
Control sites in the sequence at the beginning of T7 gene 1

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ABSTRACT

The DNA sequence of the fragment Hind.30, 378 bases long, from the beginning of gene 1 of T7 is presented. It contains the C promoter, two in vitro transcriptional terminator sites and a sequence of 171 bases which probably codes for the N terminus of the T7 RNA polymerase. The sequence also codes for the RNase III cleavage site before gene 1. This overlaps with the transcriptional terminators. The RNA transcript of the sequence about the terminators can be arranged in a set of alternative double-stranded hairpin structures. It is suggested that conversion between these structures may have a role in termination; this may be influenced by interactions with ribosomes and RNase III. The region of the C promoter between genes 0.7 and 1 thus contains several sites which may be involved in the control of transcription and translation.

INTRODUCTION

The bacteriophage T7 has been extensively studied and reviewed (1,2). The early region, transcribed by the host RNA polymerase is located at the conventional left-hand end of the genetic and physical maps and extends from about position 1 to position 20 containing five structural genes 0.3, 0.7, 1, 1.1 and 1.3 (3,4). Gene 1 codes for the T7 RNA polymerase (5) and the region at the beginning of this gene which is at position 8 approximately is of considerable interest. It contains a promoter called C (6,7), an RNase III cleavage site (8) and possibly a terminator. Rho-dependent (9) and rho-independent termination (6) have been observed here in vitro, and there is also evidence for termination in this region from in vivo studies (10).

These observations suggested that this region might play a complex role in modulating the expression of the T7 early genes. In this paper we report the DNA sequence between genes 0.7 and gene 1. Several control sites can be identified in this sequence as well as the code for the first 57 amino acids of the T7 RNA polymerase. Two transcriptional terminators overlap with the

code for the RNase III cleavage site between genes 0.7 and gene 1, which in turn overlaps with the C promoter. In addition there is a potential ribosome binding site sequence just before the RNase III cleavage site. The juxtaposition of these sequences suggests various functional interactions may occur between transcriptional and translational systems during the expression of sequences from this region.

MATERIALS AND METHODS.

Methods for the growth of T7 and the purification of T7 DNA have been previously described (11). Restriction digests were carried out as described before (7). DNA sequencing was performed according to Maxam and Gilbert (12). Restriction enzymes Hind II, Hpa I and II, Hae III, Tag I and Tha I were prepared as described by Humphries et al. (13) and McConnell et al. (14). E.coli RNA polymerase and T4 polynucleotide kinase were kind gifts of R.B. Simpson and A.M. Maxam respectively. RNA synthesis on purified restriction fragments was carried out as described by McConnell (7).

Nomenclature: Restriction fragments are named as described by Gordon et al. (15). For example, Hind II.30 is the fragment in band 30 after electrophoresis on a polyacrylamide gel of a Hind II digest of T7. This is shortened for convenience to Hind.30. Digestion of Hind.30 by for example Hpa II gives three fragments and these are called Hind.30. Hpa A, Hind.30. Hpa B, and Hind.30.Hpa C in decreasing order of size. Digestion of Hind.30.Hpa.B by Hae III gives two fragments called Hind.30.Hpa.B.Hae.A. and Hind.30.Hpa.B.Hae.B.

RESULTS.

(a) The T7 cleavage map at the beginning of gene 1.

The cleavage map of the left hand end of T7 for the enzymes Hind II, Hpa I and II, and Hae III has been described previously (15), and the part of it at the beginning of gene 1 is shown in Figure 1. The map is based on the sizes of the restriction fragments and on the changes in the fragment patterns caused by deletions C5, C42, C63, C93 and D111 (3,4,16,17). These deletions have been analysed in detail. None of these affects the T7 gene 1 polypeptide although C5 deletes the RNase III cleavage site which lies just to the left of gene 1 (3,4,16), and the Hae III site between Hae 31 and Hae 48 (15). The C5 deletion extends farther to the right than any of the others in this group according to biochemical studies, electron microscopy and

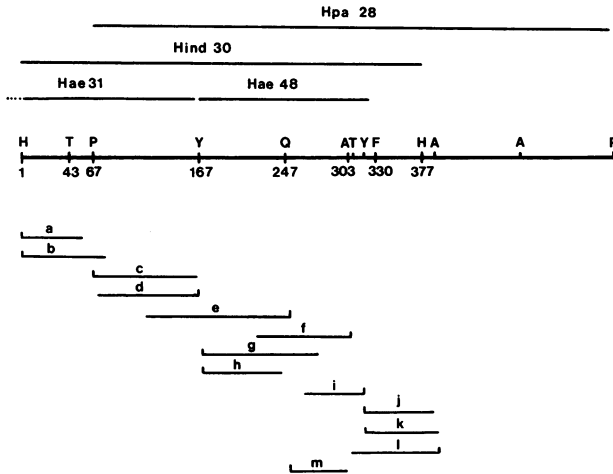


FIGURE 1. Cleavage map of the DNA at the beginning of gene 1 and the extent of sequences obtained.

The cleavage map taken from McConnell (7) extends from the left end of Hind.30 to the right end of Hpa.28. The positions of Hae 31 and Hae 48 are also shown. Cleavage sites for the enzymes Hind II, Tha I, Hpa II, Hae III, Taq I, Alu I and Hinf I are denoted by H, T, P, Y, Q, A and F respectively. Sequencing runs were carried out on fragments:

- | | |
|--------------------------|--------------------------|
| (a) Hind.30.Hae.A.Hpa.B. | (b) Hind.30.Taq.A.Hae.A. |
| (c) Hpa.28.Hae.C. | (d) Hind.30.Hae.A.Hpa.A. |
| (e) Hind.30.Taq.A.Hpa.A. | (f) Hpa.28.Alu.A.Hae.A. |
| (g) Hpa.28.Hae.B.Alu.A. | (h) Hpa.28.Hae.B.Taq.A. |
| (i) Hpa.28.Hae.B.Taq.B. | (j) Hpa.28.Hae.A.Alu.A. |
| (k) Hpa.28.Hae.A.Hind.B. | (l) Hind.30.Taq.B.Alu.A. |
| (m) Hind.30.Taq.B.Alu.B. | |

These fragments had one 5' end labelled with ^{32}P as indicated in the figure. The extent of the sequence data obtained for each is shown. The labelled end has a vertical bar.

restriction analysis (4,15,16). It has been established that the Hpa I and II fragment 28, the Hind II fragment 30 and the Hae III fragments 31 and 48 lie at the beginning of gene 1 (15).

(b) DNA sequences.

The cleavage map (Figure 1) provided the basic data needed for sequencing this region. Thirteen sequencing runs provided the sequence between the two Hind II sites, a distance of 378 bases. The relationship of these sequences to each other and to the cleavage map are shown in Figure 1. A representative

sequencing gel is shown in Figure 2 and the complete sequence is in Figure 3, All, except the sequence 1-67 has been confirmed by sequencing both strands of the DNA. The data from the two strands have been complementary except in one case. The G at position 355 is clearly present in sequencing gels for Hpa.28.Hae.A.Alu.A. and Hpa.28.Hae.A.Hind.B. but the complementary C is not distinguishable in the sequencing gel for Hind.30.Taq.B.Alu.A. The next largest fragment in this gel is intensely labelled but at present there is no explanation for these observations. The complementary base to the G may be modified.

The sequence 1-67 is not firmly established. Although it has been sequenced twice from the left hand end, the complementary sequence was not obtained. The purines are probably correct but there is doubt about the pyrimidines denoted by Y.

(c) Transcription in vitro from Hpa.28 and Hind.30.

The sequence contains the C promoter (6,7) and fragments Hind.30 and Hpa.28 act as efficient templates for RNA synthesis by E.coli RNA polymerase in vitro (7). A feature of transcription from these fragments is the appearance of partial transcripts in relatively large amounts (7). This is illustrated in Figure 4.

Two fragments, Hpa.28 carrying the C promoter and Hpa.25 carrying the A1 and D promoters, were used in separate reactions as templates for RNA synthesis as described in the legend to Figure 4. The reactions were at 37°C for 90 minutes in the presence of γ -³²P labelled ATP. At the end of the reaction the RNA was precipitated, dissolved and electrophoresed on a 12% polyacrylamide gel containing 7 M urea. The autoradiograph (Figure 4) shows that the RNA made from each template falls into two sets of discrete size classes. The major labelled products on Hpa.25 are the transcripts from the A1 promoter which is known to initiate with A (8) and to be located 39 base pairs from the end of the Hpa.25 fragment (18). Most of these transcripts terminate at a series of 6 positions marked A at the end of the molecule. The series is seen more easily on a shorter exposure. A major band labelled B may represent partial transcripts of length about 27 nucleotides which initiate at the A1 promoter and terminate prematurely before the end of Hpa.25. Most transcripts from A1 proceed past this site, as is clearly evident from the relative intensities of the bands at A and B. The transcripts from the D promoter on Hpa.25 are not labelled since they initiate with GTP (19).

The transcripts of Hpa.28 are distributed differently. The great majority are short molecules of two size classes 26 and 30 nucleotides long. These

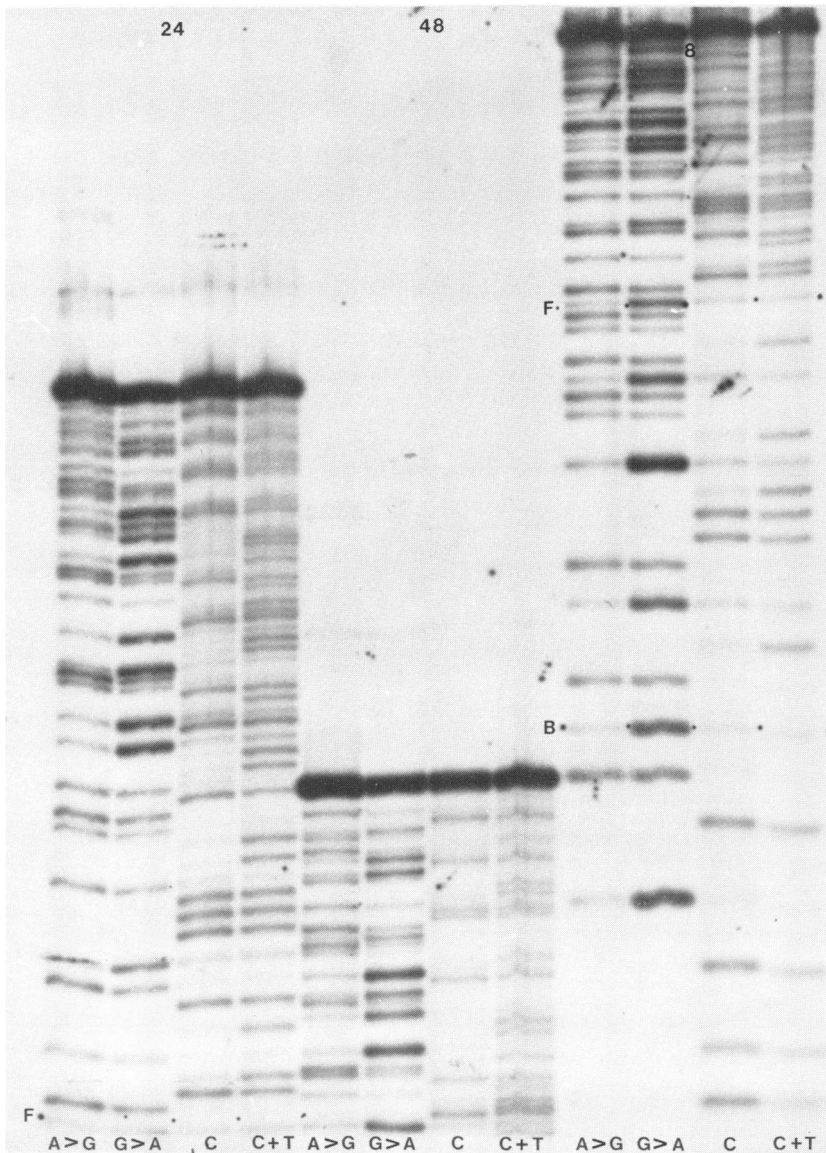


FIGURE 2. DNA sequencing gel of Hind.30.Hae.A.Hpa.A.

The products of cleavages at A G, G A, C and C + T were each electrophoresed in three different channels for 48 hours, 24 hours and 8 hours at 1000 V on a 40 x 40 x 0.15 cm 20% polyacrylamide gel. The positions of the dyes xylene cyanol FF and bromophenol blue are shown by F and B respectively.

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R A C A Y G Y T C T C T G G G G A C C T T A A G G Y G C C T
                                                                30
G A G G A A C G A A T C G C G C C G C A Y Y G G Y G Y Y A A
                                                                60
Y G Y Y G A C C G G A T G G C T A T C G C T A A T G G T C T
                                                                90
T A C G C T C A A C A T T G A T A A G C A A C T T G A C G C
                                                                120
A A T G T T A A T G G G C T G A T A G T C T T A T C T T A C
                                                                150
A G G T C A T C T G C G G G T G G C C T G A A T A G G T A C
                                                                180
G A T T T A C T A A C T G G A A G A G G C A C T A A A T G A
                                                                210
A C A C G A T T A A C A T C G C T A A G A A C G A C T T C T
                                                                240
C T G A C A T C G A A C T G G C T G C T A T C C C G T T C A
                                                                270
A C A C T C T G G C T G A C C A T T A C G G T G A G C G T T
                                                                300
T A G C T C G C G A A C A G T T G G C C C T T G A G C A T G
                                                                330
A G T C T T A C G A G A T G G G T G A A G C A C G C T T C C
                                                                360
G C A A G A T G T T T G A G C G T C

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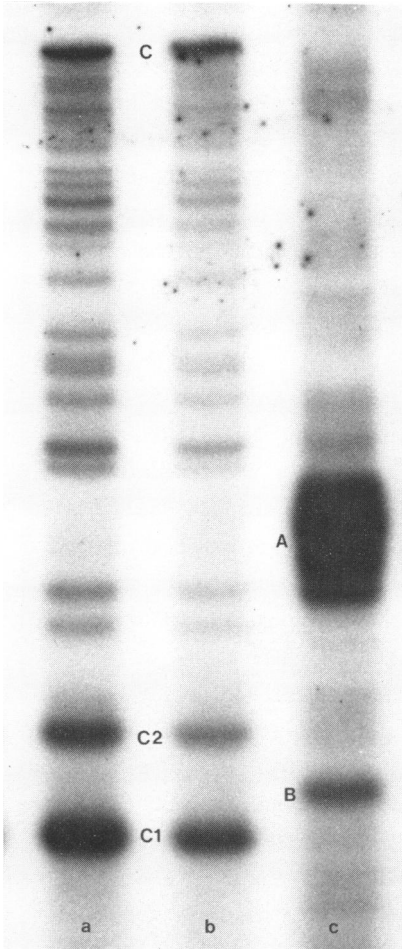
FIGURE 3. The sequence of Hind.30.

The sequence is that of the left strand of Hind.30. According to the standard nomenclature for T7 DNA, the left strand is that which would be transcribed leftwards when the early region of T7 is at the left hand end (1,2). There is some doubt about the pyrimidine residues labelled as Y in the first 67 bases.

are indicated by the letters C1 and C2. Full length transcripts of Hpa.28 initiated at C are about 380 nucleotides long and these are indicated by C. Many other size classes are seen but most of these occur in small amounts.

The partial transcripts could be considered as due to termination or pausing. A paused RNA polymerase has not dissociated from the DNA and is capable of continuing to extend the partial transcript after a delay. This is not however the case in this system, since the incubation was carried out for a sufficiently long period (90 minutes) to allow a paused molecule to begin synthesis. Moreover in a different experiment the amount of the partial transcripts was not changed if heparin or rifampicin was added and the incub-

FIGURE 4. RNA synthesised on Hpa.25
and Hpa.28.



RNA was synthesised at 37°C for 90 minutes in reactions (10 ul) containing 0.15 M KCl, 0.01 M MgCl₂, 0.01 M tris pH 7.9, 0.1 mM EDTA, 0.1 mM dithiothreitol, 12.5 uM ATP, UTP, CTP and GTP, 1 ug *E. coli* RNA polymerase, and DNA fragments Hpa.28 (a and b) or Hpa.25 (c). After ethanol precipitation the samples were dissolved in 7 M urea and electrophoresed on a 12% polyacrylamide slab gel (20 x 20 x 0.15 cm) at 300 V for 4 hours. The reservoir buffer was 0.05 M tris borate pH 8.3, 1 mM EDTA; the gel buffer contained in addition 7 M urea. The label was γ ³²P ATP. The figure shows an autoradiograph of the gel.

ation continued for 20 minutes. The conclusion is that under the conditions of this experiment RNA polymerase terminates transcription at two positions 26 and 30 base pairs from the C promoter at relatively high frequencies.

DISCUSSION.

(a) Location of the sequence on the T7 map.

The sequence in Figure 3 can be located relative to the genetic map of T7 by making use of the known effects of the deletions C5, C42, C63 and C93 on the pattern of early gene expression (3,4,16) and on the pattern of restriction fragments (15).

The deletions C5, C42, C63 and C93 have right hand end points in the region close to the end of gene 0.7. C5 extends farthest to the right because it is the only one which eliminates the Hae III cleavage site at position 167. C63 eliminates the Hpa II site at position 67 and so must at least enter this site. C42 eliminates the Hind II site at the beginning of the sequence. Of these deletions only C5 which extends the farthest to the right eliminates the RNase III cleavage site sequence which lies between gene 0.7 and gene 1 (4,8). A polypeptide of a size corresponding to the normal T7 RNA polymerase coded by gene 1 is present after infection by C5 (16) so that C5 apparently does not delete any of the T7 RNA polymerase structural gene.

The left hand end of the fragment Hae.48 has been mapped to the position 7.88 on the T7 physical map (15). This is the Hae III site at 167 in the sequence in Figure 1 and it is eliminated by C5. The beginning of gene 1 has been mapped to position 8 on the T7 physical map (16) and this is not affected by C5. Although these physical positions are subject to errors they suggest that the beginning of gene 1 lies about 50 base pairs to the right of the Hae III site at 167, that is at or about position 217 (Figure 3).

(b) Transcriptional signals: promoters and terminators.

There have been several reports that RNA polymerase initiates RNA synthesis at a promoter close to the beginning of gene 1 (6,20,21) and this has been confirmed by direct sequence analysis of the DNA in this region and of RNA made in vitro from restriction fragments Hpa.28 and Hind.30 (7). This RNA is initiated close to a sequence which resembles other promoters for E.coli RNA polymerase between positions 112 and 149 in Figure 3 and RNA synthesis initiates at the A at 149 (7) in the sequence TACA as predicted by Minkley and Pribnow (6). This sequence 112-149 is the C promoter.

There have also been reports that RNA polymerase terminates at the beginning of gene 1. Minkley and Pribnow (6) observed termination in this region in the presence of rifampicin and in the absence of rho factor. This has been confirmed by Stahl and Chamberlin (21) who have suggested that it is caused by the presence of inactivated RNA polymerase - rifampicin complexes at the C promoter, and there is other evidence for this proposal (22,23,24).

On the other hand there is evidence that termination may occur in this region in the absence of rifampicin. The relative amounts and decay rates of the early gene products have been measured in vivo (10). The results suggested that one half of the RNA polymerases which transcribe gene 0.7 terminate before gene 1. Experiments carried out in vitro showed that

at high ionic strength and at high concentrations of rho termination occurred at the beginning of gene 1 at about the same frequency as suggested for the *in vivo* situation (9,25). In this study site-specific rho-independent termination has been observed *in vitro* when Hpa.28 is transcribed (Figure 4).

One possible explanation of termination distal to the C promoter is that it is caused by secondary structure of the RNA which has been transcribed from the DNA preceding the termination sites. The first 38 bases of RNA initiated at C can be arranged in a variety of secondary structures (Figure 5). There are three pairs of related complementary sequences which can be arranged to form one, two or three hairpins. These sequences or their complements in the DNA might undergo structural rearrangements during transcription of this region. Complex secondary structures have been suggested

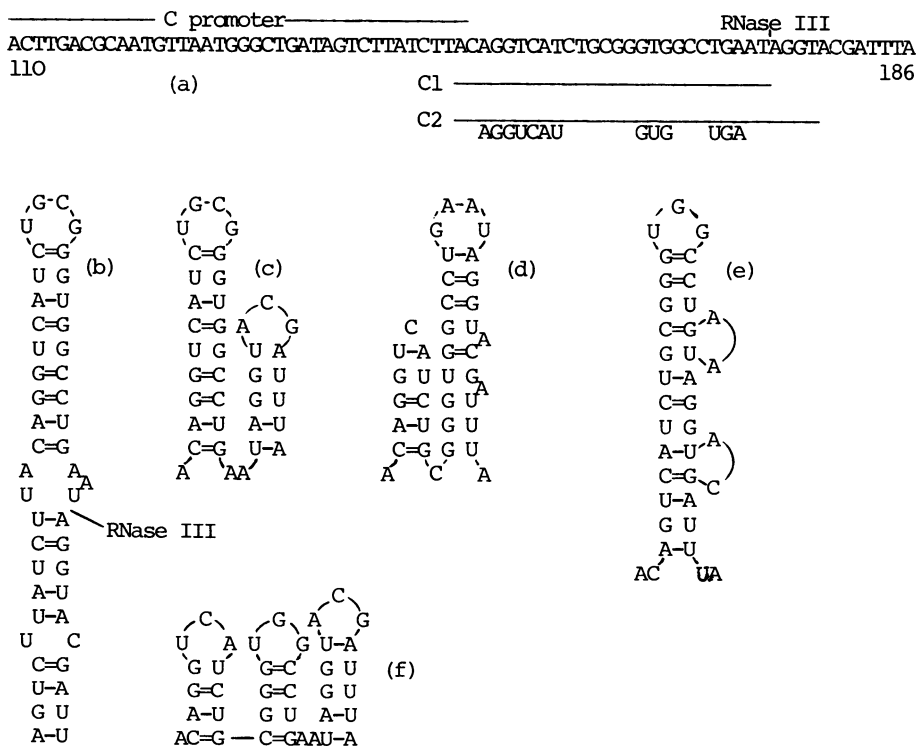


FIGURE 5. Sequences near the C promoter before gene 1. (a) Location of the C promoter RNase III site and terminators at the ends of C1 and C2 transcripts. Sequence resembling a ribosome binding site, a GUG and UGA are indicated under C2. (b) Sequence around the RNase III site. (c,d,e,f) Sequence of the transcript from the C promoter - various arrangements.

as important in other systems, for example, in the trp and phe attenuators (26-28) and preceding a rho-dependent termination site to the right of λp_R (29).

(c) The RNase III cleavage site.

An RNase III cleavage site should be coded in the sequence in Figure 3. This prediction follows from observations of two kinds, the physical limits of certain deletions and the effects of the same deletions on this RNase III cleavage site. For example, from cleavage maps for Hind II, Hpa II and Hae III the right hand ends of the deletions C93, C42, C63 and C5 lie respectively to the left of position 1, between 1 and 67, between 67 and 167, and between 167 and 318 relative to the sequence in Figure 3 (15). However gene 1 mRNA is present after infection by C93, C42 or C63 (4,16) so that a complete RNase III recognition site must be intact in each of these mutants and must therefore be coded in the sequence to the right of 67 (Figure 3). However gene 1 mRNA is not observed in C5, so that it must remove an essential part of the RNase III sequence to the left of gene 1. The best estimate of the end point of C5 is at position 215 with an error of about ± 50 base pairs (15). Taking the most extreme positions the data predict that critical elements of an RNase III site lie in the sequence coded between 67 and 267.

The RNase III cleavage site between gene 0.7 and gene 1 mRNAs has been sequenced (J. Dunn and W.F. Studier, personal communication). The 53 base RNA sequence determined around the site corresponds exactly to the sequence 135 - 187 inclusive in Figure 3. RNase III cleaves the RNA transcript between nucleotides corresponding to 174 and 175 as shown in Figure 5. It has previously been reported that the 3' end sequence of gene 0.7 mRNA was $C_{2-3}UUUUAU_{OH}$ (30). This sequence does not appear in the sequence in Figure 3, nor are there any sequences resembling it and we assume some error arose in the earlier study.

The RNA transcript of Hind.30 initiated at the C promoter is not cleaved by RNase III (J. Dunn and W.F. Studier, personal communication). This is explained because sequences coded to the left of the C promoter contribute to the RNase III cleavage site. This observation raises the possibility that the gene 1 mRNA sequences initiated at the C promoter may have different properties (for example, in half life, or frequency of translation) from the same sequences cleaved by RNase III from the large precursor molecules initiated at the A1 A2 or A3 promoters. Gene 1 mRNA sequences initiated at C would have an extra 26 nucleotides on the 5' end compared to the gene 1 mRNAs cleaved from the large precursor molecules.

(d) Translational signals: the beginning of T7 gene 1.

The beginning of T7 gene 1 must lie to the right of the RNase III cleavage site located at 174-175 in the sequence in Figure 3. There are two possible sites the ATGs beginning at 207 and 366. Each is preceded by a sequence resembling a ribosome binding site (193-200 and 354-349) and each is followed by a sequence without any stop codons in phase.

There are a number of reasons for believing that the ATG at 207 is the beginning of gene 1. It is close to position 217 predicted by the physical map (15,16). The sequence of the ATG at 207 and the preceding potential ribosome binding site sequence are separated by 6 bases which is common (31) whereas the ATG at position 366 is separated from its putative ribosome binding site coding sequence by 16 bases. The longest reported separation between a verified ribosome binding site and an AUG or GUG is 11 (31). Finally the N terminal amino acid sequence of met - asn - thr predicted if the start is at 207 is in agreement with preliminary sequencing data on the T7 gene 1 protein (J. Oakley, personal communication).

The sequence of the first 57 amino acids of the T7 RNA polymerase is expected to be as given by the DNA sequence starting at position 207.

(e) RNase III sites, transcriptional terminators and possible ribosome binding sites are coded by overlapping sequences.

It has been noted before that RNase III sites and weak transcriptional termination sites may be coded by sequences from the same region and it has been suggested that there may be a structural relationship between them (32). The data in this paper agree with this proposal. The RNase III cleavage site coding sequence preceding gene 1 mRNA at 174-175 corresponds to one transcriptional termination site (Figure 5) and precedes a second termination site by four nucleotides.

The sequence data of this paper and of J.Dunn and F.W. Studier (personal communication) also reveal that RNase III cleavage sites are associated with sequences which resemble ribosome binding sites. Five T7 RNase III sites are just distal to sequences resembling ribosome binding sites. For example, RNase III cuts at position 174-175 in the sequence in Figure 3. The sequence of 8 bases AGGUCAUC (151-158) has 7 which are complementary to the sequence at the 3' end of *E.coli* 16S rRNA which is involved in binding mRNA to ribosomes (31,33). For each of the RNase III sites there is a GUG just to the 3' side of the potential ribosome binding site which suggests that fmet tRNA might be involved in an interaction between ribosomes and these RNA sequences and that a small peptide might be synthesised.

Although a small peptide of two or three amino acids could be coded by the sequence between the GUG and the RNase III cleavage sites, there is no evidence that this is the functional significance of the juxtaposition of these sequences. A different reason for the relationship is suggested by reports showing that the rate of transcription is affected by elements of the translational system (reviewed in 34,35). In the case of the trp operon there is very strong in vivo evidence that translation modulates the frequency of termination at the attenuator (27,28) and the sequence data suggest similar interactions in the his and phe leader sequences (36,37). In addition it has been known from several lines of evidence that transcriptional terminators suppressible by suA are "revealed" by polar mutations affecting the translation of the preceding sequence (reviewed in 35).

These known interactions between transcriptional termination and translation suggest that interactions at ribosome binding sites on the RNA before RNase III cleavage sites could modulate the rate of transcriptional termination in the DNA sequences which code for the region around the RNase III site.

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