Volume 13 Number 17 1985 Nucleic Acids Research

A novel transcription property of SP6 and T7 RNA polymerases: dependence on template structure

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Received 19 February 1985; Revised and Accepted 9 August 1985

ABSTRACT

The in vitro synthesis of extraneous RNA sequences by SP6 and T7 RNA polymerases from specific DNA templates is described. Transcription of templates prepared by digestion with restriction enzymes that leave 3' protruding ends resulted in the production of significant amounts of long, template-sized RNA transcripts which hybridized to vector DNA. Sequences copied from the noncoding template strand were among the extraneous transcripts. The presence of these sequences in probe preparations were detected in Southern and RNase protection hybridization assays. In contrast, transcription of DNA templates with blunt or 5' protruding ends yielded few RNA products as extraneous sequences.

INTRODUCTION

A powerful approach for the analysis of gene structure and function has become possible with the development of systems that provide the efficient synthesis of defined RNA sequences in vitro from cloned DNA. The RNA polymerases encoded by the Salmonella typhimurium bacteriophage SP6 and Escherichia coli phage T7 have characteristics suitable for such a system. These enzymes are very efficient, exhibit stringent promoter specificity (1), and are able to copy long heterologous DNA sequences (2,3). In practice, templates to be transcribed by SP6 RNA polymerase are most often constructed using the available high copy number cloning vectors pSP64 and pSP65 (2). These plasmids have a SP6 promoter located immediately upstream from a region of multiple cloning sites into which selected DNA sequences are inserted. For transcription, this recombinant DNA is linearized by a restriction enzyme which cleaves downstream from both the SP6 promoter and DNA insertion. This template is transcribed by SP6 RNA polymerase in an in vitro runoff transcription reaction, yielding many copies of homogeneous RNA corresponding to the cloned DNA sequence. Transcription by T7 RNA polymerase is analogous to the system described for SP6 RNA polymerase except that templates have a T7 promoter.

RNA probes of high specific activity can be synthesized by SP6 or T7 RNA

polymerases when limiting concentrations of radioactive ribonucleotides are used in the transcription reaction mixture. Such single-stranded RNA probes have been used successfully to detect DNA and RNA sequences by Southern and Northern blotting techniques (2), or by solution hybridization followed by treatment with RNase (2,4-6). The RNA probes have the advantage of being up to 10 times more sensitive than conventional DNA probes (2).

An additional feature of this transcription system is that it can be used to synthesize large amounts (typically tens of micrograms) of low specific activity or unlabeled RNA in the presence of non-limiting concentrations of ribonucleotides. Such RNAs have been used as substrates to study RNA splicing reactions (2,7,8), translation (9-11) and modification (12-14).

Practical applications for SP6 or T7-synthesized RNA usually rely on the production of specific, single-stranded RNA transcripts. In this communication we describe extraneous transcripts that were synthesized in vitro from DNA templates prepared by digestion with restriction enzymes that produce 3' protruding ends. These transcripts appeared in addition to the expected runoff transcript synthesized by SP6 or T7 RNA polymerases. The extraneous RNAs contained sequences which were complementary to the expected RNA transcript, as well as sequences corresponding to vector DNA. The presence of these extra RNA species could affect results of studies using unfractionated transcription products as probes for Southern blotting, in situ hybridization or mapping analyses.

MATERIALS AND METHODS

Materials

Riboprobe™ SP6 and T7 RNA polymerases, pSP64 DNA, RNasin® ribonuclease inhibitor, restriction enzymes, and the Klenow fragment of DNA polymerase I were obtained from Promega Biotec, Madison, WI. Unlabeled ribonucleotide triphosphates were purchased from Sigma and Pharamacia P-L; [α-^{32}P]GTP and [α-^{32}P]CTP (410 Ci/mmol) were purchased from Amersham. RNase-free DNases were obtained from Promega Biotec (RQ1™ DNase) and Worthington. Ribonuclease A was purchased from Millipore and ribonuclease T_1 was from Calbiochem. Rifampicin was purchased from Sigma.

Plasmids

The recombinant plasmid pSP64/-U1 (Fig. 1B) was constructed by inserting human U1 RNA coding region sequences into the pSP64 vector. The 206 bp BglII-BamHI fragment (positions -6 to +200) of pHU1-1D (15) was cloned into the BamHI site of pSP64. The fragment was oriented in the 3' to 5' sense so

that the SP6 RNA polymerase would synthesize RNA complementary to authentic U1 RNA. Another recombinant plasmid was constructed by inserting a 237 bp PstI-ScaI fragment from pBR322 into PstI-SmaI digested pSP64 (kindly provided by Ken Lewis, Promega Biotec).

A third recombinant plasmid was constructed by inserting a 1.37 kbp HindIII-EcoRI fragment from bacteriophage λ into a HindIII-EcoRI digested pGEM™ plasid construction. The pGEM vectors (patent pending, Promega Biotec) are transcription plasmids that contain both SP6 and T7 promoters in opposite orientations flanking a region of multiple cloning sites. Figure 1A shows a map of the pGEM-1 vector as an example.

Reaction conditions for SP6 and T7 RNA polymerase transcriptions

Plasmid DNAs were digested to completion with appropriate restriction enzymes, extracted with phenol and precipitated with ethanol. The linear DNA (0.2-1μg) was added to a transcription reaction mixture similar to that described by Melton et al. (2). Reaction volumes were 10 μl to 50 μl and contained 40 mM Tris-HCl (pH 7.9), 6 mM $MgCl_2$, 2 mM spermidine, 0.5 mM each of rATP, rUTP, rGTP and rCTP, 10 mM dithiothreitol and RNasin ribonuclease inhibitor (1U/μl). We routinely added 10 mM NaCl to the reaction and noted that the inclusion of NaCl, up to 20 mM, increased transcriptional activity. When high specific activity probes were synthesized using [α-^{32}P]GTP or [α-^{32}P]CTP, the final concentration of the labeled ribonucleotide was 12μM to 20μM. The reaction was initiated by adding SP6 or T7 RNA polymerase (2 to 10 units per μg DNA) and incubated at 37°C or 40°C for 1 hour. After the transcription reaction, the synthesized RNA was either analyzed directly or incubated an additional 10 min with DNase (250 μg/ml) and extra RNasin ribonuclease inhibitor (1u/μl). The RNA was extracted with phenol:chloroform and precipitated twice with ammonium acetate (2M final concentration) and 2.5 volumes ethanol in the presence of carrier RNA. The precipitates were washed with 70% ethanol, dried and resuspended in water.

DNA blots

"Slot-blots" were prepared by immobilizing linear DNA onto nitrocellulose with a Slot-Blotter (Schleicher and Schuell), according to the manufacturer's directions. Filters were pre-hybridized at 37°C for 2 hr in 50% formamide, 6XSSC, 1% SDS, 0.1% Tween 20 and 50 μg/ml carrier RNA. The pre-hybridization solution was replaced with fresh solution containing the RNA probe and the filters were hybridized at 50°C for 20 hr. Filters were washed at room temperature twice in 1 X SSC, 0.1% SDS (20 min each) and at 65°C twice in 0.1 X SSC, 0.1% SDS (20 min each). Processed filters were air-dried and exposed

to X-ray film at -70°C for 36 hr.

Solution hybridization and RNase protection

Unlabeled human U1 RNA prepared from MCF-7 cells (16) by polyacrylamide gel fractionation (E. Lund, personal communication) of total nucleic acids (17) was dissolved in 28 µl of 80% formamide containing 40 mM PIPES (pH 6.7), 0.4 M NaCl and 1 mM EDTA. Control tubes contained the same hybridization solution without human U1 RNA. The RNA probe (2 µl) was added to each tube, the mixtures were heated at 85°C for 10 min and were incubated overnight at 50°C. After hybridization, 300 µl RNase digestion buffer (10 mM Tris-HCl (pH 7.5), 300 mM NaCl, 5 mM EDTA, RNase A (40 µg/ml) and RNase T_1 (2 µg/ml)) was added and the incubation continued at 30°C for 30 min. The RNase digestion was terminated by addition of 50µg Proteinase K and 20 µl of 10% SDS (37°C, 15 min). The RNA-RNA duplexes were extracted with phenol:chloroform and precipitated with ethanol. The precipitates were washed with 70% ethanol, dissolved in loading buffer containing 7M urea, and applied to a 8% polyacrylamide gel containing 7M urea. Following electrophoresis, the gel was exposed to X-ray film at -70°C in the presence of an intensifying screen.

Procedures used for RNA fingerprinting included RNase T_1 digestions of gel-purified RNAs and separation of the oligonucleotides (18), as previously described (15).

Host vs. SP6 RNA polymerase activity

RNA polymerase was purified from uninfected Salmonella typhimurium using a modification of the procedure described by Burgess and Jendrisak (19). Rifampicin-sensitivity was assayed by including 20 µg/ml of rifampicin in the transcription reaction mixture.

RESULTS

Transcription of large RNA species from linear DNA templates having 3' protruding ends

The pGEM™ plasmid containing λ DNA flanked by the SP6 and T7 promoters was digested with restriction enzymes to prepare DNA templates with blunt or 3' or 5' protruding ends (overhangs) (see Fig. 1C). The linear DNAs were used as templates by SP6 or T7 RNA polymerase under identical conditions in in vitro transcription reactions. The length of runoff transcripts expected from templates linearized by PstI, HincII, EcoRI, or HindIII are indicated on the map shown in Fig. 2C. These transcripts are shown in the autoradiogram in Fig. 2A. A very small amount of high molecular weight RNA

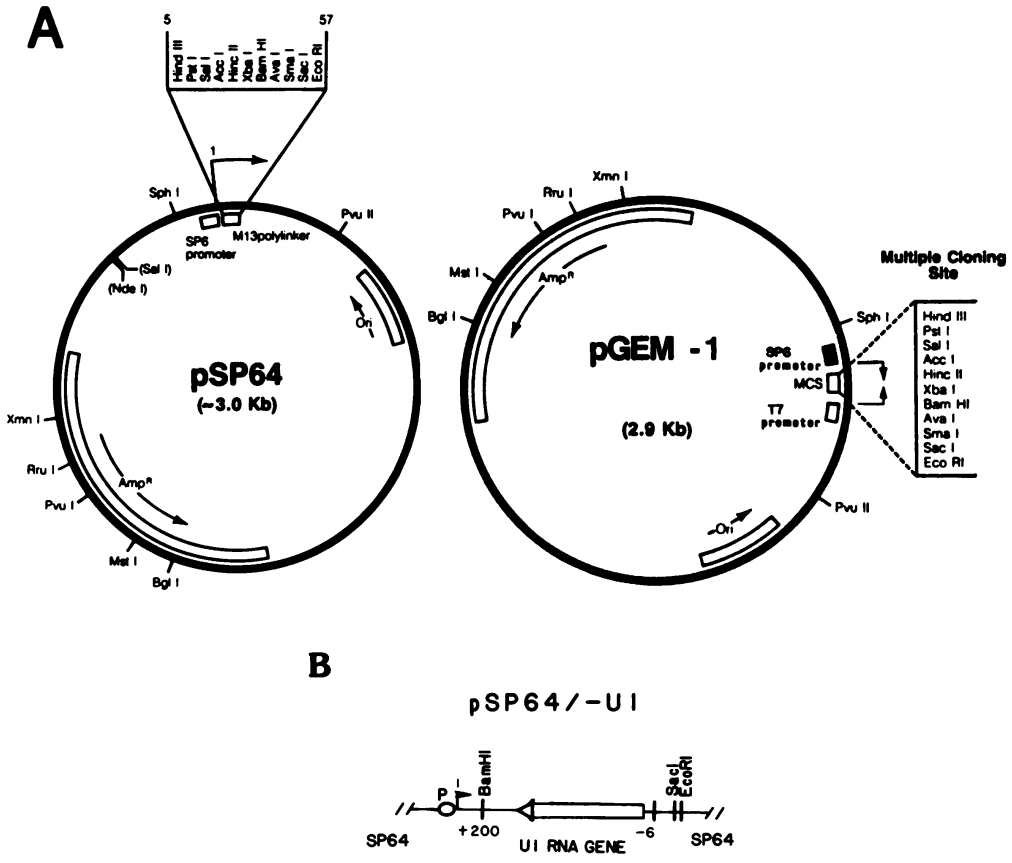


Fig. 1. Structures of pSP64, pGEM-1 and pSP64/-U1 recombinant DNAs, and of the ends generated by various restriction enzymes. A. Maps of the pSP64 and pGEM-1 cloning vectors are illustrated and include the restriction sites in the multiple cloning site regions. The arrows starting from the promoters indicate the direction and start of SP6 or T7 RNA polymerase-dependent transcription. Amp denotes the β-lactamase gene which confers ampicillin resistance, and ori is the plasmid origin of replication. B. Diagram is shown of the U1 RNA gene sequences in pSP64/-U1 recombinant DNA. The 206 bp fragment of a human U1 RNA gene that includes the coding region sequences was inserted into the BamHI site of pSP64 DNA. The positions -6 and +200 refer to the U1 RNA gene map (15). The large arrow indicates the usual direction of transcription and size of the U1 RNA coding region sequences (164 bp); the small arrow and position 1 denote the direction and initiation point of SP6 RNA polymerase-directed transcription. BamHI, SacI and EcoRI restriction sites are located in the multiple cloning site region of pSP64 DNA; P denotes the SP6 promoter. C. The DNA ends generated by restriction enymes PstI, SacI, HindIII, EcoRI and HincII are shown.

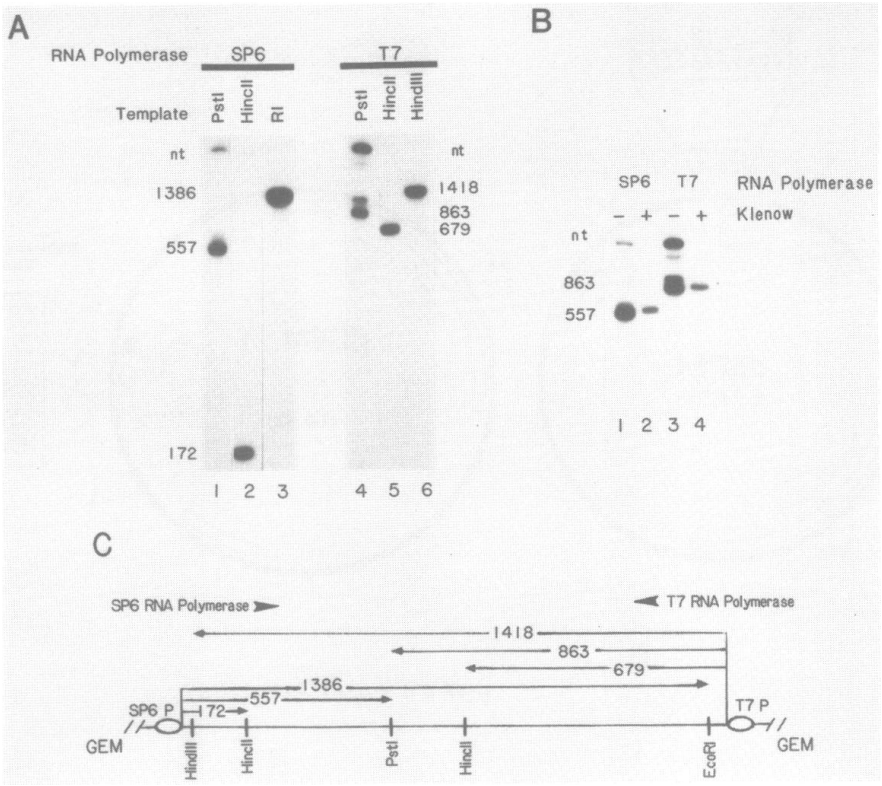


Fig. 2. Extraneous transcription from DNA templates linearized by various restriction enzymes. A. The pGEM recombinant DNA was linearized by PstI (lanes 1 and 4), HincII (lanes 2 and 5), EcoRI (lane 3), or HindIII (lane 6) and used as a template by SP6 (lanes 1-3) and T7 (lanes 4-6) RNA polymerases. The transcription reaction contained [α-^{32}P]CTP and the RNAs synthesized in vitro were analyzed directly after transcription by electrophoresis in a denaturing gel of 5% polyacrylamide -7 M urea. An autoradiogram is shown. Sizes of the RNA products (nt) are shown at the sides of the figure. B. Linear templates were prepared by cleavage of the same plasmid with restriction enyzme PstI. The DNAs were incubated in transcription buffer at 22°C for 15 min with the Klenow fragment of DNA polymerase I (5U/µg DNA) before adding ribonucleotides and SP6 RNA polymerase (lanes 1 and 2) or T7 RNA polymerase (lanes 3 and 4). Transcription proceeded under the conditions described in Materials and Methods. The reaction mixtures were applied to a 5% polyacrylamide -7M urea gel and following electrophoresis the gel was exposed to X-ray film. The + and - symbols indicate, respectively, whether the DNA was or was not treated with Klenow DNA polymerase. C. Map of the pGEM recombinant DNA indicating sizes of run-off transcripts made by SP6 or T7 RNA polymerase on linearized templates. Sizes are given in nt. SP6 P and T7 P indicate positions of the SP6 and T7 promoters, respectively.

transcribed from templates linearized by HincII (lanes 2 and 5) or by restriction enzymes that produced 5' end overhangs (lanes 3 and 6) was observed in the wells of the gel. In contrast, a substantial amount of high molecular weight RNA was produced from DNA having 3' protruding termini (PstI,

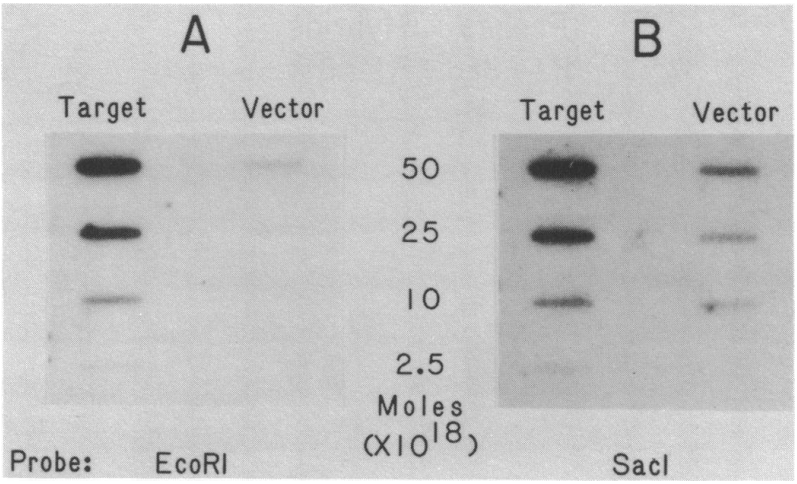


Fig. 3. Hybridization to target and vector sequences using RNA probes synthesized from templates linearized by EcoRI (5' protruding end) and SacI (3' protruding end). A. U1 RNA coding region (Target) and SP64 (Vector) sequences were hybridized to labeled RNA synthesized from a EcoRI-cut pSP64/-U1 template (5' overhangs). Linear DNAs were applied to nitrocellulose filters such that an equimolar amount of Target and Vector sequences were spotted opposite each other. The amount of DNA (moles) in each row is indicated. The filter was hybridized and washed, and the autoradiogram is shown. B. Same as panel A, except the filter was probed with RNA synthesized from the pSP64/-U1 DNA cut by SacI (3' overhangs).

lanes 1 and 4). Other experiments (not shown) indicated that only low levels of spurious transcription by SP6 RNA polymerase occurred from templates lacking the SP6 promoter, such as pBR322, even when the templates had 3' overhanging ends. This low level of transcription indicates that at least SP6 RNA polymerase can initiate transcription at sites other than the SP6 promoter, but with a very low frequency.

Production of large extraneous RNA species from templates having 3' end overhangs was effectively eliminated after modification of the DNA ends. Prior to transcription in vitro, the 3' to 5' exonuclease activity of DNA polymerase I Klenow fragment was used to trim the 3' end tails generated by PstI. As shown in Fig. 2B, treatment with the Klenow DNA polymerase dramatically reduced the amount of large transcripts synthesized from the PstI-cut template by both SP6 and T7 RNA polymerases.

The extraneous transcripts synthesized from templates having 3' end overhangs contained sequences copied from vector DNA upstream of the promoter. This was demonstrated using transcripts labeled to a high specific activity as hybridization probes for DNA blots. For this study, pSP64/-U1 recombinant DNA (cf. Fig. 1B) was cleaved by EcoRI (5' end overhangs) and used

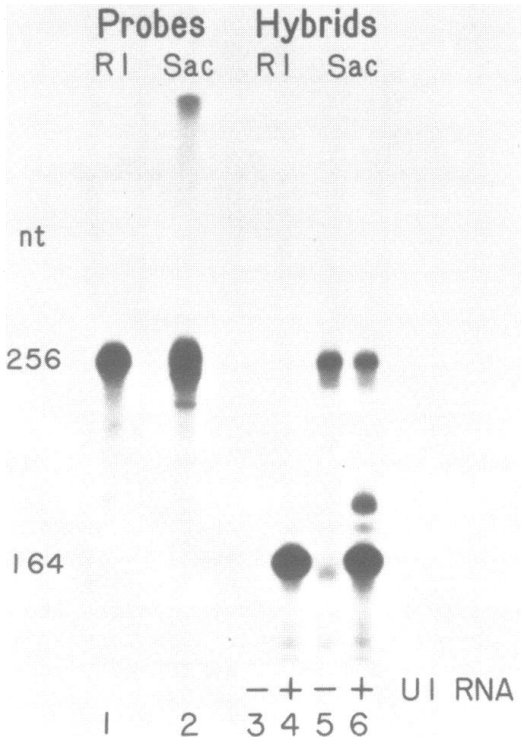
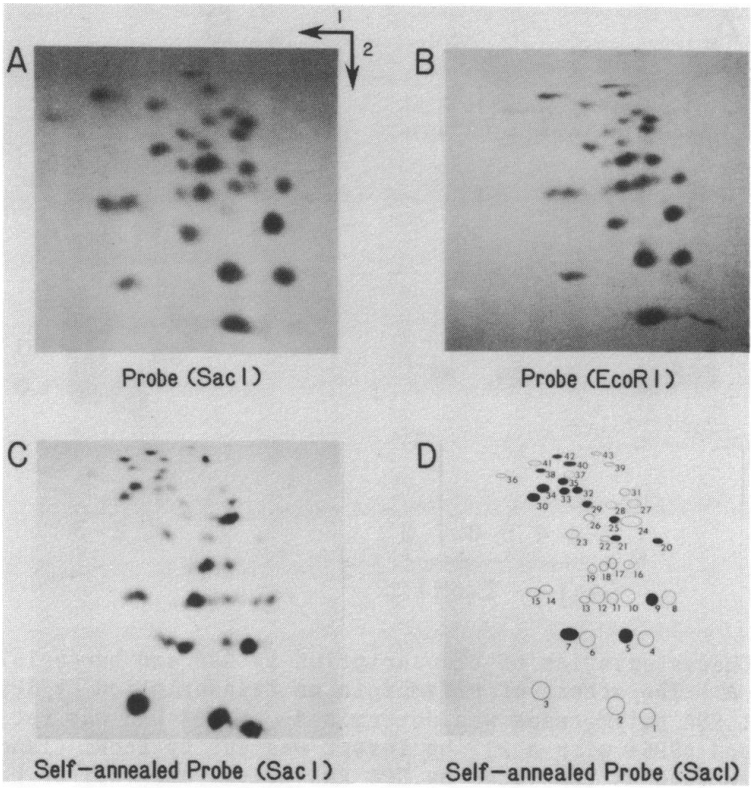


Fig. 4. RNase-protection of U1 RNA hybrids. Labeled RNA was synthesized from pSP64/-U1 templates digested by EcoRI (RI;lane 1) or SacI (Sac;lane 2). The "anti-U1" RNAs were hybridized in solution with or without unlabeled human U1 RNA, and treated with RNases. The original probes (lanes 1,2) and the protected RNA duplexes (lanes 3-6) were electrophoresed in an 8% polyacrylamide-7M urea gel. The + and - symbols indicate the presence or absence of human U1 RNA. An autoradiogram of the gel is shown.

as a template for in vitro synthesis of a RNA probe complementary to human U1 RNA. When the synthesized RNA was used to probe slot blots for U1 gene or vector sequences, the hybridization signal to U1 gene target sequences on the nitrocellulose filter was much more intense than it was to the vector sequences (Fig. 3A). In contrast, a similar-sized probe synthesized from the same recombinant DNA, but linearized by SacI (3' end overhangs), hybridized strongly to both target and vector sequences (Fig. 3B). This DNA blot also demonstrates that the RNA probe can detect as little as 0.3 pg of target DNA sequences in a Southern-type blot.

Synthesis of RNA from the noncoding template strand

In order to determine if one or both strands of DNA with 3' end overhangs was transcribed by SP6 RNA polymerase, we performed solution hybridization and RNase protection assays. Labeled RNA was synthesized from linear pSP64/-U1 templates, hybridized to authentic U1 RNA and treated with RNases. If only



E Primary transcript:

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        28    14       6  4    6    26        17    15  1  18      36
pppG AAUACAAG CUUG G G CUG CAG G UCG ACUCUAG G AUCCG G UUAG CG UACAG UCUACUUUUG

    31    11  12          11  1  1  10  4              43            4  6  2
AAACUCCAG AAAG UCAG G G G AAAG CG CG AACG CAG UCCCCCACUACCACAAAUUAUG CAG UCG AG

       40              22    19     12    27      2  3  18     19   16   16
UUUCCCACAUUUG G G G AAAUCG CAUUG G UCAG CACAUCCG G AG UG CAAUG G AUAAG CCUCG CCCUG G

      39      3  23          37       8    13  15  2   24          1  2
G AAAACCACCUUCG UG AUCAUG G UAUCUCCCCUG CCAG G UAAG UAUG AG AUCCCCG G G G CG AG CU
```

Complementary sequence:

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       16              42          4          2   29     21   5   3
..CG CCCCG G G G AUCUCAUACUUACCUG G CAG G G G AG AUACCAUG AUCACG AAG G UG G

    32        1  2          38          24      7  3  6     25   1       40      3
UUUUCCCAG G G CG AG G CUUAUCCAUUG CACUCCG G AUG UG CUG ACCAAUG CG AUUUCCCCAAAUG UG G

    25    12     34     3    7  3           12  1  14  1      35        30
G AAACUCG ACUG CAUAAUUUG UG G UAG UG G G G G ACUG CG UUCG CG CUUUCCCCUG ACUUUCUG G

 2    33     7   12  12     24          26  2  6   17   4     20   14
AG UUUCAAAAG UAG ACUG UACG CUAACCG G AUCCUAG AG UCG ACCUG CAG CCCAAG CUUG UAUUC
```

Fig. 5. RNase T_1 fingerprints of gel-purified RNA transcripts and the self-annealed duplex shown in Fig. 4. The fingerprints shown in panels A and B were from the [α-^{32}P]GTP-labeled transcripts (~256 nt) from SacI- and EcoRI- cut pSP64/-U1 templates (the RNAs were from lanes 2 and 1, respectively, shown in Fig. 4). Panel C is the fingerprint of the self-annealed duplex (see Fig. 4, lane 5). Panel D is a schematic diagram of the fingerprint shown in panel C. The solid black spots are the unique oligonucleotides contributed by RNA which is complementary to the expected run-off transcript. The first dimension of each fingerprint was electrophoresis on cellulose acetate at pH 3.5 and the second dimension was homochromatographpy on polyethyleneimine thin-layer plates using homomix C (24). Panel E lists the sequences of the primary transcript and its complement. The numbering system in panels D and E matches each oligonucleotide with its position in the fingerprint. Assignments were based on size and base composition of the oligonucleotides.

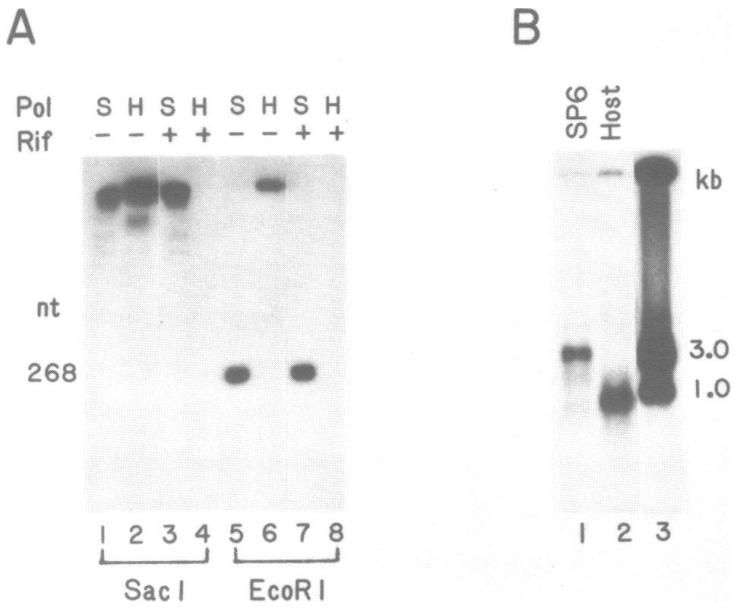

Fig. 6. Characteristics of transcription by SP6 and bacterial host RNA polymerases. A. The effect of rifampicin on transcription by SP6 and bacterial host RNA polymerases was determined. pSP64 DNA was cut by SacI (lanes 1-4), and pSP64 with a 237 bp insert was cut by EcoRI (lanes 5-8). These DNAs were used as templates by SP6 RNA polymerase (4U) or bacterial host RNA polymerase (2.5U) in the presence or absence (+ or -) of rifampicin (20 μg/ml), as indicated. Abbreviations used are: Pol, polymerase; S, SP6 RNA polymerase; H, Host RNA polymerase; Rif, rifampicin. Transcription products were run on a 5% polyacrylamide-7M urea gel and an autoradiogram is shown. B. Large RNA products were sized from SP6 and bacterial host RNA polymerase transcriptions of SacI-cut pSP64 template DNA. The labeled RNA synthesized by SP6 (lane 1) or host (lane 2) RNA polymerases were obtained from the same reactions shown in lanes 1 and 2 of panel A. Size markers were run in lane 3. The RNA was size-fractionated on an 1.4% agarose-1.1 M formaldehyde gel and transferred with 20xSSC to a nitrocellulose filter. An autoradiogram of the filter is shown.

the coding strand was transcribed, we would expect only a single U1 RNA-sized hybrid (164 nt) to survive RNase treatment. But if the opposite DNA strand was transcribed, in addition to the coding strand, then we would observe an additional protected RNA extending the entire length of the probe (256 nt). As shown in Figure 4, lane 6, solution hybridization of the probe (generated from SacI digested template DNA) with purified human U1 RNA and RNase treatment resulted in the protection of two major labeled RNAs: the expected U1 RNA-sized hybrid (164 nt) and the full-length probe hybrid (256 nt). A small amount of high molecular weight RNA was also RNase-resistant, and may represent template-length transcripts (see also lane 2). Self-annealing of this RNA probe also occurred in the control which contained no added human U1 RNA (lane 5). Presence of the 256 nt hybrid was specific for RNA synthesized

from the SacI-cut template DNA and did not occur with probe prepared from the SP64/-U1 template cut by EcoRI (lanes 3 and 4).

We verified that the RNA sequences responsible for self-annealing were complementary to the expected transcript by analyzing RNase T_1 oligonucleotides ("fingerprints"). The expected runoff transcripts from SacI- or EcoRI-cut templates (~256 nt) were gel-purified and digested with RNase T_1. The expected oligonucleotide patterns of these "anti-U1 RNA" probes were observed for both of these RNAs (Fig. 5, panels A and B). However, RNase T_1 digestion of the gel-purified, 256 nt "self-annealed RNA" (seen in Fig. 4, lane 5) generated additional unique oligonucleotides which matched the predicted pattern for RNA that was complementary to the expected runoff transcript (Fig. 4E). Therefore, SP6 RNA polymerase transcribed the noncoding strand of the template having 3' end overhangs.

Extraneous transcription from templates with 3' protruding ends is a characteristic of SP6 RNA polymerase

We tested the possibility that the extraneous transcripts formed *in vitro* were due to a contaminating bacterial host RNA polymerase activity. Since SP6 RNA polymerase was purified from phage-infected *Salmonella typhimurium* cells, it was possible that bacterial RNA polymerase activity could account for the extraneous transcription. Therefore, the transcription characteristics of the purified host enzyme were compared with those of SP6 RNA polymerase, under identical conditions. Figure 6 shows the transcripts produced by SP6 and *S. typhimurium* RNA polymerases from two different templates, and in the presence or absence of rifampicin. Rifampicin is known to preferentially inhibit bacterial RNA polymerases (1,20). As shown in Fig. 6A, large RNAs were synthesized by both enzymes from the SacI-cut pSP64 template, but only the host RNA polymerase activity was inhibited by rifampicin. Using the EcoRI-cut DNA template (pSP64 with a 237 bp insert), a homogeneous RNA of the expected length, 268 nt, was synthesized by SP6 RNA polymerase; synthesis was rifampicin-resistant. The bacterial enzyme again produced a large transcript, and the enzyme activity was inhibited by rifampicin.

While the size of the large RNAs synthesized by both enzymes could not be determined from the 5% polyacrylamide gel shown in Fig. 6A, electrophoresis of the same transcription products on an agarose-formaldehyde gel did reveal a difference in the size of these transcripts (Fig. 6B). The transcripts produced by SP6 RNA polymerase from the SacI-cut pSP64 were predominantly the size of template DNA (3 kb). The transcripts synthesized by the bacterial RNA polymerase were smaller, approximately 1 kb, and could represent transcripts of the plasmid β-lactamase gene (21) which contains transcription signals

recognized by the bacterial enzyme. These results demonstrate that transcription characteristics of the bacterial host and phage RNA polymerases were distinctly different, and that the SP6 RNA polymerase preparation was free of host enzyme activity.

DISCUSSION

We describe here a previously unreported property of transcription by SP6 and T7 RNA polymerases, which is the synthesis of extraneous RNA transcripts from promoter-containing DNA templates with 3' overhanging ends. Transcription on these templates proceeds from both strands of DNA, leading to production of vector sequences and RNA which is complementary to the expected runoff transcripts. Significant amounts of extraneous transcripts were synthesized *in vitro* by SP6 or T7 RNA polymerase only when DNA templates with the respective promoter had 3' protruding ends. The background of spurious transcription was less than 1% from all DNA templates with blunt or 5' overhanging ends. This observation is in agreement with the 0.2% background previously reported for SP6 RNA polymerase (2).

The presence of RNA transcribed by SP6 RNA polymerase from both DNA strands was revealed in RNase protection studies. Self-annealing of the probe produced a high background which interfered with RNase-protection or mapping analyses. In addition, the presence of RNA sequences which hybridize to vector DNA could be problematical when screening for target DNA associated with homologous vector sequences. Studies are underway to further characterize the large RNA products synthesized by T7 RNA polymerase from templates with 3' overhanging ends. We expect that the transcripts, like those synthesized by SP6 RNA polymerase, will self-anneal and hybridize to vector sequences.

Practical difficulties stemming from the synthesis of extraneous transcripts from templates with 3' protruding ends can be circumvented in several ways. The most obvious, of course, is to prepare templates for transcription by digesting DNA with enzymes that do not make 3' end overhangs. However, if the recombinant DNA must be linearized with a restriction enzyme that leaves 3' protruding tails (e.g. PstI or SacI), these ends can be modified by treatment with Klenow DNA polymerase prior to the transcription reaction. For applications where hybridization to pBR322 or vector sequences might occur (e.g. Southern blots or *in situ* hybridizations), the background hybridization can be eliminated by prehybridizing the filter with excess unlabeled vector DNA. Alternatively, the labeled RNA preparation

could be prehybridized with vector DNA, thus removing RNA complementary to the vector sequences. Finally, RNA synthesized in vitro could be gel-purified prior to use. This purification step would ensure a high purity of the desired transcript.

Synthesis of large RNA species from template DNA with 3' protruding ends appeared to be an intrinsic property of SP6 RNA polymerase. SP6 and T7 RNA polymerase preparations analyzed on SDS-polyacrylamide gels showed no contamination by host polymerases (unpublished results). In addition, extraneous transcription occurred with SP6 and T7 RNA polymerases from various sources (commercial and non-commercial) and therefore was not an artifact of any one preparation (data not shown). Thus, in vitro synthesis of these transcripts from templates having 3' end overhangs is a common characteristic shared by at least two bacteriophage-encoded RNA polymerases.

It is unclear how these RNA polymerases synthesize RNA from both DNA strands when the templates have 3' end overhangs. A low background of template-sized RNA apparently arose from initiation of transcription at the ends of DNA templates. However, these transcripts were only a very minor proportion of RNA synthesized from DNA templates with the SP6 or T7 promoter and 5' protruding or blunt ends. Several models could account for these observations. For example, the promoter could serve to "attract" polymerases, but some of these enzymes might initiate transcription at the 3' end just downstream from the promoter. Alternatively, the DNA configuration in the actively transcribed region might make the 3' overhanging single-stranded tail a better site for transcription initiation. Finally, some RNA polymerases may initiate transcription correctly but, rather than "falling-off" at the end of the template, will swing around and continue transcription of the opposite DNA strand.

Although transcription by phage RNA polymerases has been well studied (for reviews see 22,23), the type of extraneous transcription described here has not previously been reported. One reason is that in most of the previous studies, phage DNA templates having natural transcription termination sequences were used; templates with both a promoter and 3' overhang were not examined. Only the advent of in vitro systems for runoff transcription of cloned DNA sequences, along with highly sensitive assays, has revealed the dependence of extraneous transcription on the end structure of DNA templates. Further studies are now needed to determine how SP6 and T7 RNA polymerases interact with promoters and various end structures on DNA templates, and to determine the mechanism of termination on truncated templates.

Acknowledgements

We would like to extend special thanks to James E. Dahlberg and Jerry Jendrisak for stimulating and helpful discussions throughout the course of these investigations, and to Jerry Jendrisak for purifying the host RNA polymerase. We also thank E. Lund for critically reading, and P. Tregloan for typing, this manuscript. E.T.S. was supported by the Damon Runyon-Walter Winchell Cancer Fund Fellowship DRG-715. This work was partially supported by NIH grant PCM-8309618 to J.E. Dahlberg.

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