybalski, 1973).

To measure the im + to im - phase shift during heating at 41 °C, the uninduced 594(λN7cI857Odef3) which is rex + was grown up at 30 °C in tryptone broth. When the culture reached 2 x 10^8 to 3 x 10^8 cells/ml, tryptophan was added to permit T4 phage adsorption. After taking one sample at 30 °C, the culture was divided into eight fractions. One fraction which served as a reference was cultured continuously at 30 °C for 14 h without exposure to 41 °C; this would remain in the im + phase. The other seven fractions were transferred into a 41 °C water bath. Since the aeration was vigorous, rapid temperature equilibration was obtained. At 41 °C, the thermolabile repressor is inactivated, and the cro gene product can be made (Spiegelman et al., 1970; Nijkamp et al., 1971). Six fractions were given exposure to 41 °C for 5, 10, 15, 20, 30 min or 60 min and then returned to 30 °C culture for 13 to 14 h so that the cells had enough time to decide between the im + phase and the im - phase. The last fraction was retained at 41 °C for the entire 14 h of culture to serve as a reference for the im - phase. Although the synthesis of the rex gene product and the lambda repressor can be terminated by the action of the cro gene product, the removal of the existing rex gene product and the lambda repressor in the cell require many generations of growth to dilute them out (Mark & Szybalski, 1973). This was done by maintaining the cultures for 14 h in the exponential phase of growth at a concentration of 2 x 10^8 to 3 x 10^8 cells/ml by regular dilution with prewarmed tryptone broth supplemented with tryptophan. Samples were taken out at various times to measure the rex gene activity by the infective centre method. The rex - phenotype was used as the indicator for im - phase, and the rex + phenotype for the im + phase.

From Fig. 1, it can be seen that the control grown at 30 °C without exposure to 41 °C maintained a steady level of rex gene activity throughout the 14 h of culture. On the other hand, the control grown at 41 °C continuously for 14 h lost the rex gene activity (becoming rex -) after 3 to 5 h of culture at 41 °C, as observed previously (Mark & Szybalski, 1973). The cultures with short exposure to 41 °C followed by a long period of culture at 30 °C appeared to have a fraction of the cells becoming rex -. The fraction of the cells becoming rex - increased with increasing duration of exposure to 41 °C (Fig. 1 and 2). Each of these cultures reached a steady level of the rex gene activity after the cultures had been grown at 30 °C for 2 to 3 h. This indicated that a fraction of the cells became rex - due to their im - phase shift instead of reducing the rex gene activity in all the cells. This was also confirmed by testing individual colonies for rex gene activity.

Using the level of the rex gene activity from the cultures grown only at 30 °C or 41 °C as references, the percentage of cells that converted to rex - phenotype in other cultures with short exposure to 41 °C could be calculated. Since the rex gene activity of these cultures reached a steady level after 3 h of culture at 30 °C, and the rex gene activity of the control culture grown all the time at 41 °C dropped to about 1% after 5 h of culture at 41 °C, the average values taken from 5 to 14 h of culture were used for this calculation. Fig. 2 shows the correlation between the percentage of cells converted to rex - and the length of exposure time to 41 °C. It appears from this graph that 5 min of heat treatment produced almost no rex - cells or cells in the im - phase. It takes about 17 min heating at 41 °C to change half the cell population into rex - cells or cells in the im - phase. Therefore, it seems to take a considerable amount of time for the cro gene to establish dominance over the lambda repressor.

In trying to explain these observations, one should first point out that the cI857 mutation confers a reversible type of thermosensitivity on the repressor protein which can renature upon transfer back to low temperature (30 °C) (Susman & Jacob, 1962; Naono & Gros, 1966). Therefore, upon returning the culture to 30 °C after the short 41 °C exposure the
Fig. 1. The loss of rex gene activity following short exposure to 41 °C. Uninduced lysogen 594(λN7cI857Ode3) with rex+ phenotype was grown up at 30 °C in tryptone broth. They were divided into eight fractions. One fraction remained at 30 °C as a reference for im+ phase. Six of the fractions were given short exposure to 41 °C for 5, 10, 15, 20, 30, and 60 min respectively, and then returned to 30 °C for 13 to 14 h culture. The last fraction was transferred to 41 °C, and kept at 41 °C for the whole 14 h of culture to serve as a reference for the formation of the im- phase. For details see Methods. Samples were taken out at various times to measure rex gene activity by the infective centre method. ○, Grown only at 30 °C without exposure to 41 °C; ●, exposure to 41 °C for 5 min; ◆, exposure to 41 °C for 10 min; ▲, exposure to 41 °C for 15 min; ○, exposure to 41 °C for 20 min; □, exposure to 41 °C for 30 min; ▼, exposure to 41 °C for 60 min; ●, grown at 41 °C for the entire period without returning to 30 °C.

Fig. 2. The correlation between the percentage of cells converted to rex- phenotype and the length of heating at 41 °C. The data in this figure are calculated from the results shown in Fig. 1. The level of the rex gene activity from the culture grown only at 30 °C, the control for im+ phase, was used as 100% for rex+ cells or 0% for rex- cells, and the rex gene activity of the culture grown at 41 °C for 13 to 14 h the control for im- phase, was used as 100% for rex- cells or 0% for rex+ cells. Using the average values of the rex gene activity obtained from 5 to 14 h of culture, the level of rex gene activity of the cultures with short exposure to 41 °C was convened into percent of cells with rex- phenotype, and these values were plotted against the length of time of heating at 41 °C.

existing repressor will renature and will inhibit further expression of the cro gene. Thus the cro gene can express itself only during the heating period. The longer the heating period, the greater the amount of cro gene product is likely to be made. A similar situation was observed with W3350 (λcl857O29). When this lysogen was grown at 42 °C for 15 min both early and late genes were transcribed, but such transcription could be turned off by returning the lysogen to 33 °C (Kourilsky et al., 1971).

Kourilsky et al. (1971) also measured the length of heating at 45 °C required to induce the lysogenic cells of λcl857. They found that 5 min heating induced only 5 to 10% of the cells while 10 min heating induced 75% of the cells. Since heating (42 °C) the λcl857 lysogen terminated the cl-rex transcription almost completely within 3 min (Heinemann & Spiegelman, 1970; Szybalski et al., 1970), the cro gene must be quick to express itself and to inhibit the expression of the cl-rex operon (Hayes, 1972). However, terminating the cl-rex expression at high temperature does not guarantee that the cell will shift to the im- phase. To establish dominance by the cro gene product would take more than just temporarily blocking the expression of the cl-rex operon by the cro gene product. Therefore, a certain level of cro
gene product must be accumulated, so that the expression of the cl-rex operon can be blocked permanently. A similar conclusion has been drawn from molecular binding studies between cro protein and lambda repressor on the rightward operator OR (Furth, 1980; Ptashne et al., 1980). Consequently the long period of heating at 41 °C required for shifting the lysogen from the im+ phase to the im− phase must be for build up of the cro gene product in the cells, rather than inhibition of expression of the cl-rex operon.

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REFERENCES


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