Transcriptional Mapping of the Bacteriophage Mu DNA

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SUMMARY

The transcription of temperate phage Mu throughout lytic development was analysed quantitatively by hybridization of pulse-labelled RNA to full-length Mu DNA and to plasmids that define Mu DNA segments covering the whole phage genome. The transcription rate (i.e. binding data corrected for the incorporation rate of the radioactive precursor, for the size of the DNA template, and for the number of phage genomes present in the bacterium at the time of analysis) revealed three defined phases of Mu transcription: early (0 to 9 min), intermediate (between 9 and the interval 14 to 17 min) and late (from the interval 14 to 17 min onward). The analysis also revealed that the region comprising the genes involved in phage morphogenesis was organized into two independent 'late' transcription units.

INTRODUCTION

The temperate Escherichia coli phage Mu lysogenizes its host by stable integration of its DNA at one of many possible sites in the host chromosome. The lytic cycle is initiated either by infection or by heat inactivation of a temperature-sensitive repressor (for review, see Toussaint & Réshibois, 1983). Most of the genes of phage Mu (see top of Fig. 1) have been characterized genetically, but the pattern and the sequence of their expression during the lytic cycle is only partially known. Early experiments have shown that Mu RNA is synthesized in two phases (early and late) and is mostly transcribed from one DNA strand from left to right on the conventional map of the phage (Bade, 1972; Wijffelman et al., 1974; Wijffelman & van de Putte, 1974).

The leftmost transcription unit consists of the repressor gene c. This gene is transcribed to the left (van Meeteren et al., 1980; Priess et al., 1982) from a promoter initiating transcription at 1063 to 1064 bp (Goosen et al., 1984) or 1066 bp (Krause & Higgins, 1986) from the left end of the Mu genome. A second, weaker promoter which initiates transcription at 884 to 885 bp has also been reported (Goosen et al., 1984). To the right of c lies the early operon, which comprises the genes involved in replication, integration, transposition, control of early gene expression and inhibition of cell division (van de Putte et al., 1980; Goosen et al., 1982; Boeckh et al., 1986). Transcription of the early operon is initiated from a single promoter (Pe) at 1028 bp (Krause et al., 1983) and terminated approximately 8700 bp from the left end (J. Engler, cited in Giphart-Gassler et al., 1981 a). The synthesis of the early transcript begins immediately after induction of a temperature-sensitive prophage, is inhibited from the 4th min by the her gene product (gpner), and after a reactivation phase (from the 9th min) it remains at low levels until the end of the lytic cycle (Wijffelman & van de Putte, 1974).

The next transcription unit covers the C gene, which is located approximately 10 kb from the left end (Marrs, 1982) and is transcribed from the interval 15 to 20 min onward independently of the early operon (van Meeteren, 1980). The sequence of this gene has been determined and evidence has been presented that supports the existence of both a rightward-oriented promoter for C at least 0.5 kb upstream from the gene and a transcription terminator at the 3' end of the gene (Heisig & Kahmann, 1986; Margolin & Howe, 1986). Expression of C is required for

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transcription of the late genes (located to the right of C) (van Meeteren, 1980) and gpC is required for transcription of mom (mom transactivation function) (Hattman et al., 1985; Heisig & Kahmann, 1986; Margolin & Howe, 1986).

The late genes involved in phage morphogenesis are organized into two genetically defined clusters: the first comprises genes D to J (head structure and assembly) and the second comprises genes K to U (tail structure and assembly) (Giphart-Gasser et al., 1981b). Evidence for independent promoters for the genes S (unpublished observations of H. Breepoel & J. Mellenla, cited in Giphart-Gasser et al., 1981b) and U (Clayton et al., 1981; R. Kahmann & D. Kamp, cited in Toussaint & Résoibois, 1983) has been presented.

To the right of U lie the two rightmost transcription units: one is the gin gene, and the other is the mom operon, which comprises the genes com and mom. The base sequence and the promoter of gin are known (R. Kahmann & D. Kamp, unpublished observations; Plasterk et al., 1983). The sequence of com-mom, the corresponding promoter and the transcript initiation position have also been determined (Kahmann, 1983; Hattman & Ives, 1984; Plasterk et al., 1984). In addition, sequence analyses showed that both units are transcribed in the rightward direction. Gene gin is expressed both in the lysogenic state and during lytic development (Kamp et al., 1978; Symonds & Coelho, 1978; Bukhari & Ambrosio, 1978) but expression of mom has been demonstrated only during lytic development (Toussaint, 1976). Hattman & Ives (1984) detected transcripts for the gin and mom regions from the 20th min and demonstrated a rapid increase of their synthesis after the 25th min of the lytic cycle.

The data reported here present the general pattern of Mu transcription and the sequence of expression of different Mu DNA regions throughout the lytic cycle. They also provide evidence for new transcription units.

**METHODS**

*Bacteria, phage and plasmids.* W3110 thy· (from J. Cairns) and a Mu cts62 lysogenic derivative (7566; Bade, 1972) are E. coli K12 strains. Strain MH3185 (MH3184/RP4::Mu cts62, from M. Howe) is a *Proteus mirabilis* derivative. The plasmids containing Mu restriction fragments cloned in pBR322 were pKN50, pKN13, pKN35 (Staufenbiel & Schumann, 1983), pKN56 (Schumann & Bade, 1979) and pKN104 (Boeckh, 1980). The vector for pKN80 (Schumann, 1979) and pKN48 (Staufenbiel & Schumann, 1983) was pRSF2124 (Fig. 1). Phage DNA was prepared by heat induction from strain MH3185 as described (Bade, 1972). Plasmid DNA was prepared by standard methods (Birnboim & Doly, 1979) and further purified by three cycles of CsCl-ethidium bromide equilibrium centrifugation.

*Labelling and isolation of RNA.* Cultures of strain 7566 were grown at 28 °C in LSTG medium (Schröder et al., 1974). At 2.7 × 10⁸ cells/ml, 7.5 ml samples were distributed in 50 ml Erlenmeyer flasks and incubated at 28 °C until they reached 3 × 10⁸ cells/ml. The prophage was induced by simultaneously shifting all the samples to 44 °C. The cultures were kept at this temperature until lysis. Pulse-labelled RNA was prepared before induction (lysogenic RNA) and at various times after it (lytic RNA) by incubating the samples for 1 min with 7.4 MBq [5-³H]uridine (37 MBq/ml, 1 TBq/mmol; Amersham). Incorporation was stopped by adding 2 ml of ice-cold stop solution (200 µg/ml chloramphenicol, 20 mM-NaCl, 1 mg/ml uridine). Under these conditions incorporation was linear for 1 min or longer in all cases. The RNA from the frozen cell pellets was extracted (Bade, 1972) and stored at -20 °C in 4 × SSC (SSC is 0.15 M-NaCl, 0.015 M-sodium citrate pH 7.0) saturated with phenol. The yield of 3H-labelled RNA was about 10⁶ TCA-precipitable c.p.m. per 10⁸ bacteria labelled for 1 min. Pulse-labelled RNA from the non-lysogenic parental strain W3110 (non-lysogenic RNA) was prepared from culture samples at 28 °C (3 × 10⁸ cells/ml) and at 5 and 25 min after shifting to 44 °C.

*RNA-DNA hybridization.* Plasmid DNA linearized with EcoRI or PsiI, extracted with phenol and heat-denatured was adsorbed to 47 mm diameter nitrocellulose membrane filters (BA85; Schleicher & Schüll) to obtain 0.01 pmol plasmid DNA loaded on each "minifilter" of 6 mm diameter punched from the large filter. Minifilters loaded with 0.01 pmol of full length, denatured Mu DNA were prepared in the same way. A standard filter hybridization assay (Barev & Szybalski, 1971; Kourilsky et al., 1971) was used except the hybridization mixtures contained 50% formamide, 4 × SSC and phenol at 20% saturation, and were prepared with the labelled RNA for all assays in a series of experiments using only 20 µl of hybridization mixture per minifilter. Incubations were performed at 38 °C for 10 days in small glass vials, each with one minifilter. For RNA preparations with a low content of Mu-specific RNA (0 to 17th min after induction) inputs in the range of 1 × 10⁸ to 10 × 10⁸ c.p.m. per assay were used, while reactions for RNAs with high contents of Mu RNA (20th to 46th min) were carried out at inputs in the range of 1 × 10⁹ to 10 × 10⁹ c.p.m. Under these conditions the DNA on the filter was always in excess and hybridization with all RNAs studied reached completion (not shown).
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Fig. 1. Genetic and physical map of Mu DNA (top), the restriction fragments cloned in pKN plasmids (middle) and the seven non-overlapping segments, I to VII (bottom). The Mu DNA map is divided into 5 kb intervals and shows the Mu genes, relevant restriction sites, the invertible G segment (parentheses) and the variable host DNA sequences (open box).

Fig. 2. Specific binding of pulse-labelled RNA to Mu DNA during phage development. RNAs were prepared from strain 7566 as described in Methods, at different times after heat induction. All RNAs were hybridized to filters loaded with 0.01 pmol total Mu DNA thus guaranteeing an excess of DNA.

Fig. 3. Transcription rate of Mu DNA during phage development. The TR values were calculated as described in Methods using the sb values from the experiment described in Fig. 2, as well as the available data for the number of Mu DNA copies per bacterium (1-00, 1-02, 1-07, 1-10, 1-13, 1-24, 1-45, 1-79, 2-56, 3-88, 6-74, 10-08, 16-79 and 35-56 Mu DNA copies corresponded to 0, 2, 4, 5.5, 7, 9, 11, 14, 17, 20, 23, 26, 31 and 41 min respectively).

Calculations. The Mu-specific binding (sb) of a given lysogenic or lytic RNA to a given filter type (carrying a defined DNA) corresponds to the fraction of the input radioactive RNA that binds to the filter minus the fraction of input non-lysogenic RNA that binds to the same filter type (non-specific binding). For example, phage 7566 RNA from the 11th rain was hybridized to pKN90 filters at 778600, 464600 and 117300 c.p.m. The fraction of input that bound to the filter, as calculated from the input binding curve, was 48.80 × 10^{-5}. After subtracting the non-specific binding (W3110 RNA to pKN90 filters) the sb value 46.58 × 10^{-5} was obtained. The use of filters with a series of pKN plasmids (middle of Fig. 1) allowed us to determine the sb values for seven non-overlapping Mu segments I to VII (bottom of Fig. 1) which cover the whole phage genome. These values were determined as follows: the sb values for filters carrying pKN80, pKN50, pKN13, pKN35 and pKN104 corresponded to the sb values for segments I, II, III, IV and VII respectively; the difference in the sb values of pKN48 and pKN56 filters corresponded to the sb value for V and the difference in those of pKN56 and pKN104 filters corresponded to that of VI. In the previous example 46.58 × 10^{-5} corresponds to the sb value for segment II.

The cellular concentration (C) of the newly synthesized segment-specific RNA is the product of the sb value for the segment and the total incorporation rate of [3H]uridine per bacterium at the time of analysis. In our example, the calculated C of segment II (C-II) for the 11th min was 52.17 × 10^{-7} segment-specific TCA-precipitable c.p.m. detected per bacterium after 1 min incorporation. The transcription rate (TR) of a segment results from dividing the corresponding C value by the size of the segment (kb) and by the number of Mu DNA copies present per
Fig. 4. Concentration of newly synthesized, segment-specific RNA at different times during phage development. Samples of the RNAs used in the experiment described in Fig. 2 were hybridized to filters containing the plasmids pKN80, pKN50, pKN13, pKN35, pKN48, pKN56 and pKN104 (middle Fig. 1). As described in Methods, the binding data were used to calculate the C values for the seven non-overlapping Mu DNA segments indicated at the bottom of Fig. 1: in (a) I, ●; II, △; III, ■ and in (b) IV, ▽; V, ○; VI, △; VII, □.

RESULTS

To obtain information on transcription during the lytic cycle, RNA labelled before and at different times after heat induction of a Mu cts62 prophage was hybridized to total Mu DNA and to pKN plasmids (middle of Fig. 1). These plasmids define the seven non-overlapping Mu
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segments I to VII, which are indicated at the bottom of Fig. 1, as described in Methods. The sb value, C and TR for total Mu DNA and for every segment at every time were calculated as described in Methods.

The sb values for total Mu DNA (Fig. 2) reproduced the two-phase pattern of Mu transcription (Bade, 1972; Wijffelman et al., 1974). The same pattern resulted from the sum of the sb values for the seven Mu segments (not shown). The TR values, which describe transcription more realistically than the sb values as they are independent of changes in the incorporation rate of $[^3]H$uridine and of the number of Mu DNA copies, revealed three defined phases (Fig. 3): early (0 to 9 min), intermediate (between 9 and the interval 14 to 17 min) and late (from the interval 14 to 17 min onward).

Fig. 5. Transcription rate of the Mu DNA segments at different times during phage development. The C values shown in Fig. 4 were used to calculate the TR values for the seven segments indicated at the bottom of Fig. 1. Symbols as for Fig. 4.
Segment I consists of the repressor gene and the left half of the early operon. The changes of C-I and TR-I during the first 11 min (Fig. 4a, 5a) reflected the known transcriptional pattern of the early operon, characterized by activation, inhibition and reactivation of the early promoter Pe (Wijffelman & van de Putte, 1974). From the 11th min to the end of the lytic cycle we observed two simultaneous phenomena: a constant level for C-I (Fig. 4a), indicating the maintenance of the early transcript concentration at a constant low level, and a gradual decrease of TR-I (Fig. 5a), reflecting a progressive inhibition of transcription from Pe. Both phenomena suggest that the progressive inhibition of the Pe promoter is a mechanism that compensates accurately for the permanent increase in the number of Pe copies (a consequence of phage replication) to ensure a constant early transcript concentration.

Transcription of segments II and III

During the first 17 min, the transcription of segment I was followed, after a short delay, by that of segment II (Fig. 4a, 5a) in agreement with the transcription of the early operon starting from a promoter located in segment I (Pe) and extending into segment II. From the 17th min the transcription of II increased and became uncoupled from transcription of I (Fig. 4a, 5a). The C promoter, known to be activated between the 15th and 20th min (van Meeteren, 1980), is probably the principal, or only, promoter responsible for the observed increase in II (hypothetical promoters in segments III to VII cannot be involved because Mu late transcription does not proceed leftward). From the 17th min onward we also observed that transcription in II and III ran parallel (Fig. 4a, 5a), suggesting that transcription directed by the C promoter was coupled to the transcription of the genes to the right of C, located in II and III. With the exception of lys, these genes belong to the head gene cluster (Giphart-Gassler et al., 1981b).

Transcription of segments IV and V

The low TRs of the middle segments III, IV and V during the first 14 min of the lytic cycle (Fig. 5a, b) do not allow a final conclusion and require additional experiments.

Our results showed a coupled transcription increase in IV and V, beginning at 14 min (Fig. 4b, 5b). Late transcription proceeds rightward and therefore one might postulate that the coupled transcription in IV and V was an extension of the transcription coming from II and III. However, this hypothesis is not acceptable because the transcription increase in IV and V appeared earlier than in II and III (at 14 and 17 min, respectively). We conclude that the promoter involved in transcription of IV and V has to be different from, and independent of, the promoter(s) responsible for the transcription of II and III, consequently defining a new transcription unit in the region of IV and V. This region comprises the major part of the tail gene cluster. Also, the lack of a TR gradient from III to V during the increasing phase (Fig. 5a, b) is not consistent with a long transcription unit stretching from segment II to V and transcribed from left to right.

Transcription of segment VI

The low levels of segment-specific RNA and the low TR of segment VI observed during the early and intermediate phases (Fig. 4b, 5b) can be attributed to gin, as this gene is the only one within this segment which would be expected to be transcribed during these phases (see Introduction).

During the late phase, an increase in the TR of VI occurred at the same time as in segments IV and V, but the decrease was delayed with respect to these segments (Fig. 5b). In addition, a gradient from IV to VI was not formed; the opposite would have been expected if the transcription in VI were only an extension of the transcription from IV and V. This pattern is consistent with transcription starting from one or more promoters within or close to segment VI, partially superimposed on an extension of the transcription of IV and V. Evidence for independently regulated promoters within segment VI that are active during the late phase has been presented for the genes gin, S and U (see Introduction). The extension of the transcription of IV and V could involve the right portion of the tail gene cluster (genes Q to R).
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Transcription of the end segment VII

The end segment VII consists of the right portion of gin and the whole mom operon (Plasterk et al., 1983). The observed increase in transcription of this segment during the late phase appeared to be coupled to the transcription of VI (Fig. 4b, 5b) and supports the hypothesis that the overlapping of the termination codons of gin with the promoter region of mom could allow coordinated expression of these transcription units (Plasterk et al., 1983).

DISCUSSION

We have analysed the transcription of Mu DNA throughout the whole lytic cycle, independent of changes in incorporation rates of the radioactive precursor and changes in gene dosage (number of Mu DNA copies) occurring with phage development. The course of the TRs for whole Mu DNA during the lytic development revealed three transcriptional phases: early (0 to 9 min), intermediate (between 9 and the interval 14 to 17 min) and late (from the interval 14 to 17 min onward), instead of only two (early and late) detected by the conventional percentage specific hybridization.

The results confirmed the activation and inhibition of the early operon during the early phase (Wijffelman & van de Putte, 1974). The reactivation, detected in the intermediate phase, is independent of gpA, gpB, or any late gene product, because it was observed in Mu cts Aam, Mu cts Bam, and Mu ΔC-β phages (Wijffelman et al., 1974; Wijffelman & van de Putte, 1974). Moreover, it is not due to an increase of Mu DNA copies because it was detected by the TR, which is independent of gene dosage effects. Therefore, it can only result from the reduction of the gpner concentration (which would be expected as a consequence of the reduction of the early transcript synthesis between the 4th and 9th min), and/or from an increase in the concentration of the integration host factor complex (IHF), which enhances the early transcription by binding in a region adjacent to the RNA polymerase-binding site at Pe (Goosen & van de Putte, 1984; Goosen et al., 1984; Krause & Higgins, 1986). The progressive inhibition of the early operon from the 11th min (detected with TR) probably occurs to compensate for the permanent increase of the early operon copies, and leads to the maintenance of a low constant concentration of the early transcript (detected with C) and probably of the proteins for which it codes. A continuous increase of these proteins [gpA, gpB, gparm, gplig (Paolozzi et al., 1980) and gpkil] is lethal for the cell. The protein gpner is probably not responsible for the progressive inhibition because the constant level of early transcript would not be sufficient to produce the permanent increase of gpner required to inhibit an increasing number of Pe copies. On the contrary, an IHF-related mechanism becomes plausible because the gradual inhibition of E. coli transcription observed from the 16th min (results not shown) would lead to a gradual reduction of the active IHF concentration, and consequently to a reduction of the IHF-positive effect on the Pe promoter.

The data presented indicate that late genes involved in phage morphogenesis are probably organized into two independent transcription units. The left one was detected on segments II and III, and therefore could consist of the head gene cluster D to J. Our results suggest that the transcription of this unit is coupled to C transcription; this could occur either by C-mediated control of the hypothetical promoter for D to J or by extension of the C transcript into D to J. The second transcription unit, on the right, was detected on segments IV and V, and therefore could consist of the tail gene cluster K to R. Since RNA from the right unit was detected earlier than RNA from the left unit, and considering that both RNAs are synthesized from left to right, we conclude that transcription of the right unit cannot be an extension of transcription from the left. Consequently, the right unit transcription has to be directed by an independent promoter.

The genes for the structural proteins and assembly of the particles of phages λ and T7 are organized into only one transcription unit. In contrast, the genes for Mu particles appear to be organized according to their functions into at least four transcription units, D to J (head), K to R (tail), S (fibre) and U (fibre). This functional clustering of structural genes, also observed in other complex phages like T4, assures the independent synthesis, local accumulation and therefore assembly of proteins for only one constituent, avoiding premature interactions with proteins for other constituents. In addition, an independently regulated expression of genes S and U, which determine the host specificity of the particles, is essential for the wide spectrum of host ranges of phage Mu.
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REFERENCES


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