Patterns of Transcription in Bacteriophage P22-Infected *Salmonella typhimurium*

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Received for publication 17 May 1976

Two peaks of RNA synthesis (early and late) are directed by bacteriophage P22 in lytic infections of *Salmonella typhimurium*. Late RNA synthesis is not seen in P22 23\(-\) infections; neither early nor late RNA synthesis occurs in P22 24\(-\) infections. Genes 23 and 24 of P22 appear to be analogous to genes Q and N of \(\lambda\), respectively.

Bacteriophage P22 is a temperature phage of *Salmonella typhimurium*. The genetic structure and regulatory circuits of P22 are similar to those of coliphage \(\lambda\) (2, 4, 6). Lytic development of the phage is under the positive control of the products of genes 24 and 23 (5, 7; S. Hilliker and D. Botstein, personal communication). The lysozyme pathway is controlled by a number of genes including \(c_1\), \(c_2\), \(c_3\), and \(c_{\text{ly}}\) (6). We have undertaken the examination of the kinetics of phage RNA synthesis in *S. typhimurium* infected with various regulatory mutants of P22. In this study, we hope to correlate what is known about some phage regulatory gene functions with patterns of transcription seen in the infected cells. The patterns we see for the clear-plaque mutants reflect what is known about the role of the \(c_1\), \(c_2\), and \(c_3\) genes in the lysogenic response (6, 9, 11). The roles of the products of genes 24 and 23 in their positive control of gene action (5, 7) are also reflected in patterns of phage-directed RNA synthesis consistent with the idea that these gene products act at the transcriptional level.

The kinetics of phage RNA synthesis in P22 H1 (wild type)-infected cells is shown in Fig. 1a. In this infection there are no progeny phage produced, and nearly all the cells are lysogenized. Phage-specific mRNA synthesis reaches a maximal rate at 6 min after infection. At this time, phage RNA synthesis is shut off and the cells become lysogenized. The RNA synthesis at 6 min is also apparent in lytic infections (see below). This phage-directed RNA synthesis probably corresponds to transcription of genes whose products have been partially characterized as early proteins by Lew and Casjens (7).

The kinetics of phage RNA synthesis in P22 \(c_{1+}\), \(c_{2-}\), or \(c_{3-}\)-infected cells is shown in Fig. 1b-d. Two distinct peaks in the rate of RNA synthesis are seen in these lytic infections. In P22\(c_{1+}\), and \(c_{3-}\) infections (Fig. 1b and d, respectively) the first peak occurs at 6 min after infection, and the second occurs at about 25 min. These are termed the early and late peaks of RNA synthesis, respectively. It is of interest to note that in \(c_{1-}\) and \(c_{3-}\) infections, the early RNA synthesis lasts only a short time and is shut off (reduced to 50% of its maximal rate) before late RNA synthesis begins. In the case of P22\(c_2\) infections (Fig. 3c), the appearance of the late RNA peak is delayed until about 35 to 40 min after infection. This is consistent with the belief that \(c_1\) and \(c_3\) gene products, when present in their active forms, act together to delay the appearance of phage gene products involved in the lytic response (9, 11).

Figure 2 shows that the appearance of lysozyme activity corresponds temporally to the appearance of the late peak of RNA synthesis in \(c_{1+}\), \(c_{2+}\), and \(c_{3+}\)-infected cells. Activity does not appear in lysogenic infections (P22 H1). Lysozyme is the product of gene 18, which maps to the right of gene 23 and is under its positive control (6; S. Hilliker, personal communication). It is the enzyme primarily responsible for cellular lysis. Note that the appearance of lysozyme activity is delayed about 10 min in P22 \(c_2\) \((c_{1+}, c_{3+})\) infections.

If cells are infected with P22 \(c_{2-}5\) 23\(-\) phage, the late peak of RNA synthesis does not appear (Fig. 3). Furthermore, lysozyme activity cannot be detected in extracts from these infected cells for up to 60 min after infection. The early RNA peak is present, and DNA synthesis as determined by pulse-label kinetics with \([\text{H}]\)thymidine is normal (data not shown). It should be pointed out that in infections leading to lysogeny neither the late peak of RNA synthesis nor lysozyme activity appears (Fig. 1a.

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FIG. 1. Kinetics of phage-directed RNA synthesis after infection with various P22 clear-plaque mutants. In all experiments, S. typhimurium LT2 (provided by J. Roth) was infected in logarithmic growth at a multiplicity of infection of 20. Cells in E medium (12) at 37°C were infected at an absorbance at 650 nm (A650) of 0.2 (10⁸ cells per ml). Under these conditions, phage c7-7 and c7-32 give eclipse periods of 20 min and latent periods of 55 min, with a burst size of about 300 phage. Both of these times are extended 8 to 10 min in infections with c7-5. At various times after infection, 5-ml aliquots were removed and labeled for 1 min at 37°C with 100 μCi of [³H]uridine. Labeling was stopped by adding 5 ml of ice-cold E medium plus 0.5 M NaCN. The cells were collected by centrifugation, and the labeled RNA was extracted with diethyl pyrocarbonate (10), treated with 50 μg of DNAse I per ml at 37°C for 30 min, and reextracted with diethyl pyrocarbonate. RNA isolated from each pulse had a specific activity of about 40,000 cpm/μg, although this value is somewhat variable due to a partial shutoff of host transcription after P22 infection (J. M. Pipas and R. H. Reeves,
At various times after infection, 5-ml aliquots were removed and chilled by mixing with 5 ml of ice-cold water. The cells were disrupted by sonication for 90 s at 0°C. Cellular debris was removed by centrifugation (30,000 × g for 20 min), and the supernatant was assayed directly for lysozyme activity with sensitized Escherichia coli B (9). Usually, 0.2 ml of extract was mixed with 0.8 ml of sensitized cells at 25°C to give an initial A40 of 0.6. Lysis was followed for up to 20 min by the decrease in A40. One unit was defined as the amount of activity that produced 0.01 unit of decrease in A40 in 1 min (initial rate). All values were corrected for background lysis of the sensitized cells alone (0.002 to 0.004 unit of decrease in A40 per min), and are plotted as specific activities—units per A40 unit of extract. Symbols: (○) P22 c7-7, (△) P22 c7-32, (□) P22 c5-5, (■) P22 H1 (wild type) and uninfected cells.

FIG. 2. Synthesis of lysozyme after infection with various P22 regulatory mutants. Cultures of S. typhimurium LT2 were infected as described in Fig. 1. At various times after infection, 5-ml aliquots were removed and chilled by mixing with 5 ml of ice-cold water. The cells were disrupted by sonication for 90 s at 0°C. Cellular debris was removed by centrifugation (30,000 × g for 20 min), and the supernatant was assayed directly for lysozyme activity with sensitized Escherichia coli B (9). Usually, 0.2 ml of extract was mixed with 0.8 ml of sensitized cells at 25°C to give an initial A40 of 0.6. Lysis was followed for up to 20 min by the decrease in A40. One unit was defined as the amount of activity that produced 0.01 unit of decrease in A40 in 1 min (initial rate). All values were corrected for background lysis of the sensitized cells alone (0.002 to 0.004 unit of decrease in A40 per min), and are plotted as specific activities—units per A40 unit of extract. Symbols: (○) P22 c7-7, (△) P22 c7-32, (□) P22 c5-5, (■) P22 H1 (wild type) and uninfected cells.

FIG. 3. Kinetics of phage RNA synthesis in P22 c5-5 23- infected and P22 c5-5 23+ infected cells. The procedure was exactly as in Fig. 1, except that the infecting phage were P22 23- am H316 c5-5 (○) and P22 c5-5 (△) (reproduced from Fig. 1c). P22 23- am H316 c5-5 was kindly provided by S. Hilliker.

and 2). We conclude that the late peak of RNA synthesis observed in lytic infections is primarily due to transcription of genes under the positive control of the gene 23 product. These genes probably correspond to most of the morphogenic and lysis functions of the phage (1, 6).

Gene 24 of bacteriophage P22 is a positive regulator of many phage functions, including those for recombination, DNA replication, morphogenesis, and lysis (5). When infections are carried out with phage carrying mutations in gene 24, little phage mRNA is made, and the

manuscript in preparation). The RNA had an A260:A280 ratio of 0.49 to 0.50 and was free of protein, as determined by the method of Lowry et al. (8). RNA from each pulse (0.25 μg, about 10,000 cpm) was hybridized in 1.0 ml of 2 × SSC (1 × SSC = 0.15 M NaCl plus 0.0015 M sodium citrate) for 20 h at 66°C to 10 μg of purified P22 DNA (J. M. Pipas and R. H. Reeves, manuscript in preparation) immobilized on a nitrocellulose filter as described (3). Radioactivity on each filter was counted in 10 ml of Aquasol (New England Nuclear Corp.) in a Packard scintillation spectrometer. Under these conditions, labeled RNA from uninfected cells showed no hybridization to P22 DNA (<25 cpm), and [3H]RNA from either infected or uninfected cells did not bind (<25 cpm) to blank filters or to filters containing bound T7 phage DNA. The amount of radioactivity retained on a filter is directly proportional to the rate of RNA synthesis at a given pulse time. Each experiment was repeated at least twice in its entirety, and hybridizations within each experiment were done in triplicate (deviations were less than 10%). The average values for hybridizations from one experiment are plotted. Infections were carried out with the following phage (provided by J. Roth and M. Levine): (a) P22 H1 (wild type), (b) P22 c7-7, (c) P22 c5-5, (d) P22 c7-32.
early peak of RNA synthesis does not appear (Fig. 4). In such infections, no viral DNA synthesis or lysozyme activity can be detected, and no progeny phage appear (data not shown). The small amount of phage transcription that does occur at 5 min is probably derived from gene 24 itself or other genes not under its control. We conclude that the early peak of RNA synthesis is derived from genes under the positive control of gene 24 but not under the control of gene 23.

It thus appears that P22 genes 24 and 23 act in a manner analogous to coliphage λ genes N and Q, respectively, by positively regulating waves of gene expression at the level of transcription (4). Similar experiments have been carried out by E. Jackson (personal communication), who has reached the same general conclusions.

We wish to thank J. Roth, M. Levine, and S. Hilliker for providing bacterial and phage strains used in this study. We thank E. Jackson, S. Hilliker, and D. Botstein for helpful discussions.

This work was supported by Public Health Service grant GM 21994-01, from the National Institute of General Medical Sciences and by an institutional ERDA grant, E-(40-1)-2690. Robert H. Reeves was supported by Public Health Service Career Development Award GM 00049 from the National Institute of General Medical Sciences.

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