Effect of Multiplicity of Infection on Transcription in *Escherichia coli* Cells Infected by Bacteriophage Lambda

By KA-CHEUNG LUK and KAI-KEUNG MARK*

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

(Accepted 16 July 1982)

**SUMMARY**

The effect of multiplicity of infection (m.o.i.) on transcription was studied by infecting *Escherichia coli* with bacteriophage λcI47, λcI47O29P3 and λcI857cro27P3. DNA–RNA hybridization with λcI47 l-strand DNA, φ80imm1 r-strand DNA, and λimm80 r-strand DNA were used to measure mRNA transcription from the l-strand, the r-strand of the early x-P-Q region and the late A-J-b2 region of λ bacteriophage respectively. In λcI47croO−P−-infected cells, transcription from the l-strand, r-strand of the early x-P-Q region and the late A-J-b2 region all decreased with increasing m.o.i. The response in the x-P-Q region was less marked than in other regions, but the pattern looked similar to that described above. When phage DNA replication was permitted, as in the case of λcI47, the response was similar to that observed in λcI47croO−P−-infected cells, but the level of transcription was increased two- or threefold. In λcI857croP−-infected cells, the leftward transcription and the rightward transcription from the early x-P-Q region and the late A-J-b2 region all increased with increasing m.o.i., but the extent of change was less drastic than with λcro+. This result demonstrated clearly that the decrease in transcription from various regions at increasing m.o.i. of λcro+ was due to the inhibitory action of the cro gene product. The results obtained with cro− strongly support the view that gene dosage is a significant controlling factor for the extent of gene expression.

**INTRODUCTION**

In eukaryotes, the presence of repeating genes is correlated with untranslated gene products and structural proteins that are required in large amounts (Edstrom & Lambert, 1975; Tartof, 1975). In prokaryotes, there is a correlation between DNA replication and production of structural proteins (Dove, 1966; Mark, 1970). These observations lead us to believe that gene dosage might play a significant role in regulating the amount of gene product produced. We have successfully used the lambda phage system, which has many advantages for gene dosage experiments, to demonstrate a good correlation between the number of genes present, or multiplicity of infection (m.o.i.), and the extent of translation of a particular gene (*Escherichia coli* β-galactosidase or phage endolysin) in the absence of negative controlling factors (Tsui & Mark, 1976; Luk & Mark, 1980, 1982).

This communication describes experiments designed to correlate gene dosage or m.o.i. with the extent of transcription in the lambda phage system, and the role of the cro gene in suppressing gene expression at high m.o.i.

**METHODS**

*Bacteria.* *E. coli* strain 594 (non-permissive for λsus mutants; Campbell, 1961) was used as the host for λ 3H-RNA preparation. TC600 (permissive for λsus mutants) was used for growing phages.

*Bacteriophages.* The following phage strains were used for preparing λ 3H-RNA: λcI47 (Kaiser, 1957) was obtained from Dr A. D. Kaiser; λcI47susO29susP3 (Campbell, 1961) from Dr W. F. Dove and λcI857cro27susP3 (Eisen *et al*., 1970) from Dr W. Szybalski.

The DNA strands of the following phages were used for hybridization mapping of λ 3H-RNA; λcI47 (Kaiser,
1957); \lambda\text{imm}80 (Franklin \textit{et al.}, 1965), referred to as \lambda\text{imm}80, was obtained from Dr N. Franklin and \phi80\lambda\text{imm}80 (Signer, 1964), referred to as \phi80\lambda\text{imm}80, from Dr E. Signer.

The media, solutions and the methods for the DNA–RNA hybridization experiments have been described previously (Mark, 1973).

RESULTS

Effect of multiplicity of infection on transcription from the 1-strand

The effect of the m.o.i. of \lambda\text{I}47 on total 1-strand transcription is shown in Fig. 1. It is clear that the extent of transcription was inversely related to m.o.i. The rate and the final level of transcription was significantly reduced at high m.o.i.

To keep a constant number of phage DNA molecules present in the infected cells and proportional to m.o.i., we infected the su– host with \lambda\text{I}47\text{O29P3}, which carries amber mutations in the O and P genes, essential for phage DNA replication (Ogawa & Tomizawa, 1968). As observed with \lambda\text{I}47 in Fig. 1, the rate and final level of 1-strand transcription from \lambda\text{I}47\text{O29P3}-infected cells again decreased with increasing m.o.i. (Fig. 2).

Since the 1-strand transcription from the promoter P1 is repressed by the product of the gene \text{cro} (Kourilsky \textit{et al.}, 1971), \lambda\text{I}857\text{cro27P3} was used to explore the role of \text{cro} in the m.o.i. effect. In contrast to \lambda\text{I}47 and \lambda\text{I}47\text{O29P3}, the rate of 1-strand transcription in \lambda\text{I}857\text{cro27P3}-infected cells increased with increasing m.o.i. (Fig. 3). These results show that the decreasing synthesis of 1 1-strand RNA with increasing m.o.i. was due to the action of the \text{cro} gene product. In the absence of the inhibiting \text{cro} gene product, transcription increased with m.o.i.

A comparison of the results shown in Fig. 2 and 3 demonstrates that the \text{cro27} mutation, besides abolishing the negative effect of m.o.i. on 1-strand transcription, also released the repression of transcription from the promoter P1. When the phage was \text{cro}+ (Fig. 1, 2), the rate of 1-strand transcription was higher at early times, but reduced to a lower level during the period 15 to 25 min after infection. Conversely, with \text{cro}– phage (Fig. 3), the rate of transcription was low at early times, but continued to increase and reached a much higher level 15 to 25 min after infection.

Fig. 1. The effect of multiplicity of infection on transcription from the 1-strand of \lambda\text{I}47 DNA. \textit{E. coli} 594 cells grown in MCGB medium at 37 °C were infected with \lambda\text{I}47 at the indicated m.o.i. Pulse-labelling was carried out with [3H]uridine for 3 min, the indicated times being the centres of this period, and 3H-RNA extracted from the infected cells was hybridized to the 1-strand DNA of \lambda\text{I}47. The background counts obtained without DNA have been subtracted, and the values represented are the percentage of the total input 3H-RNA (both \textit{E. coli} and 4) that hybridized to the 1-strands of \lambda\text{I}47 DNA. Less than 0.01% of the uninfected \textit{E. coli} 3H-RNA hybridized with the 1-strand DNA of \lambda\text{I}47.
Effect of multiplicity of infection on transcription from the r-strand

The total synthesis of mRNA from the λ r-strand can be separated into two major parts: the early x-P-Q transcription and the late A-J-b2 transcription which accounts for the head and tail genes. In order to separate these two segments experimentally, we utilized two λ-ph80 hybrid phages (Szpirer et al., 1969). Lambda mRNA does not hybridize to the r-strand of ph80 (less than 0.005% of the total RNA present in an infected cell). The r-strand DNA from the hybrid phage ph80imm2 was used to hybridize the mRNA from the early x-P-Q region of λ and the r-strand of the hybrid phage λimm80 was used to hybridize the mRNA from the late A-J-b2 region of λ.

x-P-Q region

Figs. 4 and 5 show that in λcI47- and λcI47O29P3-infected cells the transcription of the x-P-Q region decreased with increasing m.o.i. Conversely, in λcI857cro27P3-infected cells (Fig. 6) the rate of transcription of that region increased progressively with m.o.i. This result shows that the decrease in transcription from the x-P-Q region with increasing m.o.i. is due to the inhibitory effect of the cro gene.

The results also show that the transcription from the x-P-Q region quickly reached a constant level about 5 min after infection in cro+ (Fig. 5), but that it continued to increase 5 min after infection in cro− (Fig. 6).

In addition to the above observation, the most prominent feature in this region is the mild response to m.o.i.-mediated reduction in transcription. When the m.o.i. was varied between 2 and 20, the reduction of transcription in this region is small in comparison with other regions.

A-J-b2 region

Transcription from the A-J-b2 region occurs at a later time than the early x-P-Q transcription.
Fig. 4. The effect of multiplicity of infection on transcription from the r-strand x-P-Q region of λc147 DNA. E. coli 594 cells grown in MCGB medium at 37 °C were infected with λc147 at the indicated m.o.i. Pulse-labelling was carried out with [3H]uridine for 3 min, the indicated times being the centres of this period, and ³H-RNA extracted from the infected cells was hybridized to the r-strand of φ80immλ. The background counts obtained without DNA have been subtracted, and the values represented are the percentage of the total input ³H-RNA (both E. coli and λ) that hybridized to the r-strands of φ80immλ DNA. Less than 0.01 % of the uninfected E. coli ³H-RNA hybridized with the r-strand DNA of φ80immλ.

Fig. 5. The effect of multiplicity of infection on the transcription from the r-strand x-P-Q region of λc147O29P3 DNA. (Details are otherwise as outlined for Fig. 4.)

Since A-J-b2 region is transcribed from the late promoter Pk (Nijkamp et al., 1970) and is activated by the Q gene product (Roberts, 1975; Sklar et al., 1975), the steady increase of A-J-b2 transcription was probably due to the accumulation of Q gene products (Fig. 7 to 9). The rate of transcription from the A-J-b2 region in λc147-infected cells was significantly reduced at high m.o.i. (Fig. 7) compared to that of the x-P-Q region. The same negative effect of m.o.i. on A-J-b2 transcription was also found in λc147O29P3-infected cells (Fig. 8). On the other hand, transcription from the A-J-b2 region in λc1857cro27P3-infected cells increased with increasing m.o.i. (Fig. 9). This observation again suggests that the reduction of transcription from the A-J-b2 region with increasing m.o.i. was due to an inhibitory effect of the cro gene.

DISCUSSION

The effect of gene dosage on the extent of gene expression can be clearly observed only when the function of any interfering controlling genes has been removed or in simpler systems where there is no negative controlling element. The results presented in this communication show that the cro gene product can interact with the multiplicity of infection in controlling the extent of transcription from various regions of the lambda genome.

We have shown that, in the absence of interfering cI and cro gene products, the rate of transcription from various regions in the genome is a function of m.o.i., i.e. of gene dosage. Other simplified systems have also demonstrated a good correlation between the rate of gene expression and gene dosage (Ray & Pearson, 1976; Luk & Mark, 1982). The transcription results reported in this communication are consistent with the corresponding translation experiments reported previously (Court et al., 1975; Takeda, 1975; Tsui & Mark, 1976).

Tsui & Mark (1976) found that the expression of both endonuclease and endolysin synthesis
Fig. 6. The effect of multiplicity of infection on the transcription from the r-strand x-P-Q region of λcl857cro27P3 DNA. (Details are otherwise as outlined for Fig. 4.)

Fig. 7. The effect of multiplicity of infection on transcription from the r-strand A-J-b2 region of λcl47 DNA. E. coli 594 cells grown in MCGB medium at 37°C were infected with λcl47 at the indicated m.o.i. Pulse-labelling was carried out with [3H]uridine for 3 min, the indicated times being the centres of this period, and [3H]-RNA extracted from the infected cells was hybridized to the r-strand DNA of imm80. The background counts obtained without DNA have been subtracted, and the values represented are the percentage of the total input [3H]-RNA (both E. coli and λ) that hybridized to the r-strands of imm80 DNA. Less than 0.01% of the uninfected E. coli [3H]-RNA hybridized with the r-strand DNA of imm80.

decreases as m.o.i. increases. In explaining these observations, it was postulated that the cro gene product might be responsible. Results presented here confirm that, at high m.o.i., the cro gene product is responsible for the decrease in gene expression from both the left and right arms of the lambda genome.

The cro gene is one of the genes that is expressed immediately after infection, and no gene other than the λ repressor is known to repress its expression but in our experiments, the cl repressor was inactive. Based on the conclusion drawn above, it is logical to expect that increasing the m.o.i. should increase the production of the cro gene product early in infection. This would enable the cro gene product to reach the level necessary to inhibit the promoters P_L and P_R at an earlier time (Takeda et al., 1977; Ptashne et al., 1980; Meyer et al., 1980). This reasonably explains the results shown in Fig. 1, 2, 4 and 5 as well as those reported earlier (Takeda, 1975; Court et al., 1975; Tsui & Mark, 1976). The late transcription (A-J-b2 region) is also repressed by increasing m.o.i. of cro+ phage (Fig. 7, 8), but this is indirectly regulated by reducing the level of Q gene product (Herskowitz & Hagen, 1980).

Previous investigators (Cohen & Chang, 1970) have shown that host mRNA and protein synthesis are both sharply reduced after λ phage infection, and the extent of reduction is m.o.i.-dependent. Also, m.o.i. has a pronounced effect on the rate of [3H]thymidine incorporation in λ-infected cells (McMacken et al., 1970). We have also shown that some β-galactosidase (large molecules) may leak out of host cells after λ or T4 phage infection at very high m.o.i., but the leakage after λ infection is much smaller than that after T4 infection (Tsui & Mark, 1976).
Therefore, one would expect that the internal environment of the infected cell at high m.o.i. is also changed, e.g. by leakage of ions. This might explain why the rate of transcription at high m.o.i. does not increase in proportion to m.o.i. in cro- conditions.

The authors wish to express their sincere gratitude to Professor W. Szybalski for helpful discussions of this manuscript. The authors also wish to acknowledge the technical assistance of Mr K. S. Ho.

REFERENCES


Multiplicity effect on transcription


(Received 26 June 1981)