UNIVERSITÉ DE GENÈVE

Archive ouverte UNIGE

https://archive-ouverte.unige.ch

Article scientifique | Article | 1989

Published version | Open Access

This is the published version of the publication, made available in accordance with the publisher's policy.

Transcription and messenger RNA processing upstream of bacteriophage T4 gene 32

Carpousis, Agamemnon James; Mudd, Elisabeth Anne; Krisch, Henry M.

How to cite

CARPOUSIS, Agamemnon James, MUDD, Elisabeth Anne, KRISCH, Henry M. Transcription and messenger RNA processing upstream of bacteriophage T4 gene 32. In: Molecular and General Genetics, 1989, vol. 219, n° 1-2, p. 39–48. doi: 10.1007/BF00261155

This publication URL: https://archive-ouverte.unige.ch/unige:147854

Publication DOI: 10.1007/BF00261155

© The author(s). This work is licensed under a Creative Commons Attribution (CC BY) https://creativecommons.org/licenses/by/4.0

Mol Gen Genet (1989) 219:39–48

MGG
© Springer-Verlag 1989

Transcription and messenger RNA processing upstream of bacteriophage T4 gene 32

Agamemnon J. Carpousis, Elisabeth A. Mudd, and Henry M. Krisch
Department of Molecular Biology, University of Geneva, 30, Quai Ernest-Ansermet, CH-1211 Geneva 4, Switzerland

Summary. Bacteriophage T4 gene 32 lies at the 3′ end of a complex transcription unit which includes genes 33, 59, and several open reading frames. In the course of an infection, four major transcripts are synthesized from this unit: two overlapping polycistronic transcripts about 3800 and 2800 nucleotides in length, and two monocistronic gene 32 transcripts about 1150 and 1100 nucleotides in length. These transcripts are made at different times in infection and the polycistronic transcripts have segmental differences in stability. Messenger RNA processing yields a 1025 nucleotide monocistronic gene 32 transcript, and a 135 nucleotide transcript containing part of the gene 59 coding sequence. Processing depends on *Escherichia coli* encoded ribonuclease E. This pattern of transcription and processing leads to the synthesis of gene 32 mRNA throughout infection, whereas transcripts encoding the upstream genes are present only early in infection. The 3800 nucleotide polycistronic transcript initiates at a promoter that does not require T4 encoded factors for activity. However, full-length synthesis of this transcript depends on the T4 *mot* gene product. The region upstream of gene 32 also contains four *E. coli*-like promoters that are active on chimeric plasmids in uninfected cells, but inactive in bacteriophage T4. The location of these cryptic T4 promoters is intriguing in that they lie near the 5′ ends of open reading frame B, gene 59 and gene 32. They could play a role in phage development under particular conditions of growth or in bacterial hosts other than those examined here.

Key words: Bacteriophage T4 transcription – Gene 32 – Messenger RNA processing – Ribonuclease E – Cryptic promoters

Introduction

Bacteriophage T4 gene 32 encodes a single-stranded DNA binding protein that is essential in DNA replication, recombination and repair (Alberts and Frey 1970; Mosig 1974; Alberts et al. 1975). Expression of this protein is autogenously regulated at the level of translation (Krisch et al. 1974; Russel et al. 1976; Lemaire et al. 1978). Polycistronic as well as monocistronic transcripts encoding gene 32 have been detected (Krisch et al. 1977; Young and Menard 1981). Gene 32 monocistronic mRNA 5′ ends have been mapped to −71, −145 and −205 relative to the gene 32 translation initiation codon; and the polycistronic transcripts have been shown to initiate from further upstream (Belin et al. 1987). The 5′ end at −71 arises by RNase E dependent processing of the primary transcripts (Mudd et al. 1988).

Gene 32 and 3 kb of upstream DNA have been sequenced (Krisch and Allet 1982; Hahn et al. 1986). The upstream region encodes two essential genes (33 and 59) and three open reading frames (ORFs A, B and C). These genes and ORFs are transcribed in the same direction. Gene 33 encodes a transcription factor required for late gene expression (Bolle et al. 1968; Horvitz 1973; Williams et al. 1989) and mutations in gene 59 lead to premature arrest of DNA replication (Wu et al. 1972). ORF A may encode gp*das*, a suppressor of mutations in gene 46 or 47, and ORF B may encode a small peptide associated with RNA polymerase in T4 infected cells (Hahn et al. 1986; however, see Orsini and Brody 1988). Since the products of genes, 33, 59 and 32 are required at about the time DNA replication starts, the organization of this unit may reflect a need for coordinate expression of these genes early in infection. In this paper we characterize transcription and mRNA processing in the region upstream of gene 32.

Materials and methods

Materials. SP6 RNA polymerase, RQ1 DNase and placental RNase inhibitor were from Promega Biotec or Boehringer Mannheim; AMV reverse transcriptase and T4 polynucleotide kinase from Genofit; pancreatic RNase from Calbiochem; proteinase K from Merck; γ-[^{32}P]ATP (3000 Ci/mmol) and α-[^{32}P]UTP (400 Ci/mmol) from Amersham International.

DNA primers were made with an Applied Biosystems DNA synthesizer (Model 381A). The products were deprotected, desalted and used without further purification. The following primers were used in this study: −2813, TTCTTTGTAATCTTCATCCAA; −1932, CCTCAAAA-TTCATTAATGCTA; −1286, AAACTGATTTACCAT-CAATGT; +21, AGTAGATTTACGTTTAAACAT. The sequences are shown in the 5′ to 3′ orientation and the numbers indicate the position of the 5′ end of the primer relative to the gene 32 initiation codon.

The construction of the plasmid TAK64 has been described (pSP64-TAK: Belin et al. 1987). The remainder of

Abbreviations and symbols: am, amber mutation; *gal,* galactose operon; gp, gene product; *lac,* lactose operon; *su,* suppressor; t, transcription terminator; *ts,* temperature sensitive

Offprint requests to: A.J. Carpousis

Table 1. Plasmid templates for complementary RNA probes and position of the T4 inserts relative to the gene 32 translation initiation codon

Plasmid	Vector	Insert			
		From	(site)	To	(site)
AC71	SP64	−2232	(*Rsa*I)	−1580	(*Hin*dIII)
AC72	SP64	−2232	(*Rsa*I)	−1796	(*Eco*RI)
AC53	SP65	−1580	(*Hin*dIII)	−886	(*Taq*I)
AC55	SP65	−1580	(*Hin*dIII)	−1257	(*Nde*I)
AC54	SP65	−886	(*Taq*I)	−196	(*Rsa*I)
AC56	SP65	−886	(*Taq*I)	−569	(*Msp*I)
TAK64	SP64	−340	(*Msp*I)	+3	(*Bam*HI)[a]
HAT64	SP64	+630	(*Taq*I)	+994	(*Hin*dIII)

See Fig. 5A for DNA restriction map of the region
[a] The *Bam*HI site in TAK64 is from a synthetic linker inserted after the first codon in the gene 32 coding sequence (Belin et al. 1987)

the plasmids listed in Table 1 were constructed by subcloning DNA restriction fragments from the plasmids KSK12A or KSK239 (Krisch and Selzer 1981) into the SP64 or SP65 riboprobe vectors (Melton et al. 1984).

Bacteria and phage strains. The *E. coli* strain MC1061 (*su*$^-$, Casadaban et al. 1980) was used in most experiments. T4 infections were performed as described previously (Belin et al. 1987). In one experiment, we used *E. coli* N3433 and N3431 (Goldblum and Apirion 1981) which are an isogenic pair of RNase E^+ and E^- (*ts*) strains. Infection of these strains were performed as described by Mudd et al. (1988). M9S medium containing 0.2% casamino acids was used throughout (Champe and Benzer 1962). Phage and bacterial growth was at 30° C except for infections of the RNase E strains at 43° C. T4D wild type and T4 *mot*$^-$ *am*G1 were from the Geneva collection.

General methods. Transformation, plasmid preparation, ligations, restriction enzyme digests, ^{32}P-end-labeling of DNA primers with T4 polynucleotide kinase and gel electrophoresis were performed essentially as described in Maniatis et al. (1982).

Isolation of total RNA from T4 infected cells, preparation of ^{32}P-labeled complementary RNA probes and protection experiments were performed essentially as described by Belin et al. (1987).

5′ end mapping by primer extension. Synthetic DNA primers were labeled with T4 polynucleotide kinase and γ-[^{32}P]ATP. Approximately 1×10^6 dpm of probe and 2 µg of RNA were suspended in 20 µl of 125 mM KCl, 12.5 mM TRIS·HCl, pH 7.2, 0.625 mM sodium EDTA then heated to 95° C for 5 min, quenched on ice and annealed at 30° C. The primers were extended at 42° C for 30 min in a final reaction mixture (25 µl) containing 100 mM KCl, 50 mM TRIS·HCl, pH 8.3, 5 mM $MgCl_2$, 2 mM dithiothreitol, 400 µM deoxynucleotide triphosphate (each), and 5 units of AMV reverse transcriptase. In RNA sequencing reactions, the final concentration of dideoxynucleotide triphosphate was 200 µM. Carrier tRNA (5 µg) was added, then the reactions were extracted with CPI (chloroform:phenol:isoamyl alcohol; 24:25:1) and ethanol precipitated. The reactions were analyzed on 6% DNA sequencing gels.

Northern blot analysis. RNA (2 µg) was glyoxylated and separated in 1% agarose gels (McMaster and Carmichael 1977), then electroblotted to nylon membranes (Gene Screen, New England Nuclear) and crosslinked with ultraviolet light (Khandjian 1986). The membranes were treated briefly at 65° C with 10 mM TRIS·HCl, pH 8.3, 1 mM sodium EDTA, 10 mM NaCl, 0.1% SDS to remove the glyoxal. After rinsing the blot thoroughly with water, the 16 and 23 S rRNAs were visualized by staining with methylene blue to check the integrity of the RNA preparations and the uniformity of the transfer. The blots were probed with ^{32}P-end-labeled DNA primers using a modification of the procedure of Tiercy et al. (1988). Briefly, the membranes were prehydridized at 67° C for 2 h in 0.5 M NaCl, 10 mM sodium phosphate, pH 7.2, 1 mM sodium EDTA, 1% SDS, 5× Denhardt's solution (Maniatis et al. 1982), 100 µg/ml salmon sperm DNA (2.5 ml/30 cm^2). The probe was suspended in a small volume of water, heated to 95° C, then added to the blot (3×10^6 dpm/2.5 ml). Hybridization was at 37° C overnight. The blots were washed twice with 0.13 M NaCl, 3.3 mM sodium phosphate, pH 7.2, 0.33 mM sodium EDTA, 1% SDS at 45° C, then twice in this buffer but without the SDS.

Results

Identification and characterization of mRNA 5′ ends at −2020 and −2874, and a 3′ end at +950

A series of complementary RNA (cRNA) probes were used to map polycistronic transcripts upstream of gene 32. Using the AC71 and AC72 cRNA probes (Table 1), we detected an mRNA 5′ end at about −2000, as well as a transcript which initiated from further upstream (for a map of the region, see Fig. 5A). Figure 1A, lane 1, shows an autoradiogram in which total RNA isolated from T4 infected *E. coli* was hybridized to ^{32}P-labeled AC71 cRNA, the hybrids digested with ribonuclease to degrade single stranded RNA and the protected cRNA fragments then separated by denaturing polyacrylamide gel electrophoresis. Full-length protected probe (marked with an *asterisk*) as well as a shorter 440 nucleotide (nt) protected species were detected. Full-length protection is due to message that spans the entire length of the probe. In Fig. 1A, lane 2, full-length protected probe as well as a shorter 230 nt species was detected using the AC72 probe. Taken together, these results indicate that there is a major mRNA 5′ end at about −2000 and at least one additional transcript initiated from further upstream.

We synthesized a DNA primer to map precisely the mRNA 5′ end at about −2000. In Fig. 1C, lane 4, a ^{32}P-end-labeled primer with a binding site at −1932 was hybridized to total RNA extracted from T4 infected *E. coli*, extended with reverse transcriptase and the products then separated by denaturing polyacrylamide gel electrophoresis. In separate reactions using dideoxynucleoside triphosphates, the RNA sequence was determined. We mapped mRNA 5′ ends to −2020, −2018, −2014 and −2013. This collection of ends will be referred to as the −2020 start site.

In Fig. 1C, lane 4, we also detected larger extension products. A major mRNA 5′ end upstream of −2020 was mapped in Fig. 1B. Lane 3 shows the products of a primer extension reaction using a DNA primer with a binding site

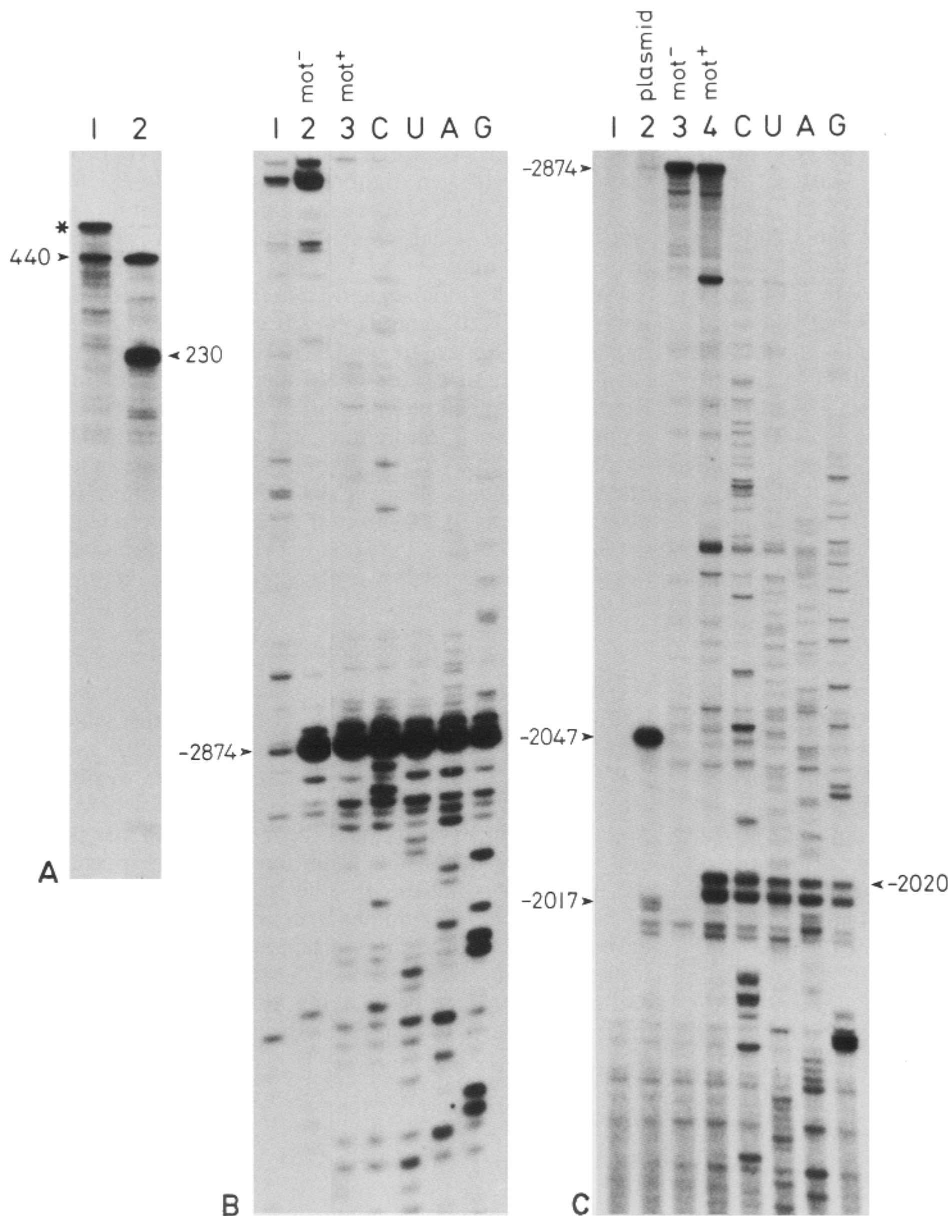

Fig. 1 A–C. Identification of mRNA 5′ ends. **A** Complementary RNA (cRNA) protection of RNA extracted 6 min after infection with wild-type T4. Lane 1, AC71 probe and lane 2, AC72 probe. The *asterisks* indicate the position of full-length protected cRNA probe and the length of the shorter protected products is indicated in nucleotides. **B, C** Primer extension using the −2813 and −1932 probes, respectively. In **B** lane 1 is a control in which RNA was extracted immediately after infection with *mot*⁻ T4; lane 2, RNA extracted 10 min after infection with *mot*⁻ T4; lane 3, RNA extracted 12 min after infection with wild-type T4. In **C** lane 1 is a control in which RNA was extracted from uninfected *Escherichia coli* harboring the plasmid pBR322; lane 2, RNA extracted from uninfected *E. coli* harboring the plasmid KSK239 which contains the T4 *Hin*dIII DNA fragment from −2503 to −1580 (Krisch and Selzer 1981; see restriction map in Fig. 5A); lane 3, RNA extracted 10 min after infection with *mot*⁻ T4; lane 4, RNA extracted 10 min after infection with wild-type T4. In **B, C** lanes C, U, A, and G are RNA sequencing reactions using dideoxynucleotide triphosphates (ddGTP, ddATP, ddTTP and ddCTP, respectively). Sequencing was performed using RNA extracted 12 min after infection (**B**) or 10 min after infection (**C**). The sequences at the mRNA 5′ ends are: −2874, AUCUCACUAAUUGAACGAGG; −2020, UAUAAAUUACCACCGCGUGGC; −2047, AAACAUUGUGAAUUAC; and −2017, AAAUUACCACCGCGUGGC. The 5′ ends at −2874, −2020 and −2017 are heterogeneous. The *underlined* nucleotides indicate each end detected by primer extension. The DNA sequence of promoters just upstream of these 5′ ends are: −2874 (immediate-early) GTTTACT-13 bp-GGTATATAAT; −2020 (*mot*-dependent) ATTGCTTCA-12 bp-TACTAT; −2047 (*E. coli*/cryptic) TTGATT-16 bp-TAATAT; and −2017 (*E. coli*/cryptic) TTGCTT-17 bp-TATAAT. The bases that match promoter consensus sequences are *underlined* (see McClure 1985; Guild et al. 1988; and Liebig and Rüger 1989 for *E. coli*, T4 *mot* dependent and T4 immediate-early consensus sequences, respectively)

at −2813. Primer extension products corresponding to ends at −2874, −2875 and −2876 were detected (−2874 start site). The −2874 and −2020 start sites each lie at an appropriate distance downstream of a DNA sequence that has a good match to a consensus promoter sequence (see below and legend to Fig. 1). Thus it is likely that these ends correspond to primary transcripts. In Fig. 1C, lane 4, several minor primer extension products were detected between

−2874 and −2020. Putative RNA 5′ ends corresponding to these primer extension products would lie within ORF A and could be due to weak transcription initiation. However, there are other possible explanations such as mRNA decay intermediates or reverse transcriptase pause products.

Gene 32 expression depends on the T4 *mot* gene product, a positive activator of transcription (Mattson et al. 1974, 1978; Uzan et al. 1985). Synthesis of the −205 monocistronic gene 32 transcript as well as mRNA initiated from upstream is strongly activated by gp*mot* (Belin et al. 1987). When RNA extracted from *E. coli* infected with a T4 mot^- strain was analyzed by primer extension, strikingly different effects on the accumulation of the −2020 versus the −2874 transcript were observed. −2020 transcription is significantly reduced in a mot^- infection whereas −2874 transcription is unaffected (in Fig. 1B compare lane 2, mot^- to lane 3, mot^+ and in Fig. 1C compare lanes 3, mot^- to lane 4, mot^+). It is interesting to note that there are transcripts initiated upstream of −2874 in the mot^- infection (Fig. 1B, lane 2) that are not present in the mot^+ infection (lane 3). These transcripts which apparently arise from within gene 34 were not further characterized.

We assayed the accumulation of the −2874 and −2020 transcripts, and their dependence on the *mot* gene product, as a function of time after infection (data not shown). The −2874 transcripts begin to accumulate as early as 2.5 min after infection whereas the −2020 transcripts begin to accumulate between 2.5 and 5.0 min. By 10 min, the level of the −2020 transcript in the mot^+ infection is about sixfold higher than in the mot^- infection. Furthermore, −2874 mRNA, but not −2020 mRNA, was detected in RNA preparations from cells treated with chloramphenicol prior to infection (data not shown). Chloramphenicol, an inhibitor of protein synthesis, blocks the production of the *mot* gene product and thus the −2020 promoter cannot be activated.

Based on DNA sequence data, Hahn et al. (1986) predicted promoters that correspond to the −2874 and −2020 start-sites (P2 and P4, respectively, in their numbering system). However, we failed to detect transcription initiation at four other potential immediate-early or *mot* dependent promoters (P2, P3, P5 and P6). Recent analyses of T4 immediate-early and *mot* dependent promoters suggests that the DNA sequence requirements for each class of promoter may be more stringent than previously thought (Liebig and Rüger 1989; Guild et al. 1988). The limited number of in vivo transcripts which we have detected is consistent with these observations.

A major rho-independent transcription terminator (t_{32}) immediately 3′ of gene 32 had been predicted by Krisch and Allet (1982) based on DNA sequence data. Efficient termination at this site (+950) has been demonstrated *in vivo* in uninfected cells harboring chimeric plasmids containing the cloned terminator, and *in vitro* in a simple system containing DNA, purified RNA polymerase and nucleotide triphosphates (Belin et al. 1987). Efficient termination of transcription in T4 infected *E. coli* was detected using the HAT64 cRNA probe (data not shown). Strong termination (greater than 95%) was observed throughout infection.

Northern blot analysis of transcripts

We determined the size and abundance of the transcripts synthesized in an infection by probing Northern blots of total RNA isolated from T4-infected cells with a set of ^{32}P-end-labeled DNA primers that hybridize to different sequences within the unit. In Fig. 2, the Northern blots reveal 11 distinct transcripts. The lengths of the transcripts are listed in Table 2 and, where possible, the position of the transcript is indicated. B_1 is a 3800 nt transcript that starts at the −2874 immediate-early promoter and ends at the gene 32 transcription terminator (t_{32}); C_1 is a 2800 nt transcript that starts at the *mot* dependent −2020 promoter and ends at t_{32}; and A_3 is a mixture of transcripts ranging in size from 1150–1000 nt that start at the −205 *mot* depen-

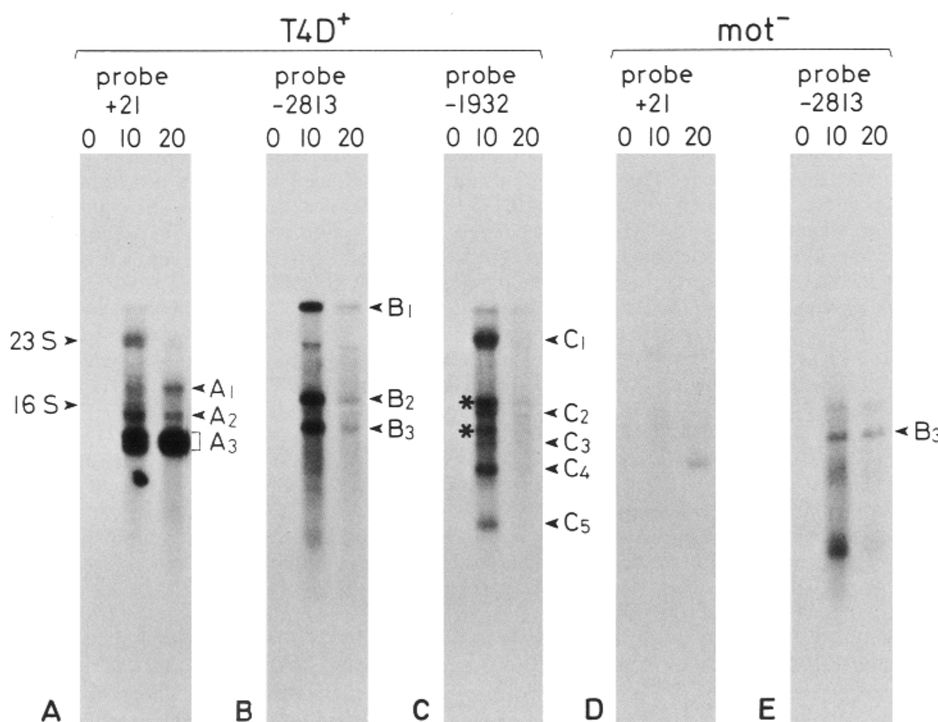

Fig. 2A–E. Northern blot analysis of mRNAs from the gene 32 transcription unit. RNA extracted from wild-type T4 infected cells was used in **A**, **B** and **C**; RNA from mot^- T4 infected cells was used in **D** and **E**. In each panel, RNA extracted from uninfected cells 0, 10 and 20 min after infection was analyzed. **A** and **D** were probed with a ^{32}P-labeled DNA primer which hybridizes to the 5′ end of the gene 32 coding sequence (+21); **B** and **E** with a probe which hybridizes to the 5′ end of the −2874 transcript (−2813) and **C** with a probe which hybridizes to the 5′ end of the −2020 transcript (−1932). In **C**, the *asterisks* indicate the positions of the B_2 and B_3 transcripts which hybridize to the −1932 probe as well as the −2813 probe. The autoradiograms in **B–E** were exposed about 4 times longer than the autoradiogram in **A** to permit visualization of the less abundant transcripts

Table 2. Transcripts detected by Northern blot analysis

Transcript	Probe	Length (nucleotides)[a]	Position[b]	
			5′ end	3′ end
A_1	+21	1800	N.D.	N.D.
A_2	+21	1300	N.D.	N.D.
A_3	+21	1150–1000	−205, −145, −71	+950 (t_{32})
B_1	−2813	3800	−2874	+950 (t_{32})
B_2	−2813	1600	−2874	−1205
B_3	−2813	1200	−2874	−1630 (t_{AB})
C_1	−1932	2800	−2020	+950 (t_{32})
C_2	−1932	1450	N.D.	N.D.
C_3	−1932	1050	N.D.	N.D.
C_4	−1932	820	−2020	−1205
C_5	−1932	480	−2020	−1630 (t_{AB})

[a] Transcript lengths were determined from their electrophoretic mobility using the endogenous *Escherichia coli* 16 and 23 S ribosomal RNA as size standards (about 1500 and 2900 nucleotides, respectively)

[b] Where possible, a best estimate of the position of the transcripts was made. We could not unambiguously determine the position of all the transcripts detected in Fig. 2 (indicated by N.D., not determined). These transcripts could be intermediates in the decay of the larger polycistronic transcripts but we cannot exclude the possibility that they arise from minor transcription initiation or termination sites within the region

dent promoter, the −145 late promoter or the −71 RNase E processing site and end at t_{32}. Note that the autoradiograms in Fig. 2B–E were exposed about 4 times longer than the autoradiogram in Fig. 2A to permit visualization of less abundant transcripts. It is possible to estimate relative abundances of the various transcripts from the blot in Fig. 2A. The A_3 gene 32 monocistronic mRNAs are the most abundant transcripts. The larger C_1 and B_1 transcripts which should hybridize to the +21 probe are much less abundant. This is not likely to be an artifact due to degradation of the RNA preparation or to inefficient transfer of larger transcripts because the 16 and 23 S rRNAs were intact and efficiently transferred in these blots (see Materials and methods). The abundance of the A_3 transcripts in Fig. 2A does not necessarily reflect large differences in the strength of the gene 32 proximal versus distal promoters. Quantitation of the peak levels of mRNA 5′ ends at −2874, −2020, −205 and −145 using cRNA protection or primer extension suggests that the corresponding promoters are comparable in strength (data not shown). The monocistronic gene 32 transcripts are apparently more stable than the larger polycistronic transcripts and thus accumulate to higher levels (unpublished results).

In Fig. 2B, two transcripts shorter than B_1 were detected using the −2813 DNA primer. Primer extension experiments showed that there are no major transcript 5′ ends upstream of −2874 in a wild-type T4 infection (Fig. 1B, lane 3). Thus the shorter products in Fig. 2B are likely to have 5′ ends at −2874, and the approximate position of their 3′ ends can be estimated from the length of the transcripts. The 1200 nt B_3 transcript would have a 3′ end at about −1674 and the 1600 nt B_2 transcript would have a 3′ end at about −1274 (Table 2). These assignments are corroborated by the detection of B_2 and B_3 with the −1932 probe (B_2 and B_3 are marked with asterisks in Fig. 2C). The B_2 3′ end probably coincides with a mRNA processing site mapped to −1205 (see below) and the B_3 3′ end with a weak terminator at −1630 (t_{AB}). Hahn et al. (1986), based on DNA sequence data, noted a potential mRNA stem-loop structure in the 5′ end of gene 33 (position 3400 in their numbering system) and suggested that it might play a role in transcription termination. Transcripts terminating at this site would encode ORFs A and B. In our experiments termination is relatively inefficient since longer polycistronic transcripts (B_1 and B_2) are present. Messenger RNA 3′ ends at t_{AB} were detected in cRNA protection experiments employing a truncated version of the AC71 probe. Quantitation indicated that 15%–30% of the transcripts terminated at this site (data not shown).

The 3800 nt B_1 immediate-early transcript arises from a promoter that does not require gp*mot* for activation. Thus, some gene 32 expression might be expected even in mutants that do not make active *mot* gene product. However, there is very little gene 32 transcription in a mot^- infection (Belin et al. 1987). Figure 2D and E show blots of RNA isolated from *E. coli* infected with mot^- T4. In Fig. 2D, the blot was probed with the +21 DNA primer which is specific for the 5′ end of gene 32. Very little gene 32 message is detected in the mot^- infection (compare Fig. 2A, D, and note that the autoradiogram in D was exposed about 4 times longer than A). In Fig. 2E, RNA from a mot^- infection was probed with the −2813 DNA primer which binds to the 5′ end of the ORF A transcripts. This autoradiogram shows that the 3800 nt B_1 transcript is not detected in a mot^- infection and that the largest discrete transcript is B_3 which corresponds to a message that initiates at −2874 and terminates at −1630 (t_{AB}). The bulk of the messages detected in Fig. 2E are in two diffuse regions below the B_3 transcript. In a mot^- infection, a significant fraction of the −2874 transcripts elongate through ORF A to at least −1932. In Fig. 1C, lane 3, extension with the −1932 primer yields a long product that corresponds to the −2874 transcript. cRNA protection using the AC72 probe which extends from −2232 to −1796 indicates that −2874 transcripts are not efficiently elongated through ORF B since full-length protection of the probe is greatly diminished in a mot^- infection (data not shown). Our Northern blot data indicate that the bulk of the transcripts do not appear to terminate at discrete sites. This is not necessarily surprising because premature termination in mot^- infections is apparently mediated by rho (Brody et al. 1983), and rho-dependent termination can occur at multiple sites spread over large regions (Platt 1986).

Characterization of a processed 135 nt transcript derived from the polycistronic transcripts

In the course of characterizing transcripts upstream of gene 32, a 135 nt RNA which accumulates late in infection was detected by cRNA protection. In Fig. 3A, lane 1, the AC53 cRNA probe gives rise to full-length protected probe due to polycistronic transcripts that span the region, and to two protected fragments 370 and 135 nt long (the 370 nt species is described below). To determine the location of the 135 nt transcript we used two cRNAs derived from the 5′ and 3′ halves of AC53 (lane 2, AC55; lane 3, AC53n). Protected species of about 80 nt and 55 nt were detected indicating that the 135 nt transcript spans the junction of

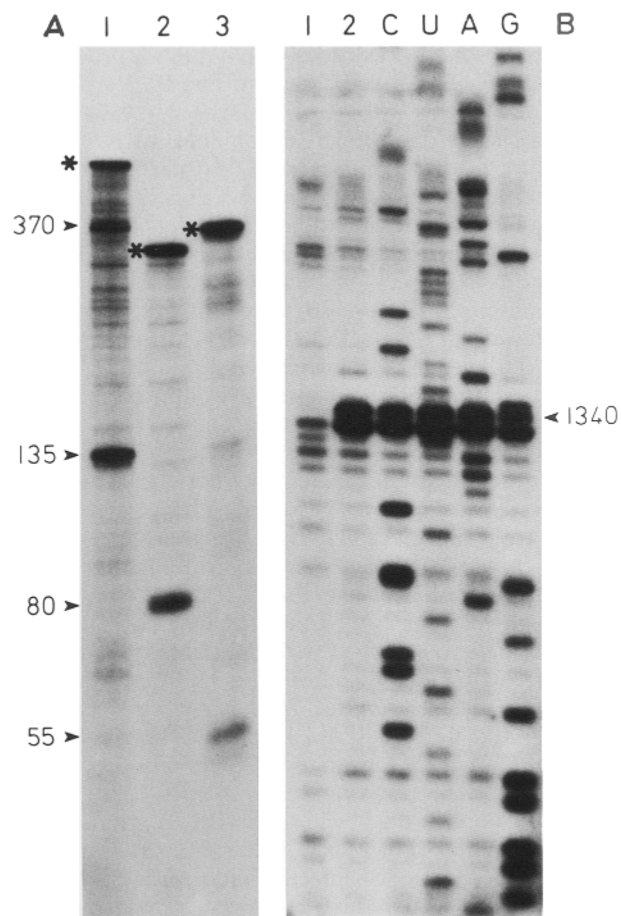

Fig. 3A and B. Identification of a 135 nucleotide transcript with a 5′ end at −1340. **A** cRNA protection of T4 transcripts in the region from −1580 to −886 relative to the gene 32 initiation codon. Lane 1, AC53 cRNA probe which spans the entire region; lane 2, AC55 probe which extends from −1580 to −1257; lane 3, AC53n probe which extends from −1257 to −886 (AC53n synthesized from AC53 plasmid linearized with *Nde*I). The *asterisks* indicate the positions of full-length protected probe and the length of the shorter protected products is indicated in nucleotides. **B** Primer extension from a DNA oligonucleotide with a binding site near the 5′ end of gene 59 (−1286). Lane 1, control in which RNA was extracted immediately after infection with wild-type T4; lane 2, RNA extracted 24 min after infection; lanes C, U, A, and G, RNA sequencing reactions using dideoxynucleotide triphosphates (ddGTP, ddATP, ddTTP and ddCTP, respectively). Sequencing was performed using RNA extracted 24 min after infection. The sequence at the −1340 5′ end is AUUAAACUCCG-CAUGCC. The 5′ ends are heterogeneous and the *underlined* nucleotides indicate the ends detected by primer extension

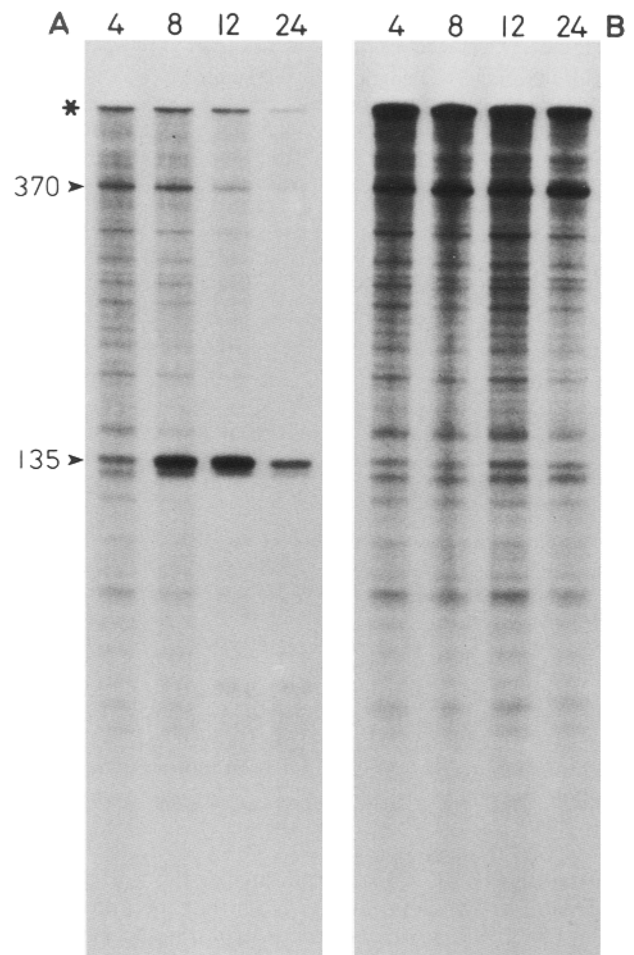

Fig. 4A and B. Processing at the −1340 site depends on RNase E. cRNA protection using the AC53 probe which extends from −1580 to −886. An isogenic pair of *E. coli* strains were infected at 43° C with T4D$^+$. **A** RNase E$^+$; **B** RNase E$^-$. RNA was extracted 4, 8, 12, and 24 min after infection. The *asterisk* indicates the position of the full-length protected AC53 probe and the length of the shorter protected products is indicated in nucleotides

the AC55 and AC53n probes. These results place the 5′ end of the 135 nt transcript at the beginning of the gene 59 coding sequence.

The exact 5′ end of the 135 nt transcript was mapped by primer extension using the −1286 DNA primer which hybridizes downstream of the 5′ end of the gene 59 coding sequence. In Fig. 3B, two prominent primer extension products corresponding to 5′ ends at −1339 and −1340 were identified (−1340 site). The 5′ end lies immediately downstream of the gene 59 translation initiation codon. Thus, the 135 nt transcript contains neither the gene 59 ribosome binding site nor an intact initiation codon. The 3′ end of the 135 nt transcript is at about −1205, based on its size.

Since there is no apparent promoter sequence immediately upstream of −1340, we tested the possibility that the 135 nt transcript was a processed RNA species. Messenger RNA processing 71 bases upstream of the gene 32 translation initiation codon depends on *E. coli* RNase E (Mudd et al. 1988). Figure 4 shows the kinetics of appearance of the 135 nt transcript in T4 infections of isogenic RNase E$^+$ or RNase E$^-$ *E. coli* strains. In the RNase E$^+$ infection (Fig. 4A), full-length protection of the probe (marked by an asterisk) due to polycistronic transcripts is detected early in infection, and the 135 nt transcript accumulates at later times. In the RNase E$^-$ infection (Fig. 4B) the polycistronic transcripts accumulate throughout the infection, and production of the 135 nt transcript is dramatically reduced. These results indicate that the 135 nt transcript is processed from the polycistronic transcripts in an RNase E dependent reaction.

The cRNA protection data in Figs. 3, 4 reveal a 370 nt as well as a 135 nt protected species. Additional protection experiments using a series of truncated probes indicated

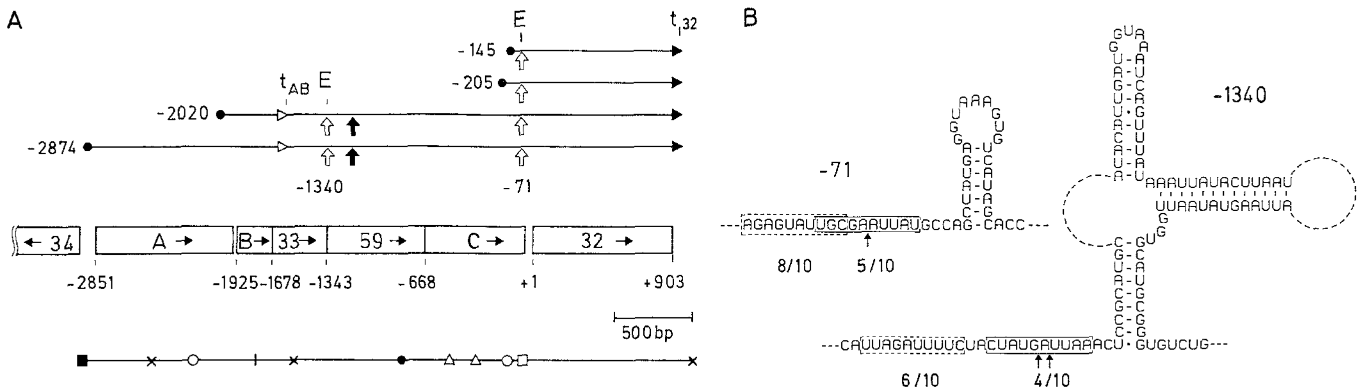

Fig. 5A and B. Major transcripts and mRNA processing sites. **A** Summary of major transcripts in the region. The 5′ ends of the primary transcripts at −2874, −2020, −205 and −145 are shown by *black circles*. The majority of the primary transcripts stop at t_{32} (*black horizontal arrows*), but there is some weak termination at t_{AB} (*open horizontal arrows*). The *open vertical arrows* show RNase E dependent mRNA processing sites at −1340 and −71, and the *black vertical arrow* shows an mRNA processing site at −1205 that apparently does not depend on RNase E. The genes and open reading frames in the region, and a DNA restriction map of the region are shown below the transcripts. The positions of the initiation codons of the upstream genes and open reading frames are numbered in base pairs using the A in the gene 32 initiation codon as +1. A selection of DNA restriction sites in the region are shown (■, *Sma*I; ×, *Hin*dIII; ○, *Rsa*I; +, *Eco*RI; ●, *Taq*I; △, *Msp*I; □, *Cla*I). **B** −71 and −1340 RNase E dependent processing sites. The sites of cleavage are indicated by the *vertical arrows*. The similarity of the sequences flanking the cut site (*box*) to a suggested RNase E recognition sequence (ACAGA/UAUUUG) is shown (Tomcsàny and Apirion 1985). The sequences in the *dashed boxes* upstream of the −71 and −1340 cleavage sites have a better match to the proposed consensus sequence than the actual site downstream. The RNA stems 3′ of the cleavage sites may play a role in RNaseE recognition (Mudd et al. 1988). Processing at the −1340 site yields a 135 nucleotide transcript which extends from −1340 to −1205. A predicted secondary structure of the 135 nucleotide transcript is shown. There is direct evidence supporting the existence of the RNA stem-loop 3′ of the −71 site (McPheeters et al. 1988)

that the 370 nt protected species arises from transcripts that start upstream of the AC53 probe and end at −1205, the same site as the 3′ end of the 135 nt transcript (data not shown). The B_2 and C_4 transcripts detected by Northern blot analysis (Fig. 2) could have 3′ ends at −1205 (Table 2), and are probably the source of the 370 nt protected species. Note that in Fig. 4B, the 370 nt protected species accumulates in the RNase E^- strain, suggesting that formation of the 3′ end at −1205 does not depend on RNase E.

Identification of T4 "cryptic" promoters

Fusion of the 5′ end of gene 32 to *lacZ* or other coding sequences leads to high-level protein synthesis in uninfected *E. coli* (Gorski et al. 1985). In an *in vivo* promoter strength assay, a T4 DNA restriction fragment extending from −340 to +21 had about 70% as much activity as the strong *E. coli galK* promoter. cRNA protection of RNA isolated from *E. coli* harboring a plasmid with a gene *32-lacZ* fusion showed that the major transcription start site was at −45 relative to the gene 32 initiation codon (Belin et al. 1987). As might be expected, the DNA sequence just upstream of −45 is a good match to the *E. coli* promoter consensus sequence. In addition, the −45 promoter has been shown to be active in a variety of gram-negative bacteria harboring chimeric plasmids (Frey et al. 1988). However, the −45 promoter is cryptic in bacteriophage T4: −45 transcripts have never been detected even as early as 2 min after infection (unpublished results).

In Fig. 1C, lane 2, primer extension analysis using RNA extracted from uninfected *E. coli* harboring the plasmid KSK239 (Krisch and Selzer 1981; see legend to Fig. 1 for details) reveals two transcription start sites: one with a discrete 5′ end at −2047, and the other with a series of ends mapping to −2017, −2016, −2014 and −2013 (−2017 start site). These mRNA 5′ ends were also detected by cRNA protection (data not shown). A comparison of lanes 2 and 4 in Fig. 1C, shows that the transcripts from the −2047 start site are not detected in T4 infected cells. This also appears to be the case for the −2017 start site which is distinct from the *mot* dependent −2020 start site. Inspection of the DNA sequence in this region indicates that the −2017 and −2020 transcripts are likely to arise from separate promoters that overlap (see legend to Fig. 1). Thus, like the −45 promoter, the −2047 and −2017 promoters are cryptic. Another cryptic promoter at −1427 was detected by cRNA protection and mapped by primer extension (data not shown). The existence of four cryptic promoters within a relatively small portion of the T4 genome (2.5%) indicates that these promoters may be widespread.

Two possible explanations for the failure to detect transcripts from the cryptic promoters in a bacteriophage T4 infection have been excluded. Briefly, neither the substitution of glucosylated hydroxymethylcytosine (glu-HMC) for cytosine in T4 DNA nor the T4-directed ADP-ribosylation of the host RNA polymerase appears to be responsible for blocking transcription at the cryptic promoters (data not shown). We failed to detect −45 transcripts in infections using T4 containing cytosine instead of glu-HMC or under conditions where the ADP-ribosylation (by the *alt* and *mod* gene products) was blocked (Mathews and Allen 1983; Snustad et al. 1983; Rabussay 1983 for reviews of glu-HMC and ADP-ribosylation in T4).

Discussion

Transcription and mRNA processing in the gene 32 region is summarized in Fig. 5A. Four primary transcripts are synthesized in the course of an infection. The −2874 transcript initiates at an immediate-early promoter, the −2020 and

−205 transcripts at *mot* activated promoters and the −145 transcript at a late promoter. A strong transcription terminator lies slightly downstream of the gene 32 coding sequence (t_{32}), and a weak terminator lies downstream of ORF B (t_{AB}). The primary transcripts are processed at −1340 and −71 in an RNase E dependent reaction. The arrangement of overlapping immediate-early and *mot* dependent transcripts is similar to the pattern of transcription in the rII region of bacteriophage T4 (Brody et al. 1983). However, the presence of the −145 late transcript in a region that is transcribed early is unusual. The involvement of RNase E in mRNA processing is novel (Mudd et al. 1988).

Gene 32 mRNA is synthesized throughout the infection. However, there is an additional level of regulation since gp32 synthesis is translationally self-regulated. In a wild-type T4 infection, the −145 late promoter is fully active at a time when the gp32 synthetic rate begins decreasing. Late in infection gene 32 mRNA is translationally repressed by the high levels of free gp32 that accumulate in the cell (Krisch et al. 1974; Russel et al. 1976). It is interesting to note that this repression can be reversed under conditions that deplete the pool of free gp32 (Krisch and Van Houwe 1976; Krisch et al. 1977; Lemaire et al. 1978). The pattern of gene 32 transcription can be viewed as a program that maintains a high level of mRNA which is available for translation in situations where the level of free gp32 is reduced. This notion has been verified by the analysis of T4 mutants in which the −205 *mot* dependent and −145 late promoters have been deleted. These mutants have significantly reduced capacity to make gp32 in situations where there is increased demand (unpublished results).

The pattern of transcription, processing and mRNA decay of the upstream transcripts may have regulatory consequences. The genes and open reading frames upstream of gene 32 are not thought to be translationally regulated. Thus, their level of expression would depend on the steady state levels of the upstream transcripts. ORF A is strictly dependent on transcription from an immediate-early promoter whereas ORF B, gene 33, gene 59 and ORF C can be synthesized from immediate-early as well as *mot* dependent promoters. Thus it is likely that ORF A is expressed earlier than the downstream genes. Furthermore, it is possible that RNase E dependent processing and subsequent decay could differentially affect the various upstream transcripts and play a role in determining the relative rates of synthesis of each gene product. It will be interesting to see if mRNA processing and decay of other bacteriophage T4 transcripts is mediated by RNase E.

Elongation of the −2874 immediate-early transcript requires mot *activity*

Surprisingly, synthesis of full-length −2874 immediate-early transcript is drastically inhibited in a *mot*⁻ infection. When *E. coli* is treated with chloramphenicol prior to infection, T4 immediate-early transcription becomes sensitive to premature, rho-dependent transcription termination. However, a few minutes after infection, T4 transcription becomes refractory to chloramphenicol induced transcription termination. Expression of gp*mot* is essential for this anti-termination effect (Daegelen et al. 1982; Brody et al. 1983). It is not clear if gp*mot* acts directly or if it activates transcription of a gene (or genes) that suppresses premature termination. It is important to note that we detected premature termination in a *mot*⁻ infection in the absence of chloramphenicol. At least in the case of the −2874 transcript, inhibition of translation by chloramphenicol is not required for premature termination, and *mot* dependent anti-termination appears to play a role in the expression of the genes in this region.

Messenger RNA processing

The primary transcripts shown in Fig. 5A are processed. There are two RNase E dependent cleavage sites. In addition, there is another site (−1205) which does not appear to depend on RNase E. Processing at these sites leads to the accumulation of gene 32 monocistronic mRNA (Mudd et al. 1988), and a 135 nt transcript that extends from −1340 to −1205. With the exception of the 135 nt transcript, the regions of the polycistronic transcripts upstream of gene 32 decay after processing. Thus, the polycistronic transcripts exhibit segmental differences in stability with the processed 135 nt and gene 32 transcripts persisting late in infection.

In Fig. 5B, the −1340 RNase E dependent processing site and the predicted secondary structure for the 135 nt transcript is shown. The previously characterized −71 site is shown for comparison (see Mudd et al. 1988 for discussion of features that may be important for RNase E recognition). The processed 135 nt transcript might fold into a relatively compact structure which could explain its apparent stability. A variety of processed short RNAs have been characterized in bacteria and their phages. These RNAs have diverse functions (for reviews see Inouye and Delihas 1988; King et al. 1986). Guo et al. (1986) have shown that a small phage-encoded RNA is required for *in vitro* packaging of bacteriophage φ29 DNA. It is possible that the 135 nt RNA could play a role in the growth and/or assembly of bacteriophage T4.

The decay of bacterial messages is thought to be mediated by endonucleolytic cleavages followed by 3′ to 5′ exonucleolytic degradation (see King et al. 1986 for a review). RNase E cleavage at −71 and −1340 could lead to rapid degradation of the upstream transcripts by 3′ exonuclease. It has been suggested that mRNAs can be stabilized, in part, by RNA secondary structures that block degradation by 3′ exonuclease (Newbury et al. 1987). Part of the resistance of the 135 nt and gene 32 transcripts to degradation may be due to double stranded RNA structures that protect their 3′ ends. The 3′ end of the gene 32 transcripts contains a potentially very stable stem-loop structure (from the rho-independent transcription termination site), and the −1205 end may be protected by the G-C rich RNA stem at the base of predicted structure for the 135 nt RNA (Fig. 5B).

Cryptic promoters

Transcripts from T4 cryptic promoters are not detected in bacteriophage infections but these promoters are active on chimeric plasmids in uninfected *E. coli*. Modification of T4 DNA (glu-HMC) or ADP-ribosylation of host RNA polymerase does not appear to be responsible for silencing the cryptic promoters. T4 immediate-early promoters are transcribed by host RNA polymerase and work in the absence of T4 encoded factors (Brody et al. 1983). Nevertheless, Liebig and Rüger (1989) have suggested that T4 imme-

diate-early promoters may be a special class of strong promoters with a consensus sequence that differs from typical *E. coli* promoters. In addition, nearly all T4 immediate-early promoters appear to be imbedded in segments of "curved" DNA and they have suggested that this structural feature may facilitate RNA polymerase binding end transcription initiation.

T4 immediate-early transcription is unusual in that the DNA template is apparently relaxed early in infection (Sinden and Pettijohn 1982; Albright and Geiduschek 1986). Torsional stress can significantly affect transcription (Sanzey 1979; Menzel and Gellert 1983). Promoters that appear to be "dead" on linear templates can become strong on negatively supercoiled templates (Borowiec and Gralla 1987). The behavior of the cryptic promoters could be explained by postulating that they are too weak on the relaxed T4 DNA template to compete with strong immediate-early promoters.

It is interesting to note that the cryptic promoters are located at positions near the 5′ ends of ORF B (−2047 and −2017), gene 59 (−1426) and gene 32 (−45). It is possible that these promoters could play a role in T4 development under particular conditions of growth or in bacterial hosts other than those examined here.

Acknowledgements. We thank R.H. Epstein for discussions and criticism of the manuscript, and for generously providing support and facilities. We also thank L. Poljak for critical comments on the manuscript. The skilful preparation of the figures by O. Jenni and F. Ebener is gratefully acknowledged. This work was supported by a grant from the Swiss National Science Foundation (no. 3.188.88). Additional support was provided by the Department of Public Instruction of the State of Geneva.

References

Alberts BM, Frey LM (1970) T4 bacteriophage gene 32: a structural protein in the replication and recombination of DNA. Nature 227:1313–1318

Alberts BM, Morris CF, Mace D, Sinha N, Bittner M, Moran L (1975) Reconstruction of the bacteriophage T4 replication apparatus from purified components. In: Goulian M, Fox CF (eds) DNA synthesis and its regulation. WA Benjamin Inc, Menlo Park, CA, pp 241–269

Albright LM, Geiduschek EP (1986) Topoisomerization of plasmid DNA in Escherichia coli infected with bacteriophage T4. J Mol Biol 190:329–341

Belin D, Mudd EA, Prentki P, Yu Y-Y, Krisch HM (1987) Sense and antisense transcription of bacteriophage T4 gene 32. J Mol Biol 194:231–243

Bolle A, Epstein RH, Salser W, Geiduschek EP (1968) Transcription during bacteriophage T4 development: synthesis and relative stability of early and late transcripts. J Mol Biol 33:325–348

Borowiec JA, Gralla JD (1987) All three elements of the lac p^s promoter mediate its transcriptional response to DNA supercoiling. J Mol Biol 195:89–97

Brody E, Rabussay D, Hall D (1983) Regulation of transcription of prereplicative genes. In: Mathews CK, Kutter EM, Mosig G, Berget PB (eds) Bacteriophage T4. American Society for Microbiology, Washington, DC, pp 174–183

Casadaban MJ, Chou J, Cohen SN (1980) *In vitro* gene fusions that join an enzymatically active β-galactosidase segment to amino-terminal fragments of exogenous proteins: *Escherichia coli* plasmid vectors for the detection and cloning of translation initiation signals. J Bacteriol 143:971–980

Champe SP, Benzer S (1962) Reversal of mutant phenotypes by 5-fluorouracil: an approach to nucleotide sequences in messenger RNA. Proc Natl Acad Sci USA 48:532–546

Daegelen P, D'Aubenton-Carafa Y, Brody E (1982) The role of rho in bacteriophage T4 development. Virology 117:105–120

Frey J, Mudd EA, Krisch HM (1988) A bacteriophage T4 expression cassette that functions efficiently in a wide range of Gram-negative bacteria. Gene 62:237–247

Goldblum K, Apirion D (1981) Inactivation of the ribonucleic acid-processing enzyme ribonuclease E blocks cell division. J Bacteriol 146:128–132

Gorski K, Roch J-M, Prentki P, Krisch HM (1985) The stability of bacteriophage T4 gene 32 mRNA: a 5′ leader sequence that can stabilize mRNA transcripts. Cell 43:461–469

Guild N, Gayle M, Sweeney R, Hollingsworth T, Modeer T, Gold L (1988) Transcriptional activation of bacteriophage T4 middle promoters by the *mot*A protein. J Mol Biol 199:241–258

Guo P, Erickson S, Anderson D (1986) A small viral RNA is required for *in vitro* packaging of bacteriophage φ29 RNA. Science 236:690–694

Hahn S, Kruse U, Rüger W (1986) The region of T4 genes 34, 33, and 59: primary structure and organization of the genome. Nucleic Acids Res 14:9311–9327

Horvitz HR (1973) Polypeptide bound to host RNA polymerase is specified by T4 control gene 33. Nature New Biol 244:137–140

Inouye M, Delihas N (1988) Small RNAs in prokaryotes: a growing list of diverse roles. Cell 53:5–7

Khandjian EW (1986) UV crosslinking of RNA to nylon membranes enhances hybridization signals. Mol Biol Rep 11:107–115

King TC, Sirdemukh R, Schlessinger D (1986) Nucleolytic processing of ribonucleic acid transcripts in prokaryotes. Microbiol Rev 50:428–451

Krisch HM, Van Houwe G (1976) Stimulation of the synthesis of bacteriophage T4 gene 32 protein by ultraviolet light irradiation. J Mol Biol 108:67–81

Krisch HM, Selzer GB (1981) Construction and properties of a recombinant plasmid containing gene 32 of bacteriophage T4. J Mol Biol 148:199–218

Krisch HM, Allet B (1982) Nucleotide sequence involved in bacteriophage T4 gene 32 translational self-regulation. Proc Natl Acad Sci USA 79:4937–4941

Krisch HM, Bolle A, Epstein RH (1974) Regulation of the synthesis of bacteriophage T4 gene 32 protein. J Mol Biol 88:89–104

Krisch HM, Van Houwe G, Belin D, Gibbs W, Epstein RH (1977) Regulation of expression of bacteriophage T4 genes 32 and 43. Virology 78:87–98

Lemaire G, Gold L, Yarus M (1978) Autogenous translational repression of bacteriophage T4 gene 32 in vitro. J Mol Biol 126:73–90

Liebig H-D, Rüger W (1989) T4 early promoter regions: consensus sequences of promoters and ribosome binding sites. J Mol Biol, in press

Maniatis T, Fritsch EF, Sambrook J (1982) Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York

Mathews CK, Allen JR (1983) DNA precursor biosynthesis. In: Mathews CK, Kutter EM, Mosig G, Berget PB (eds) Bacteriophage T4. American Society for Microbiology, Washington, DC, pp 59–70

Mattson T, Richardson J, Goodin D (1974) Mutant of bacteriophage T4D affecting expression of many early genes. Nature 250:48–50

Mattson T, van Houwe G, Epstein RH (1978) Isolation and characterization of conditional lethal mutations in the mot gene of bacteriophage T4. J Mol Biol 126:551–570

McClure WR (1985) Mechanism and control of transcription initiation in prokaryotes. Annu Rev Biochem 54:171–204

McMaster GK, Carmichael GG (1977) Analysis of single and double-stranded nucleic acids on polyacrylamide and agarose gels

using glyoxal and acridine orange. Proc Natl Acad Sci USA 74:4835–4839

McPheeters DS, Stormo GD, Gold L (1988) Autogenous regulatory site on the bacteriophage T4 gene 32 messenger RNA. J Mol Biol 201:517–535

Melton DA, Krieg PA, Rebagliati MR, Maniatis T, Zinn K, Green MR (1984) Efficient *in vitro* synthesis of biologically active RNA and RNA hybridization probes from plasmids containing a bacteriophage SP6 promoter. Nucleic Acids Res 12:7035–7056

Menzel R, Gellert M (1983) Regulation of the genes for *E. coli* DNA gyrase: homeostatic control of DNA supercoiling. Cell 34:105–113

Mosig G (1974) On the role of E. coli DNA polymerase I and of T4 gene 32 in recombination. In: Grell RF (ed) Mechanisms in Recombination. Plenum Press, New York, pp 29–30

Mudd EA, Prentki P, Belin D, Krisch HM (1988) Processing of unstable bacteriophage T4 gene 32 species into a stable species requires Escherichia coli ribonuclease E. EMBO J 7:3601–3607

Newbury SF, Smith NH, Robinson EC, Hiles ID, Higgins CF (1987) Stabilization of translationally active mRNA by prokaryotic REP sequences. Cell 48:297–310

Orsini G, Brody EN (1988) Phage T4 codes for two distinct 10-kd proteins which strongly bind to RNA polymerase. Virology 162:397–405

Platt T (1986) Transcription termination and the regulation of gene expression. Annu Rev Biochem 55:339–372

Rabussay D (1983) Phage invoked changes in RNA polymerase. In: Mathews CK, Kutter EM, Mosig G, Berget PB (eds) Bacteriophage T4. American Society for Microbiology, Washington, DC, pp 167–173

Russel M, Gold L, Morrissett H, O'Farrell PZ (1976) Translational, atuogenous regulation of gene 32 expression during bacteriophage T4 regulation. J Biol Chem 251:7263–7270

Sanzey B (1979) Modulation of gene expression by drugs affecting deoxyribonucleic acid gyrase. J Bacteriol 138:40–47

Sinden RR, Pettijohn DE (1982) Torsional tension in intracellular bacteriophage T4 DNA. Evidence that a linear DNA duplex can be supercoiled in vivo. J Mol Biol 162:659–677

Snustad DP, Snyder L, Kutter E (1983) Effects on host genome structure and expression. In: Mathews CK, Kutter EM, Mosig G, Berget PB (eds) Bacteriophage T4. American Society for Microbiology, Washington, DC, pp 40–55

Tiercy J-M, Gorski J, Jeannet M, Mach B (1988) Identification and distribution of three serologically undetected alleles of HLA-DR by oligonucleotide·DNA typing analysis. Proc Natl Acad Sci USA 85:198–202

Tomcsànyi T, Apirion D (1985) Processing enzyme ribonuclease E specifically cleaves RNA I an inhibitor of primer formation in plasmid DNA synthesis. J Mol Biol 185:713–720

Uzan M, d'Aubenton-Carafa Y, Favre R, de Franciscis V, Brody E (1985) The T4 mot protein functions as part of a pre-replicative DNA-protein complex. J Biol Chem 260:633–649

Williams KP, Müller R, Rüger W, Geiduschek EP (1989) Overproduced bacteriophage T4 gene 33 protein binds RNA polymerase. J Bacteriol 171:3579–3582

Wu R, Ma F-J, Yeh Y-C (1972) Suppression of DNA-arrested synthesis in mutants defective in gene 59 of bacteriophage T4. Virology 47:147–156

Young ET, Menard RC (1981) Sizes of bacteriophage T4 early mRNA's separated by preparative polyacrylamide gel electrophoresis and identified by in vitro translation and hybridization to recombinant T4 plasmids. J Virol 40:772–789

Communicated by H. Hennecke

Received April 24, 1989