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The isolation and characterization of bacteriophage T7 messenger RNA fragments containing an RNase III cleavage site

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ABSTRACT

We have isolated overlapping RNA fragments which contain the region surrounding the ribonuclease III cleavage site between bacteriophage T7 genes 0.3 and 0.7. Although all of these fragments contain the site of cleavage, only certain fragments are correctly recognized and cleaved by RNase III. Analysis of the cleavage products of the fragments indicates that the enzyme produces a single endonucleolytic break at this site in the T7 early RNA precursor molecule. In addition, the 3'-terminal adenylic acid residues observed previously on the in vivo T7 early RNA species were not found in these fragments and, therefore, must represent a post-transcriptional, post-processing modification of the RNA.

INTRODUCTION

When bacteriophage T7 infects Escherichia coli, the host RNA polymerase transcribes the leftmost 20% of the phage DNA (1-3) which is referred to as the early region. The resulting polycistronic RNA (with a molecular weight of about $2.2X10^6$) is cleaved into monocistronic mRNAs by a host-specified endonuclease, ribonuclease III (4,5). We have previously shown that RNase III is the only enzyme involved in the processing and that the resulting 5' termini are identical to each other as are the 3' termini (6,7). The fact that these sequences have been conserved within the T7 cleavage sites indicates that sequence specificity may be important in the processing event. In addition, the observation that RNase III has a strong specificity for double-stranded RNA molecules (8,9,10) suggests that the T7 early RNA precursor might contain specific double-stranded regions which serve as sites of recognition and cleavage by the enzyme. When Ginsburg and Steitz (20) examined the RNase III cleavage products from the E. coli 30S ribosomal RNA precursor, they found terminal sequences which differed from those of the T7 early RNAs and postulated that the identity of the T7 termini might not reflect a general property of RNase III sites. Thus, the exact requirements for RNase III cleavage of a natural substrate remained

unclear.

To approach this problem, we undertook the isolation and sequence analysis of RNA fragments derived from a region which contains a single RNase III cleavage site in the T7 early RNA precursor. This analysis should 1) determine whether the cleavage event involves hydrolysis of a single phosphodiester bond between directly adjacent messages on the precursor RNA or the excision of an oligonucleotide fragment as a result of two or more breaks, 2) help clarify the role of RNA secondary structure in substrate recognition and action by the enzyme, and 3) determine if the oligoadenylate residues found on the 3' ends of the *in vivo* T7 early messages (6) are encoded in the DNA template.

Here we report the isolation of three overlapping RNA fragments of different lengths, each of which contains the site of RNase III cleavage in the intercistronic region between the T7 early genes 0.3 and 0.7 (2,3). Analysis of the fragments following RNase III cleavage indicates that the enzyme produces a single endonucleolytic break between the 3' end of gene 0.3 and the 5' end of gene 0.7. In addition, the different fragments were used to estimate the minimum structural information required for cleavage. Sequence analysis of the fragments also indicates that the additional 3'-terminal adenosine residues are not encoded in the DNA. The complete nucleotide sequence surrounding the cleavage site will be presented elsewhere.

MATERIALS AND METHODS

(a) Phage strains

T7 deletion mutants C116, C114, C74 and H3 (11) were provided by F.W. Studier. Phage stocks were prepared as described previously (6).

(b) Isolation of RNA fragments containing the RNase III cleavage site

T7 DNA and ^{32}P-labeled *in vitro* early RNA were prepared as described by Rosenberg *et al*. (7) except that rifampicin was not added to the transcription reaction. The hybridization procedure is similar to that of Bøvre and Szybalski (12) as modified by R. Musso and B. de Crombrugghe (personal communication).

DNA was denatured in 0.2N NaOH for 10 min. at room temperature, and the reaction stopped by the addition of 0.2 volumes of 1.0 N HCl. Concentrated SSC (1X=0.15M NaCl, 0.015M NaCitrate, pH7.0) at 4°C was then added to make the final solution 4XSSC (10 ml). The denatured DNA was collected on nitrocellulose filters (Schleicher and Schuell B-6) which were then washed with 4XSSC (100 ml). The filters were dried overnight at room

temperature and then in a vacuum oven at 80°C for 2 hours.

^{32}P-labeled RNA was hybridized to the filter-bound DNA in 2XSSC. Two successive 24 hour hybridizations were carried out, each using 0.5 mg DNA from the T7 deletion mutant C116. The filters were discarded and the supernatant fraction was divided into aliquots and hybridized separately against DNA (50 to 75 µg) from each of the T7 deletion mutants C74, C114, and H3 for 18 hours.

After the final hybridization, the filter-bound hybrids were treated with either 2.5 units/ml RNase T_1 or 0.4 µg/ml pancreatic RNase in 2XSSC (2 ml) for 30 min. at room temperature. The filters were then washed with 50 ml of 2XSSC and treated for 45 min. at 54°C with 0.15M Na Iodoacetic acid, 0.1M NaCl, pH 5.6 to inactivate the RNase. After washing again, the filters were incubated for 15 min. at 90°C in distilled water to elute the RNA. The eluate was adjusted to 25mM Tris-HCl, 10mM $MgCl_2$,pH 7.5. DNase I (Worthington, DPFF) was added at 50 µg/ml and the mixture incubated at 37°C for 5 min. The sample was then phenol extracted and the RNA precipitated by the addition of 2.5 volumes of cold ethanol.

(c) Oligonucleotide mapping of isolated RNA fragments

The isolated RNA fragments which contained the RNase III cleavage site were characterized by standard sequencing techniques (13). The RNA was digested with either T_1 or pancreatic RNase, and the resulting oligonucleotides fractionated in two dimensions. The first dimension was electrophoresis on Cellogel strips at pH 3.5; the second dimension was chromatography on thin layer plates of DEAE-cellulose (Analtech) using 30 min. hydrolyzed homochromatography C buffer (13).

(d) RNase III treatment of the isolated RNA fragments

RNase III digestion of the purified fragments containing the cleavage site was carried out as described previously (7). After incubation for 10 min. at 37°C, an equal volume of T_1 RNase (1 mg/ml) or pancreatic RNase (5 mg/ml) was added and the reaction continued for an additional 10 min. The oligonucleotides were then fractionated by the procedure described in the previous section.

RESULTS

(a) Isolation of RNA fragments containing an RNase III cleavage site

A two-step hybridization procedure was used to isolate several overlapping RNA fragments derived from the region surrounding the RNase III processing site between genes 0.3 and 0.7 of the T7 early RNA precursor. Figure 1 shows the map positions of the deleted regions of the DNAs used for the hybridization. ^{32}P-labeled *in vitro* transcript synthesized from

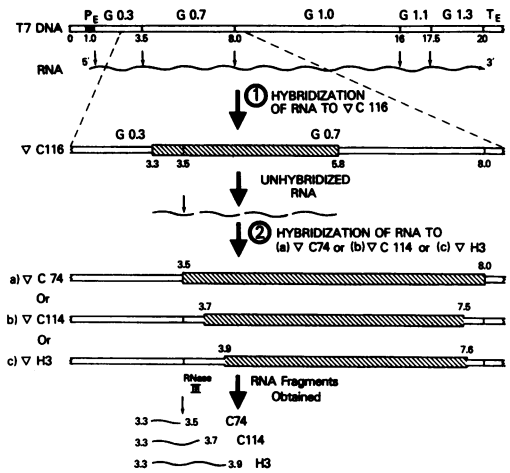


Figure 1. Isolation of RNA fragments containing an RNase III cleavage site. The T7 early RNA precursor is synthesized in vitro from wild type T7 DNA and hybridized on filters to the DNA from T7 deletion mutant C116. Under appropriate hybridized conditions (see Methods) the region of the transcript which corresponds to the deleted segment of DNA does not hybridize to the filter and remains in the supernatant portion of the hybridization mixture (step 1: unhybridized RNA). This RNA is then hybridized to DNA from one of the T7 deletion mutants C74, C114, or H3 (step 2) Only RNA derived from the leftmost segment of the C116 deletion will hybridized to these deletions. The filter bound hybrids are trimmed with ribonuclease (to remove unhybridized "tails") and subsequently eluted from the DNA. The RNA fragments obtained span the G0.3 - G0.7 boundary and contain a single RNase III cleavage site. All distances given are in T7 map units: 1 map unit $\approx$1% of T7 genome $\approx$ 380 base pairs. P_E, region of initiation for T7 early transcription; T_E, site of termination of T7 early transcription; ↓, an RNase III cleavage site; ▽, deletion mutant of T7.

wild-type T7 DNA was first hybridized to DNA from T7 deletion C116. The hybridization conditions (i.e. lengthy incubation at relatively high temperature) result in breakage of the RNA into fragments of average chain length greater than 100 nucleotides (M. Rosenberg, unpublished result). RNA fragments complementary to the deleted region (and therefore containing the gene 0.3-0.7 boundary and extending into gene 0.7) remained in the supernatant fraction while the remainder of the RNA hybridized to the filter-bound DNA. This supernatant fraction was subsequently hybridized to the DNA of another T7 deletion mutant (either C74, C114 or H3) in which the deleted region begins to the right of the 5' end of gene 0.7 and ends beyond the right end of the C116 deletion. In this step, the 0.3-0.7 boundary region hybridized to the DNA, while the segment extending into gene 0.7 did not and was removed by treatment with either T_1 or pancreatic ribonuclease. Choice of nuclease depended on which was to be used for

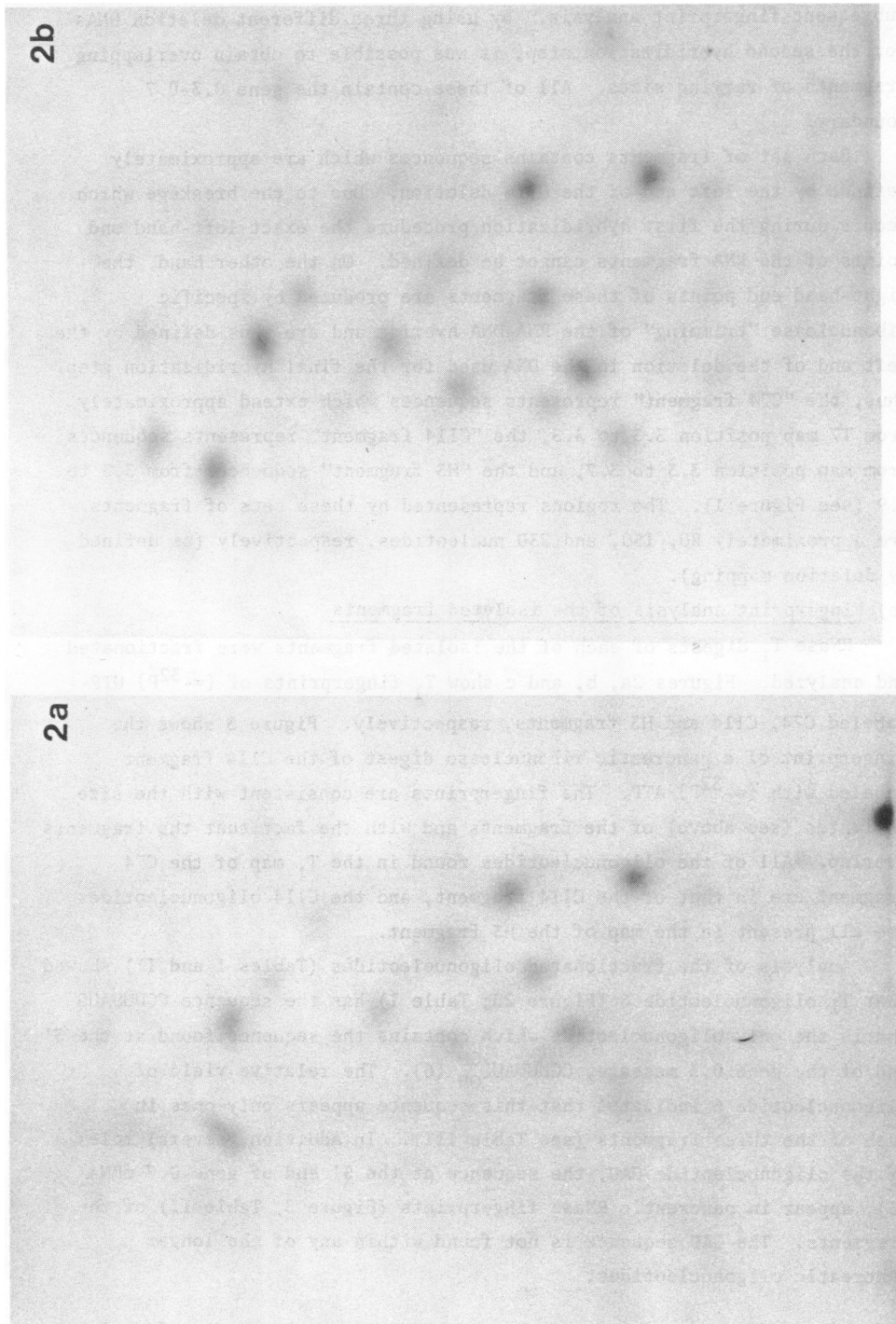
subsequent fingerprint analysis. By using three different deletion DNAs for the second hybridization step, it was possible to obtain overlapping fragments of varying sizes. All of these contain the gene 0.3-0.7 boundary.

Each set of fragments contains sequences which are approximately defined by the left end of the C116 deletion. Due to the breakage which occurs during the first hybridization procedure the exact left-hand end points of the RNA fragments cannot be defined. On the other hand, the right-hand end points of these fragments are produced by specific ribonuclease "trimming" of the RNA-DNA hybrids and are thus defined by the left end of the deletion in the DNA used for the final hybridization step. Thus, the "C74 fragment" represents sequences which extend approximately from T7 map position 3.3 to 3.5, the "C114 fragment" represents sequences from map position 3.3 to 3.7, and the "H3 fragment" sequences from 3.3 to 3.9 (see Figure 1). The regions represented by these sets of fragments are approximately 80, 150, and 230 nucleotides, respectively (as defined by deletion mapping).

(b) Fingerprint analysis of the isolated fragments

RNase T_1 digests of each of the isolated fragments were fractionated and analyzed. Figures 2a, b, and c show T_1 fingerprints of {α-^{32}P} UTP-labeled C74, C114 and H3 fragments, respectively. Figure 3 shows the fingerprint of a pancreatic ribonuclease digest of the C114 fragment labeled with {α-^{32}P} ATP. The fingerprints are consistent with the size estimates (see above) of the fragments and with the fact that the fragments overlap. All of the oligonucleotides found in the T_1 map of the C74 fragment are in that of the C114 fragment, and the C114 oligonucleotides are all present in the map of the H3 fragment.

Analysis of the fractionated oligonucleotides (Tables I and II) showed that T_1 oligonucleotide 6 (Figure 2d; Table 1) has the sequence CCUUUAUG and is the only oligonucleotide which contains the sequence found at the 3' end of the gene 0.3 message, CCUUUAU$\begin{smallmatrix}\text{OH}\\\text{OH}\end{smallmatrix}$ (6). The relative yield of oligonucleotide 6 indicates that this sequence appears only once in each of the three fragments (see Table III). In addition, several moles of the oligonucleotide GAU, the sequence at the 5' end of gene 0.7 mRNA (6), appear in pancreatic RNase fingerprints (Figure 3; Table II) of the fragments. The GAU sequence is not found within any of the longer pancreatic oligonucleotides.


2b
2a

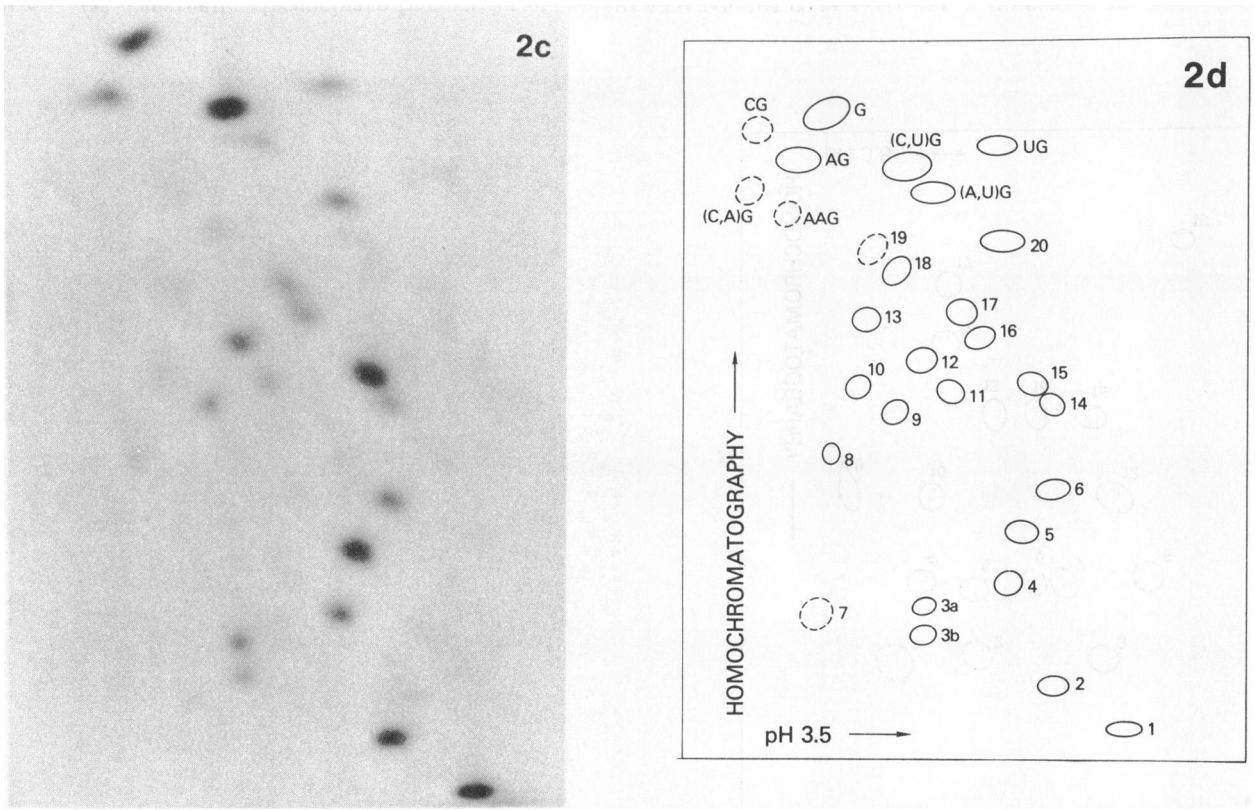


Figure 2. T_1 fingerprints of [α-^{32}P] UTP-labeled isolated RNA fragments. T_1 oligonucleotide maps were prepared as described in Materials and Methods. DNA from T7 deletion mutants (a) C74, (b) C114 or (c) H3 was used to obtain the different sized fragments as outlined in Results. A schematic representation of the fingerprints (d) gives the numbers assigned to the oligonucleotides including those not labeled by the [α-^{32}P] UTP (dotted lines).

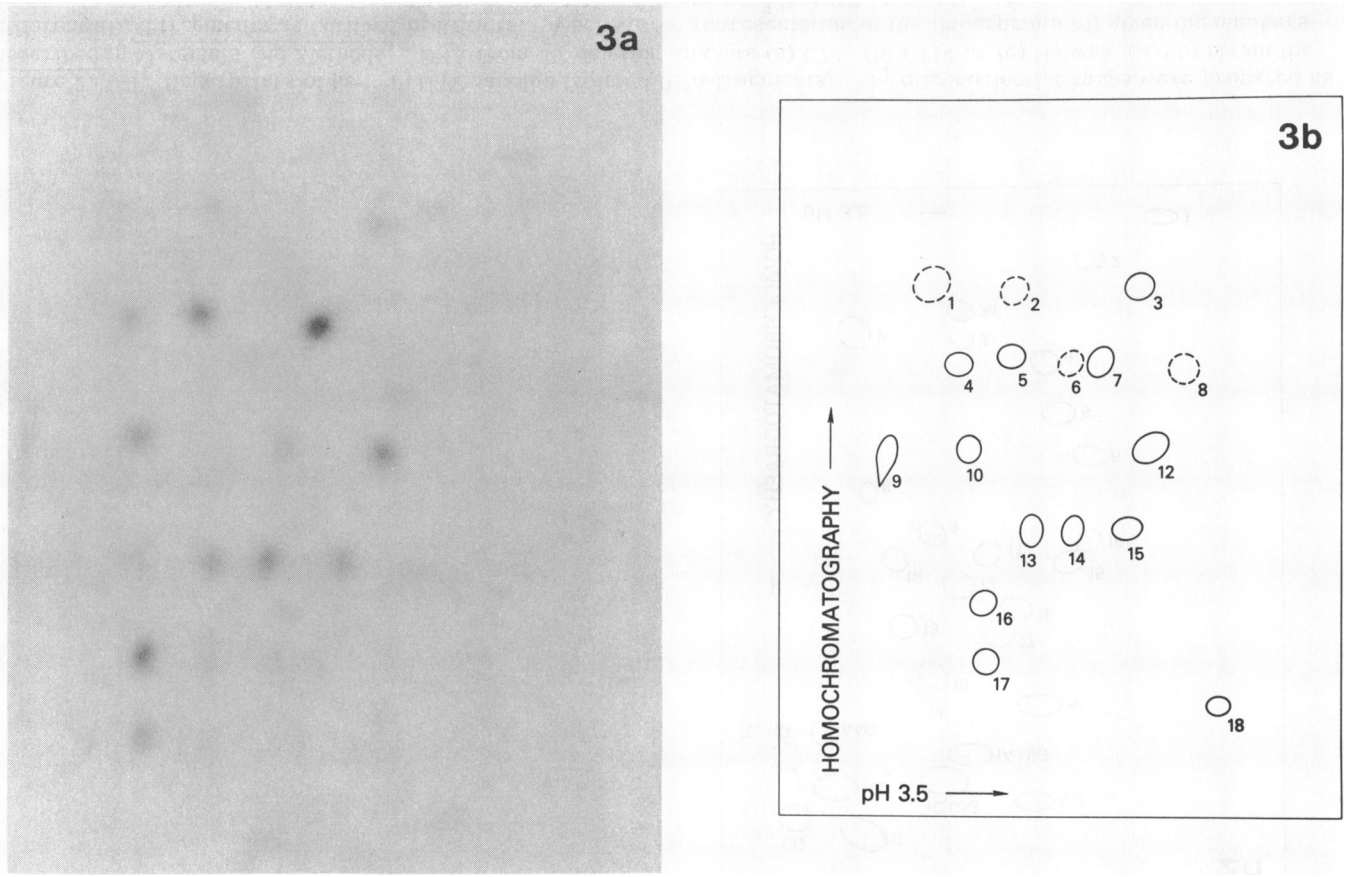


Figure 3. (a) Pancreatic ribonuclease fingerprint of [α-^{32}P] ATP-labeled C114 fragment. (b) Schematic representation of fingerprint with numbers assigned to the oligonucleotides including those not labeled by [α-^{32}P] ATP (dotted lines).

Table I. RNase T_1 Oligonucleotides Derived From Fragments C74 and C114

Oligonucleotide number[a]	Pancreatic RNase Products[b] (labeled precursor) GTP	ATP	UTP	CTP	U2 RNase Products[b] (labeled precursor) ATP	UTP	CTP	Sequence Deduced[c]
T1 (C114)	ApUp	Up(3) Cp(1) Gp(1)	ApUp(2) ApUp(1) ApCp(2.2) Up(3)	ApUp(1) ApCp(2)	UpGp UpCpAp UpUpAp CpUpUpAp CpUpUpUpAp	Ap UpUpAp CpUpUpAp CpUpUpUpAp	UpCpAp CpUpUpUpAp	A-U-U-A-U-C-A-C-U-U-U-A-C-U-U-A-U-Gp[A][d]
T6 (C74,C114)	ApUp	Up Gp	ApUp(1) Cp(1) Up(1.9)	Cp	UpGp CpCpUpUpUpAp	CpCpUpUpUpAp	-	C-C-U-U-U-A-U-Gp[A]
T7 (C74,C114)	ApApGp	ApApCp ApApGp ApApApApCp	none	ApApCp ApApApApCp	-	-	-	A-A-A-A-C-A-A-C-A-A-Gp[G][e]
T8 (C74,C114)	ApApGp	ApApGp Cp	ApCp	ApCp Up	CpUpCpAp	CpUpCpAp	CpUpCpAp Ap	A-C-U-C-A-A-Gp[G]
T9 (C74, C114)	ApGp	Up	Cp	ApGp Cp Up	CpUpCpUpUpAp	CpUpCpCpUpAp	CpUpCpCpUpAp Gp	C-U-C-C-U-A-Gp[C]
T10 (C74, C114)	ApGp	Cp ApApUp	ApApUp	ApGp	CpAp UpAp	Ap	-	C-A-A-U-A-Gp[C]
T11 (C74,C114)	Up	ApApUp	ApApUp Cp	ApApUp Gp	-	UpCpUpGp Ap	UpCpUpGp	A-A-U-C-U-Gp[C]
T13 (C74,C114)	Up Gp ApApUp	ApApCp Cp ApApUp	ApApCp Gp ApApUp	ApApCp	CpAp Ap	CpUpGp Ap	-	a)A-A-C-U-Gp[U] b)C-A-A-U-Gp[G]
T15 (C74,C114)	ApGp	ApGp Up	Cp(1) Up(1,8)	none	CpUpUpUpAp Gp	CpUpUpUpAp	-	C-U-U-U-A-Gp[A]
T16 (C114)	ApApUp	ApApUp Up	ApApUp Gp	none	UpAp	UpGp Ap	-	U-A-A-U-Gp[U]
T18 (C74,C114)	ApGp	ApUp ApGp	ApUp	none	UpAp	Ap	-	A-U-A-Gp[A]
T19 (C74,C114)	ApCp	Up Gp	none	ApCp	UpAp	-	UpAp	U-A-C-Gp[A]

Molar ratios of pancreatic and U2 products are 1:1 except where indicated in parentheses;
(a) Oligonucleotides are numbered as in Figure 2d.
(b) Further analyzed by alkaline hydrolysis.
(c) Chain length and composition of the oligonucleotides were also estimated from their relative positions after two-dimensional "homochromatography".
(d) Final structure was deduced by analysis of pancreatic RNase digestion products subsequent to modification of oligonucleotide with a carbodiimide reagent (13).
(e) Final structure deduced by analysis of pancreatic RNase product P16 (GAAAAC[A];Table II).
*Designates the radioactive phosphate.

Table II. Pancreatic RNase Oligonucleotides Derived From the C114 Fragment

Oligonucleotide number[a]	T_1 RNase Products[b] (labeled precursor) GTP	ATP	UTP	CTP	Sequence[c] deduced
P1	-	Cp	Cp	Gp	G-Cp(A,U)
P2	ApUp	-	ApUp	ApUp	A-Up(G,C)
P3	-	Up	Gp Up	Up	G-Up(A,C,U)
P4	Cp	-	Cp	ApGp	A-G-Cp(G,U)
P5	ApApUp	ApApUp	ApApUp	-	A-A-Up(G,A)
P7	-	Gp ApUp	ApUp ApUp	-	G-A-Up(A,U)
P8	Gp	-	Gp	Up	G-G-Up(C)
P9	-	ApApCp	-	ApApCp	A-A-Cp(A)
P10	ApGp	ApGp	ApCp	ApCp	A-G-A-Cp(U)
P12	ApGp	Gp	ApGp	-	G-A-G-Up(G)
P13	ApApGp	ApApGp Cp	-	Gp	A-A-G-G-Cp(A)
P14	ApGp	ApGp ApApUp	ApApUp	ApApUp	A-G-A-A-Up(C)
P15	ApApGp	ApApGp	Gp	Up	A-A-G-G-Up(C)
P16	-	ApApApApCp Gp	-	ApApApApCp	G-A-A-A-A-Cp(A)
P17	ApApApGp	ApApCp ApApApGp	ApApCp	ApApCp	A-A-A-G-A-A-Cp(U)
P18	ApGp Gp ApGp	Gp(2) Up(1)	ApGp	-	G-(A,G)-G-G-A-G-Up(A)

Molar ratios are 1:1 except as indicated in parentheses.
(a) Oligonucleotides are numbered as in Figure 3b; other products present are: Up(C,U,A,G); Cp(C,U,A,G); and ApCp(C,U,A,G).
(b) Further analysed by alkaline hydrolysis.
(c) Chain length and base composition of the oligonucleotides were estimated from their relative positions on the two-dimensional maps.
*Denotes a radioactive phosphate.

Table III. Relative Molar Yield of Oligonucleotide C-C-U-U-U-A-U-Gp in T_1 Fingerprints.

Fragment	RNase III	Appearance of gene 0.3 3'end ($C_2U_3AU<^{OH}_{OH}$)	Molar Yield* of CCUUUAUG
C74	-	-	0.78 ($\pm$0.12)
C74	+	-	0.80 ($\pm$0.10)
C114	-	-	0.91 ($\pm$0.06)
C114	+	+	0.25 ($\pm$0.01)
H3	-	-	1.10 ($\pm$0.01)
H3	+	+	0.59 ($\pm$0.01)

*The molar yield was determined relative to oligonucleotides T9 and T15 (Table I) by removing the material from the thin layer plate and counting for Cerenkov radiation. The yields given are the average of two independent isolations.

(c) Cleavage of isolated RNA fragments by RNase III

To determine whether or not the three sets of RNA fragments (C74, C114 and H3) contained sufficient information for efficient recognition and cleavage by RNase III, the fragments were treated with highly purified enzyme. Following RNase III treatment, the fragments were digested with T_1 RNase and the resulting fingerprints compared with those of the untreated fragments. Susceptibility of the fragment to RNase III cleavage can be monitored both by the reduction in yield of the T_1 oligonucleotide containing the 3' end sequence of gene 0.3 (T6, Table I) and also by the appearance of the predicted 3' terminus of the RNase III cleavage product, CCUUUAU$\begin{matrix}\nearrow OH \\ \searrow OH\end{matrix}$. This assay is extremely sensitive since it will detect even small quantities (<10%) of fragments which are susceptible to RNase III cleavage. Figure 4 shows a map of the RNase III-treated C114 fragment (compare with Figure 2b). In the case of both the C114 and H3 fragments, treatment with RNase III prior to T_1 digestion results in a change in molar yield of only a single T_1 product: approximately 70% reduction in yield of oligonucleotide T6, CCUUUAUG (Table III). Concomitantly, only one new oligonucleotide is observed (Figure 4; Table III) which corresponds to the 3'-terminal oligonucleotide of the gene 0.3 mRNA produced by RNase III cleavage of the T7 early RNA precursor *in vivo* (6) and *in vitro* (7). In contrast, RNase III treatment of the C74 fragment results in no change in the T_1 oligonucleotide pattern, even though oligonucleotide 6 which contains the site of cleavage, is present in this fragment.

Upon pancreatic ribonuclease digestion of the RNase III-treated fragments, again only a single oligonucleotide change is detected in the fingerprint of the C114 fragment, whereas none occurs in the fingerprint of the C74 fragment. Although it is difficult to document a change in the molar yield of the oligonucleotide GAU (P7, Table II), a new spot was observed in the fingerprint of the C114 fragment after RNase III treatment. The new oligonucleotide was identified as having the same sequence as the gene 0.7 5'-end, pGAU (6). Since the only T_1 and pancreatic oligonucleotides affected by RNase III treatment of the fragments are those which contain the termini of the gene 0.3 and 0.7 messages, the cleavage apparently breaks a single nucleotide linkage between directly adjacent mRNAs.

DISCUSSION

We have utilized a two-step hybridization procedure to isolate overlapping fragments of varying size from the T7 early RNA precursor. Each of these contain the region surrounding the RNase III cleavage site between

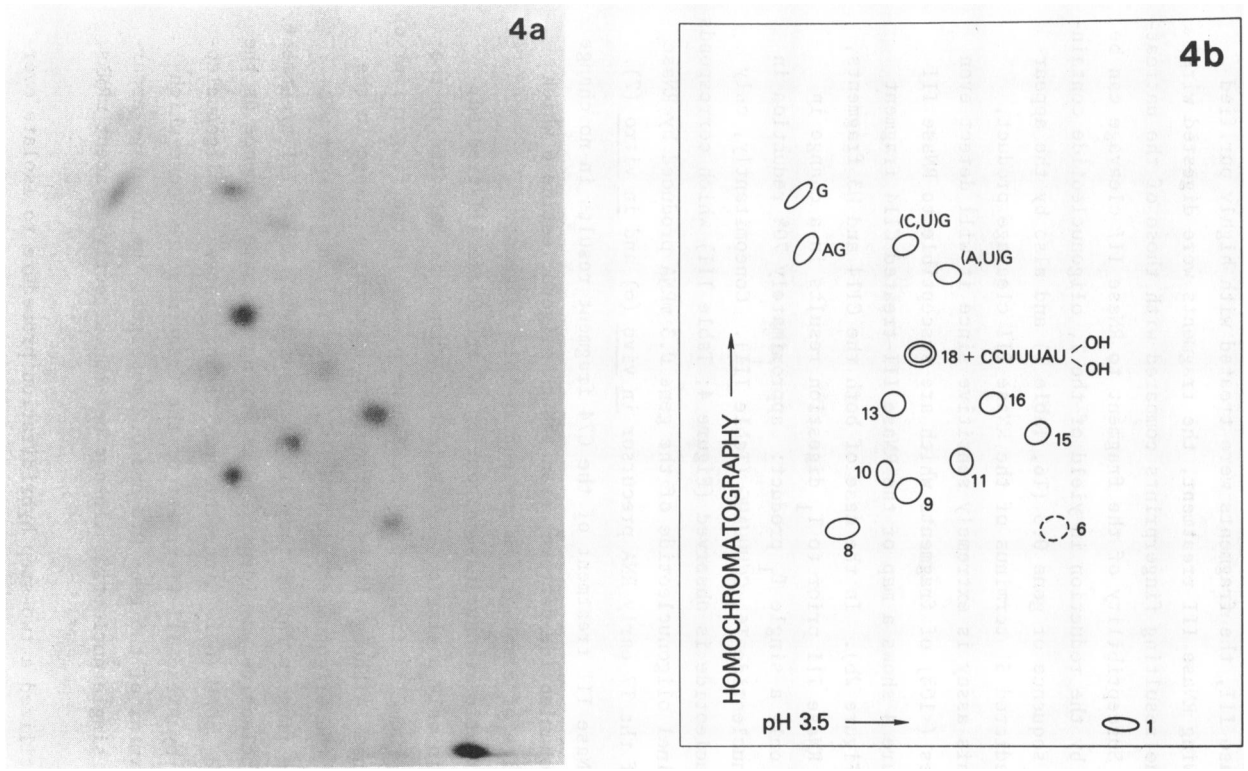


Figure 4. (a) T fingerprint of RNase III treated C114 fragment. (b) Schematic representation. The isolated RNA fragments were treated with RNase III prior to fingerprint analysis. No change was seen in the map of the C74 fragment while oligonucleotide 6, which contains the gene 0.3 mRNA 3' end sequence, was reduced in yield in the maps of both the C114 and H3 fragments (see Table III). Also, a new oligonucleotide near spot 18 appeared in the latter two maps and was subsequently shown to have the same sequence as the 3' end of gene 0.3 message.

genes 0.3 and 0.7. We have examined T_1 and pancreatic ribonuclease fingerprints of the isolated fragments and analyzed the susceptibility of each fragment to RNase III recognition and cleavage.

The oligonucleotide which contains the 3' terminal sequence of the gene 0.3 mRNA has been identified as CCUUUAUG. Since the additional adenylic acid residues found on the 3' end of the in vivo gene 0.3 message (6) are of the form CCUUUAUA$<^{OH}_{OH}$, CCUUUAUAA$<^{OH}_{OH}$, etc., they clearly are not present in the precursor RNA, and, in turn, are not encoded for in the T7 genome. Thus, they must be added either following RNase III cleavage or in a concerted reaction involving both cleavage and A addition. We do not know at this time which enzyme or enzymes are involved in adding the A's. Oligoadenylate additions have also been found on the 3' ends of bacteriophage λ transcripts prepared either in vitro (14) or in vivo (15). These A's are likewise not encoded in the DNA (15,16) and may be added by the RNA polymerase itself or a contaminating activity in the polymerase preparation. Thus, it now appears that A residues can be added post-transcriptionally to the 3' end of certain prokaryotic messenger RNAs. A similar phenomenon has been known for some time to occur with the messenger RNAs of higher organisms (17), and poly(A)-containing RNA has recently been isolated from bacteria (21, 22, 23).

Fingerprint mapping of RNase III-treated fragments indicates that the C114 and H3 fragments are recognized and cleaved in vitro by the enzyme, while the C74 fragment is not. Simon and Studier (11), using heteroduplex mapping, located the left end of the C74 deletion at map position 3.5, which should be just to the right of the 5' end of gene 0.7. They also note that the gene 0.3 mRNA is not found in cells infected with C74; a new, larger RNA is observed, suggesting either that the deletion might actually extend into gene 0.3 or that efficient cleavage does not occur at this site in vivo. Since we find the 3' end-containing oligonucleotide in the C74 fragment, the deletion does not extend into gene 0.3. Thus, we conclude that all or some of the sequence which is present in the C114 fragment but missing in C74 is necessary for processing at this RNase III cleavage site.

By comparing the T_1 fingerprint of the C74 and C114 maps (Figures 2a and b) certain oligonucleotides can be assigned to this important 3' portion of the cleavage region. The major difference is the absence of oligonucleotides 1 and 16 from the C74 map. Other differences in very small nucleotides might not be detected. In addition, a G-rich oligonucleotide of eight bases is observed in pancreatic ribonuclease fingerprints of the

C114 fragment (Figure 3) but not in C74. From the sizes of the oligonucleotides we can establish a minimum size difference of 30 nucleotides between the C74 and C114 fragments. This value is somewhat less than the 75 nucleotides predicted by mapping of the deletions but within the error of the heteroduplex method (11).

Analysis of the T_1 and pancreatic ribonuclease fingerprints of the RNase III-cleaved fragments reveals changes only in the two oligonucleotides which contain the 3' and 5' ends of the gene 0.3 and 0.7 mRNAs, respectively. Thus RNase III appears to produce only one break in the isolated fragments, and presumably at this site in the T7 early RNA precursor, giving rise to the 5' and 3' ends observed on the gene 0.7 and 0.3 mRNAs, respectively. Analysis of the products from RNase III cleavage of these fragments on polyacrylamide gels (not shown) is also consistent with the conclusion that a single endonucleolytic break occurs. This conclusion is further supported by total sequence analysis of the C114 fragment (Rosenberg and Kramer, manuscript in preparation).

It should be pointed out that results by other workers suggest that multiple breaks might occur at other sites under conditions differing from those used to reproduce the normal in vivo pattern of cleavage. Dunn and Studier (18) detected an RNA fragment of about 30 nucleotides which is released from the region between genes 1.1 and 1.3 following in vitro RNase III cleavage of the T7 early RNA precursor. However, an excess of enzyme and extremely low monovalent salt concentration are required to observe this RNA; a single scission predominates under normal reaction conditions (24). No such RNA fragments are released from the other cleavage sites. Dunn also isolated from T7 infected cells, a minor species of gene 1.1 mRNA which is about 30 bases shorter at its 3' end than the predominant species (24). It is not clear whether this second break normally occurs in vivo or simply during isolation. In addition, Paddock and Abelson (19) obtained an RNA species of 140 nucleotides from T4 phage infected cells which is cleaved in vitro at two specific sites by an enzyme shown to be RNase III (25). This cleavage does not occur in vivo and occurs in vitro only under conditions which produce aberrant cleavage of the T7 early RNA precursor. The T4 phage-specified RNA exhibits a relatively weak secondary structure in the cleavage region and yields cleavage products with terminal sequences similar to those of the T7 early RNAs. The secondary structure of the region surrounding the cleavage site in T7 and its possible involvement in recognition and action by RNase III should become evident from the complete

sequence analysis of the isolated fragments (Rosenberg and Kramer, manuscript in preparation).

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