Proc. Natl. Acad. Sci. USA
Vol. 84, pp. 1507–1511, March 1987
Biochemistry

Detection of transcription-pausing *in vivo* in the *trp* operon leader region

(attenuation/pause RNA/bacterial regulation)

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Contributed by Charles Yanofsky, November 17, 1986

ABSTRACT **To determine whether RNA polymerase pauses during transcription *in vivo*, we have examined transcripts of the *trp* operon leader regions of *Serratia marcescens* and *Escherichia coli*. Labeled RNAs synthesized in *E. coli* strains containing plasmids bearing wild-type or mutant *trp* leader regions of *S. marcescens* or *E. coli* were isolated by hybridization and analyzed by polyacrylamide gel electrophoresis. The labeled RNAs synthesized *in vivo* on the *S. marcescens* wild-type and deletion mutant plasmids were the same size as the *in vitro* pause and leader transcripts. Hybridization of the presumed *in vivo* pause RNAs, and control *in vitro* pause RNAs, to M13 phage DNA containing a *trp* leader region deletion followed by treatment with S1 nuclease produced identical protected RNA species, proving that the *in vitro* and *in vivo* RNAs were identical. The amount of labeled pause RNAs relative to leader RNAs decreased following a chase with unlabeled uridine. *E. coli* RNAs identical to the previously characterized *in vitro* pause and leader transcripts were demonstrated by electrophoretic band position and fingerprint analysis. The finding that transcription pausing occurs *in vivo* is consistent with the view that transcription pausing and ribosome release of paused transcription complexes are responsible for the coupling of translation with transcription that is crucial to attenuation.**

Both prokaryotic (1–5) and eukaryotic (6–8) RNA polymerases pause during *in vitro* transcription. Discontinuous synthesis was first inferred from the presence of nascent RNAs of discrete sizes. Kinetic analyses of *in vitro* transcription in the *Escherichia coli trp* (3, 5), *thr* (9), *ilv* (10), and *pyrBI* (11) operon leader regions have established that the paused species are indeed intermediates in the synthesis of completed transcripts and have led to proposed models in which pause events are essential to proper regulation. The sequences of several pause RNAs reveal that pausing generally occurs immediately after transcription of regions with dyad symmetry. The model most consistent with current evidence on transcription pausing is that formation of a hairpin in the nascent transcript delays one or more of the events required for transcript elongation (3, 5, 12–16).

Transcription pausing is postulated to fulfill an essential role in executing the attenuation decision in the *trp* operon of *E. coli* (5, 16–18). Pausing is thought to be necessary to synchronize movement of the ribosome synthesizing the leader peptide with movement of the polymerase molecule transcribing the leader region. Synchronization is required because the initial translating ribosome must reach the appropriate segment of the transcript before the transcribing polymerase has synthesized distal RNA segments that form decisive regulatory secondary structures (19). Synchronization is believed to be accomplished when the advancing ribosome releases the transcription complex from the paused state (18). The same basic mechanism may synchronize translation and transcription of protein-encoding genes so that ρ factor-dependent termination does not occur.

The major features of attenuation, including pausing, have been reproduced in a cell-free system (S-30) in which both transcription and translation of the *trp* operon occur (14, 18, 20). This result suggests that pausing might be detectable *in vivo* as well. Here we report that the *Serratia marcescens* pause RNA is detectable in *E. coli* strains that contain multicopy plasmids carrying either the wild-type or a deletion derivative of the *S. marcescens trp* leader region. We also have identified the *E. coli in vivo* pause RNA by RNA fingerprinting (21).

MATERIALS AND METHODS

Identification of [^{3}H]Uridine-Labeled *S. marcescens* Leader Transcripts. Plasmids were constructed that contained the *trp* leader regions of *S. marcescens* wild-type (pRL266) and deletion mutant Δ*trp307* (pRL275) by inserting appropriate *Hpa* II restriction fragments into the *Acc* I site of pUC12 so that the *lac* and *trp* promoters were present in the same orientation. Deletion 307 removes residues 18–54 of the leader transcript (Fig. 1*A*) but does not remove any of the segments responsible for the pause or termination secondary structures (18, 19, 22). Recombinant M13 phage were constructed by inserting the appropriate *Hpa* II fragments of wild-type and mutant Δ*trp307 trp* leader regions into the *Acc* I site of M13 mp8 so that the strand complementary to the transcript was present in single-stranded phage DNA.

E. coli strains *trpR tnaA2* Δ*trpEA2* (pRL266) and *trpR tnaA2* Δ*trpEA2* (pRL275) were grown in minimal medium supplemented with 0.1% acid-hydrolyzed casein, 0.2% glucose, 50 μg of L-tryptophan per ml, and 50 μg of ampicillin per ml. RNA was pulse-labeled with [^{3}H]uridine and isolated as described (23). Samples containing ≈10^5 cpm were subjected to electrophoresis through 10% polyacrylamide 7 M urea gels. Authentic ^{32}P-labeled *S. marcescens* wild-type and Δ*trp307* leader and pause RNAs synthesized in a purifed transcription system (13–15) were run as size markers. To detect the [^{3}H]RNAs, the polyacrylamide gels first were fixed in 10% acetic acid, washed for 1 hr with five successive changes of H_2O, and then soaked in 1 M sodium salicylate for 30 min (24). The gels were dried and radioautographed.

Isolation and S1 Nuclease Mapping of *S. marcescens in Vivo* Pause RNAs. About 2 × 10^6 cpm of *in vivo trpL* pause [^{3}H]RNAs were excised from a denaturing 10% polyacrylamide gel as indicated by the position of *in vitro* pause

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Abbreviation: nt, nucleotide(s).

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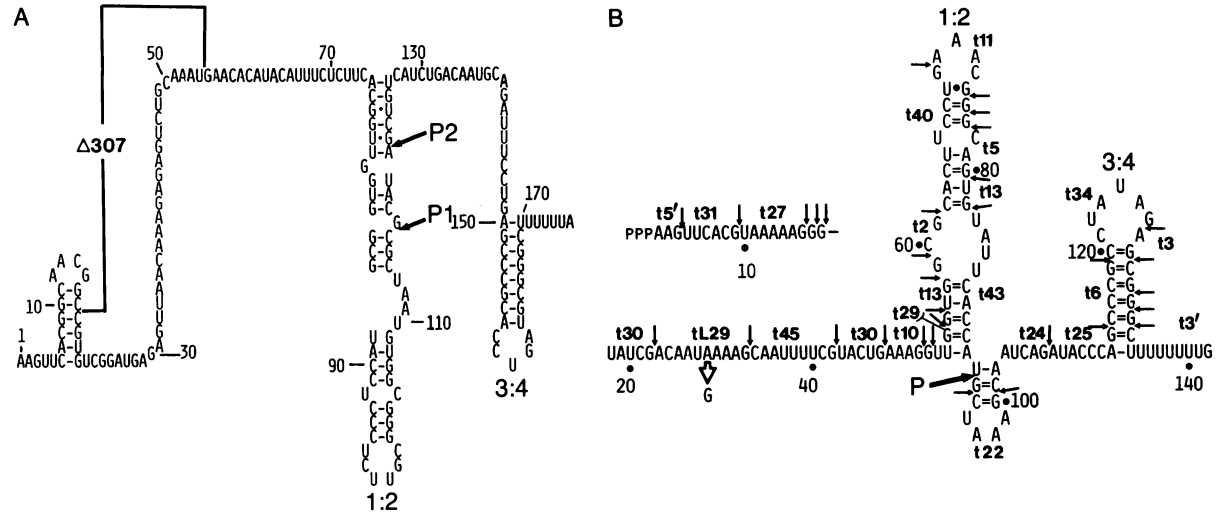

FIG. 1. Structure of the *trp* leader transcript region. The sequences are drawn showing secondary structures thought to be involved in pausing (hairpin 1:2) and termination (3:4). Pause sites P1 and P2 are indicated with arrows. (*A*) *S. marcescens* wild-type leader RNA. The nucleotides deleted in Δ*trpL307* are indicated. (*B*) *E. coli trpL29*. The sequence is numbered from +1, the start of transcription. Small arrows indicate the sites of RNase T1 cleavage. Numbers indicate oligonucleotide assignments in the RNase T1 fingerprints (14). The mutated translation initiation codon is indicated; the wild-type sequence is shown below.

[^{32}P]RNA markers and were isolated by elution and precipitation with ethanol. About 10^6 cpm of the P1 and P2 *in vivo* pause RNAs and 500 Cerenkov cpm of P1 and P2 *in vitro* RNAs were mixed with about 1 μg of M13 single-stranded DNA containing the *S. marcescens* Δ*trpL307* leader region and 100 μg of *E. coli* RNA in 35 μl of 80% formamide/40 mM Pipes, pH 6.4/0.4 M NaCl/1 mM EDTA. The samples were incubated at 65°C for 3 hr in 350 μl of ice-cold 5% (vol/vol) glycerol/250 mM NaCl/30 mM sodium acetate, pH 4.5/1 mM $ZnSO_4$ containing 2000 units of S1 nuclease (Boehringer Mannheim). The samples were shifted to 44°C and incubated for 30 min. Ethanol (1 ml) was added to each sample, and the precipitated RNAs were recovered by centrifugation. Samples were redissolved as described (18) and electrophoresed through a 7 M urea/10% polyacrylamide gel.

Analysis of ^{32}P-Labeled *E. coli* Leader Transcripts. An *rna-19* (RNase I$^-$) *trpR* strain derived from *E. coli* strain AB301 (25) was transduced to rifampicin resistance with phage P1 prepared on CY15022, which has the *rpoB7* allele (26). The resulting strain was transformed to ampicillin resistance with pAD12, a 3-kilobase (kb) pUC8-derived plasmid (A. Das and C.Y., unpublished data). This plasmid contains a *Sau*3A fragment of *E. coli trpL29* leader region DNA extending from base pair −237 upstream from the start site of transcription to +251 [90 nucleotides (nt) into *trpE*]. This fragment was inserted into the *Bam*HI site of pUC8 so that the *lac* and *trp* promoters were present in the same orientation. Transformants were grown in phosphate-limiting (28) tryptophan-free medium to late-logarithmic phase and then labeled with 0.3 mCi (1 Ci = 37 GBq) of [^{32}P]orthophosphate per ml for 2 min. Cells were killed immediately, as described (21). The lysate was adjusted to 0.3 M sodium acetate (pH 4.5) and extracted with cold phenol followed by ether. The nucleic acids were then precipitated with ethanol.

Transcripts from the *trp* leader region were purified as described (23) by hybridization to single-stranded DNA derived from recombinant M13 phage containing the same fragment of *trpL29* DNA oriented so that the packaged strand is complementary to the *trp* transcript. The isolated RNAs were loaded onto 10% polyacrylamide/7.5 M urea sequencing gels alongside markers of authentic pause and leader RNAs purified following *in vitro* synthesis in the S-30 system (18). RNAs were located by autoradiography, excised from the sequencing gel, and eluted at 37°C overnight in 0.5 ml of TE buffer (10 mM Tris·HCl, pH 7.5/1 mM EDTA) containing 20 μg of tRNA. The RNAs were precipitated with ethanol, dried, and digested with 2500 units of RNase T_1 per ml for 20 min at 37°C and then fingerprinted as described (14, 27, 28).

RESULTS

Identification of the *S. marcescens in Vivo trpL* Pause RNA. In previous studies of pausing during transcription of the *trp* leader region in a coupled transcription/translation system, we identified pause RNAs transcribed from *S. marcescens* wild-type and Δ*trpL307* DNAs. Using this system, we showed that the translationally defective leader peptide coding region of Δ*trpL307* increased pausing during transcription 10-fold (18). Accordingly, we chose these DNA templates for studies on *in vivo* transcription pausing. We labeled RNA *in vivo* with [^{3}H]uridine, using *E. coli* strains containing plasmids with the *S. marcescens* wild-type and Δ*trpL307 trp* leader regions. We isolated the RNA species transcribed from the plasmid-borne *trp* leader regions by hybridization to complementary single-stranded DNAs and compared the [^{3}H]uridine-labeled RNAs synthesized *in vivo* to authentic *S. marcescens* wild-type and Δ*trpL307* pause and leader RNAs synthesized in an *in vitro* transcription system (5, 14, 15, 18). Transcription pausing *in vitro* occurred at two adjacent sites (Fig. 1*A*) on both wild-type and Δ*trpL307* DNA templates under the reaction conditions used (37°C, with 20 μM GTP and 150 μM ATP, CTP, and UTP). *In vivo* RNA species that comigrated with the authentic *in vitro S. marcescens* wild-type and Δ*trp307* pause and leader RNAs were readily detected after a 30-sec labeling period (Fig. 2*A*, lanes 1–4). Radioactivity measurements of excised *in vivo* pause and leader [^{3}H]RNAs revealed *ca.* 2 times more pause RNA (P1 only) relative to leader RNA with the Δ*trpL307* plasmid than with the wild-type plasmid (data not shown).

To establish that the presumed pause species detected *in vivo* were kinetic intermediates rather than degradation products derived from the terminated leader transcripts, we examined the *S. marcescens trp* RNAs present both before and after a 2-min chase with unlabeled uridine in the presence of rifampicin (Fig. 2*A*, lanes 5–8). Quantification of the RNAs present after the chase period revealed that label in the presumptive wild-type pause RNAs (Fig. 2*A*, lane 6) diminished markedly relative to the amount of leader RNA. If the

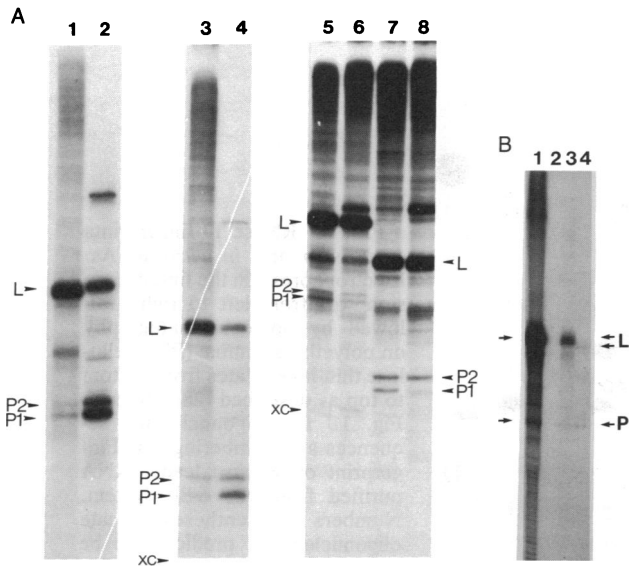

FIG. 2. Analysis of *trp* leader RNAs produced *in vivo*. (*A*) RNAs produced *in vivo* from *S. marcescens* wild-type and leader deletion templates. Lanes: 1, *in vivo* wild-type *trp* leader [^{3}H]RNAs; 2, *in vitro trp* leader [^{32}P]RNAs; 3, *in vivo* Δ*trpL307* [^{3}H]RNAs; 4, *in vitro* Δ*trpL307* [^{32}P]RNAs; 5, RNA isolated from labeled cells containing a wild-type template; 6, RNA isolated after a pulse–chase with a wild-type template; 7, RNA isolated from labeled cells containing a deletion 307 template; 8, RNA isolated after a pulse–chase with the Δ307 template. RNA containing equal radioactivity (≈100,000 cpm) was applied in each lane. L, leader; P1, first *S. marcescens* pause species; P2, second *S. marcescens* pause species; XC, xylene cyanol. (*B*) Autoradiogram of the sequencing gel displaying *in vivo E. coli* [^{32}P]RNAs purified by hybridization to *trpL29* leader DNA. XC, xylene cyanol dye marker; L, leader; P, pause. The first lane contained 90% of the recovered Cerenkov radioactivity, the second lane was left empty, and the third lane contained 10% of the radioactivity. The fourth lane contained equal amounts of *in vitro* leader and pause marker RNAs.

presumed pause bands were degradation products of leader RNA, they should be present at a constant ratio relative to the leader RNAs. The fact that the pause bands decay much faster than leader bands indicates that they are not derived from leader RNAs. Interestingly, the Δ*trpL307* P2 pause RNA did not decay during this chase period, even though the wild-type P2 RNA did. Degradation of the untranslated Δ*trpL307* leader RNA may produce a species that comigrates with Δ*trpL307* P2 (see the *Discussion*).

S1 Nuclease Mapping Proves That the *in Vivo* Pause RNAs Are Identical to *in Vitro* Standards. To establish that the *in vivo* and *in vitro* pause RNA species are identical, we eluted the wild-type pause RNAs from polyacrylamide gels (located by comparison to ^{32}P-labeled *in vitro* standards). After hybridization to single-stranded M13 DNA containing the Δ*trpL307* leader region and digestion with S1 nuclease, the RNAs were electrophoresed again through denaturing 10% polyacrylamide gels. S1 nuclease should digest residues 18–54 of the *S. marcescens* wild-type pause RNAs because they are not base-paired to the M13 DNA. Thus, RNA species 62 (P1) and 67 (P2) nucleotides long should be produced (Fig. 3). The fact that identical RNAs were recovered from both *in vivo* and *in vitro* samples proves that these RNAs contain the same 3′ ends, the authentic positions of pausing during transcription determined *in vitro*.

***E. coli trp* Leader Pause RNA Also Was Detected *in Vivo*.** To increase the likelihood of detecting *E. coli trp* pause RNA *in vivo*, we examined an *rpoB7 rna19* strain containing a multicopy, pUC8-derived plasmid with a small insert carrying the *trpL29* leader region (pAD12). The AUG → AUA change in the *trpL29* leader peptide start codon enhanced pausing 2-fold in the cell-free coupled transcription/translation system (18). We thought that the *trpL29* mutation in combination with the chromosomal *rpoB7* (RNA polymerase with enhanced pausing) and *rna19* (RNase I$^-$) mutations might increase the concentration of the pause RNA species *in vivo*. We labeled RNA *in vivo* with [^{32}P]orthophosphate and isolated RNA species from the *trp* leader region by hybridization to complementary single-stranded DNA. The RNA obtained contained species whose electrophoretic migration was similar to *trp* pause and leader RNAs synthesized in a coupled transcription/translation system (Fig. 2*B*).

The major *in vivo* RNA species detected appeared as a doublet 1 and 2 nt longer than the *in vitro*-terminated leader transcript. The major *in vitro* RNA species have been shown to be 140 and 141 nt long (28, 29). The next most abundant *trpL* RNA species detected *in vivo* migrated as though it were 1 or 2 nt longer than *in vitro* pause RNA, which is 92 nt long (14). The ratio of the 140- and 92-nt *in vivo* RNA bands, as determined by Cerenkov counting of the excised gel slices, was about 10:1.

The 140- and 92-nt *in vivo* and *in vitro* RNAs were excised, eluted, digested with RNase T1, and analyzed by two-dimensional oligonucleotide fingerprinting (27, 28). Fig. 4*A* shows the result obtained with the 140-nt *in vitro* leader RNA. The numbered spots represent the expected fingerprint pattern for the *trpL29* terminated leader transcript (3, 5, 27). The 3′-terminal oligonucleotides, Cp(Up)$_6$U and Cp(Up)$_7$U, were not detected because GTP was used for labeling. The major 5′ end for the S-30 synthesized *trpL* RNA was at position 2 of Fig. 1*B* (see discussion of Fig. 4*C* below); only a minor fraction of the transcripts began at position 1. Since most of the *in vitro* RNA was 5′-dephosphorylated, the major 5′-end oligonucleotide (ApGp) is identical to oligonucleotide t3; minor amounts of ApApGp and the 5′-phosphorylated species (t5′) were present (Fig. 4*A*).

The fingerprint of the *in vivo* 140-nt RNA was the same as that of the *in vitro* leader RNA except that, as expected, 3′-terminal oligonucleotide spots were visible (see X and Y in Fig. 4*B*). The positions of the two 3′-terminal oligonucleotide spots suggest that termination *in vivo* occurred at or near the previously identified *in vitro* 3′ termini (5), in agreement with previous studies (21). The 5′ terminal oligonucleotide(s) appeared almost completely as 5′-phosphorylated species (oligonucleotide t5′ in Fig. 4*B*). The intensity of the spots increased with oligonucleotide length, as expected, since the radioactive precursor is [^{32}P]orthophosphate.

The *in vitro* pause fingerprint (Fig. 4*C*) contained the expected subset of leader oligonucleotides. The ApGp spot in the

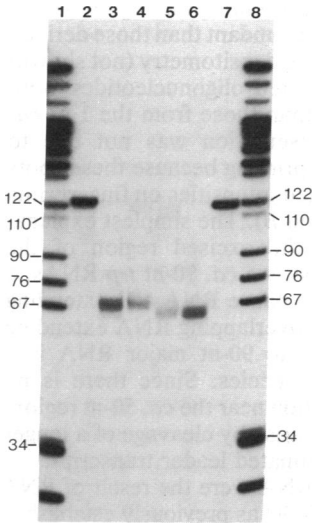

FIG. 3. S1 nuclease digestion of wild-type *S. marcescens* pause RNAs. *In vivo* and *in vitro* pause RNAs were hybridized to M13 single-stranded DNA containing the Δ*trpL307* leader region and were digested with 2000 units of S1 nuclease at 44°C for 30 min. Lanes: 1 and 8, *Hpa* II-digested pBR322 DNA size markers; 2, undigested *in vitro* P2 pause [^{32}P]RNA; 3, S1 nuclease-digested *in vitro* P2 pause [^{32}P]RNA; 4, S1 nuclease-digested *in vivo* P2 pause [^{3}H]-RNA; 5, S1 nuclease-digested *in vivo* P1 pause [^{3}H]RNA; 6, S1 nuclease-digested *in vitro* P1 pause [^{32}P]RNA; 7, undigested *in vitro* P1 pause [^{32}P]RNA.

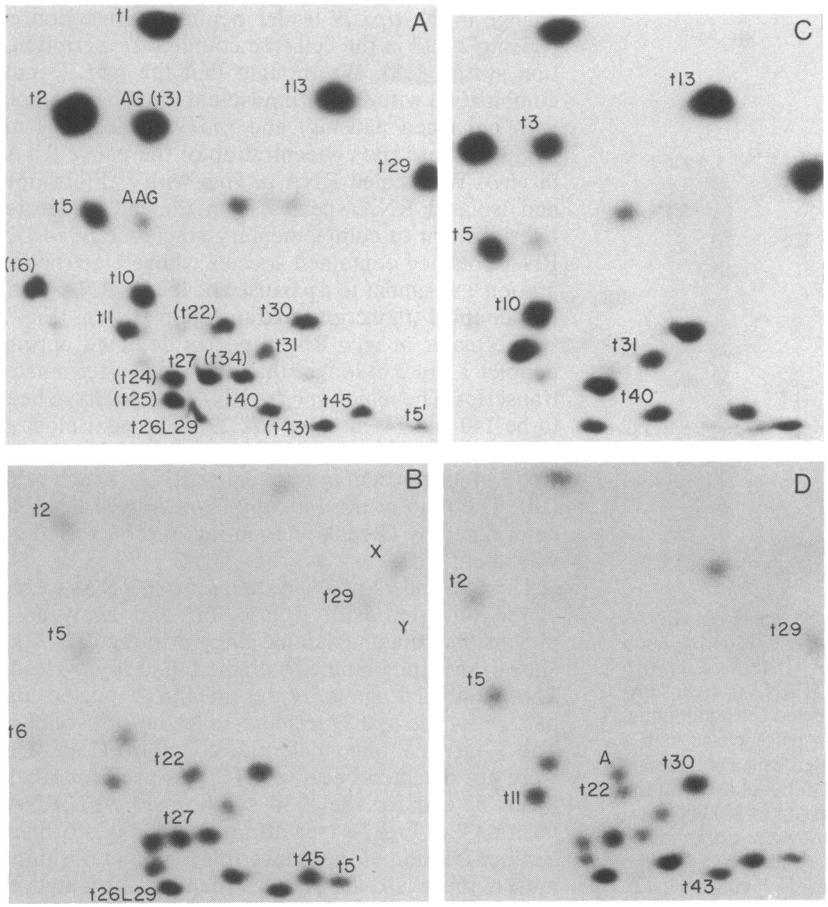

FIG. 4. RNase T1 fingerprints of *in vivo* and *in vitro* RNAs. Electrophoresis in the first dimension was from left to right, followed by homochromatography on polyethyleneimine (PEI) cellulose thin-layer plates from bottom to top as described (28). Refer to Fig. 1*B* for oligonucleotide sequences and numbering. (*A*) Fingerprint of *in vitro* leader RNA purified from the S-30 system. Numbers in parentheses indicate oligonucleotides predicted to be absent from the pause RNA fingerprints. Oligonucleotides ApG and ApApG are indicated; phosphates are omitted for clarity. (*B*) Fingerprint of 140-nt *in vivo* RNA extracted from the gel in Fig. 2. Selected spots are numbered as in *A* for orientation. Spots X and Y are discussed in the text. (*C*) Fingerprint of *in vitro* pause RNA purified from the S-30 system. (*D*) Fingerprint of 92-nt *in vivo* RNA extracted from the gel in Fig. 2. Spot A is discussed in the text.

pause fingerprint was clearly attributable to 5′-dephosphorylated RNA beginning at position 2 (Fig. 1*B*) because oligonucleotide t3 was not contained in the pause RNA sequence. Very little ApApApGp was present in the *in vitro* pause fingerprint, suggesting that almost all of the *trp* transcripts were initiated at position 2 (Fig. 1*B*) in the S-30 system. Since *in vivo trp* RNA initiates at position 1 of Fig. 1*B* (21), the observation that the S-30 *in vitro* RNAs initiated at position 2 is consistent with their slightly faster mobilities in the sequencing gel (Fig. 2*B*).

Surprisingly, the fingerprint of the 92-nt *in vivo* RNA (Fig. 4*D*) contained all of the oligonucleotides expected for the 140-nt leader RNA, except spots X and Y (Fig. 4*B*), as well as a new spot, A. However, since this RNA was excised from the 92-nt region of the sequencing gel, it cannot be 140 nt long. Inspection of the relative intensities of the spots in Fig. 4*D* showed that oligonucleotides representing the region from 1 to *ca.* 90 nt were uniformly more abundant than those derived from *ca.* 90 to *ca.* 140 nt. Scanning densitometry (not shown) indicated that the underrepresented oligonucleotides were less intense by a factor of 2–3 than those from the 1 to *ca.* 90-nt region. Their underrepresentation was not due to inefficient transfer during fingerprinting because these spots were all present with the expected intensities on fingerprints of leader RNA (e.g., Fig. 4 *A* and *B*). The simplest explanation of these results is that the excised region of the sequencing gel contained at least two *ca.* 90-nt *trp* RNAs, a major one similar to the *in vitro* pause RNA and extending from 1 to *ca.* 90 nt and a minor overlapping RNA extending from *ca.* 50 to 140 nt. The 1- to 90-nt major RNA is a candidate for the pause RNA species. Since there is no evidence for transcription initiation near the *ca.* 50-nt region, the *ca.* 50- to 140-nt RNA must arise by cleavage of a longer transcript, presumably the terminated leader transcript.

If the putative *in vivo* pause RNA were the result of RNA polymerase pausing at nucleotide 92 as previously established *in vitro*, then the 3′-terminal oligonucleotide should be present in the fingerprint pattern at the previously identified location (14). Spot A in Fig. 4*D* has the correct electrophoretic mobility to be the 3′-terminal oligonucleotide (compare ref. 14). Since this is the only oligonucleotide not clearly attributable to *trpL* sequences, it is unlikely to have arisen in the RNase T1 digest unless it was the 3′ oligonucleotide of a major RNA species in the band eluted from the gel. Furthermore, oligonucleotide T43, which ends with the 3′ phosphate of nucleotide 93, is underrepresented in the fingerprint and, therefore, undoubtedly arises from the contaminating minor RNA rather than from the pause species (compare the intensity of t43 with those of oligonucleotides tL29, t40, and t45 in Fig. 4*D*). Thus, the *in vivo* pause site appears to be at nucleotide 92, the same position found for *in vitro* transcription (14).

Careful consideration of the intensity of spot A, however, suggests that no more than 20% of the eluted RNA may be true *in vivo* paused synthetic intermediate. The 3′ oligonucleotide attributed to spot A (Fig. 4*D*) should contain nine ^{32}P-labeled residues (Fig. 1*B*) and give an autoradiographic intensity similar to t27. Scanning densitometry (data not shown) revealed an actual intensity attributable to only 10–20% of the total fingerprinted RNA. Although we are confident that the *E. coli trpL* pause RNA was detected in this experiment, a larger quantity of leader RNA degradation product apparently was present in the sample.

DISCUSSION

We have shown that *trp* RNAs indistinguishable from *in vitro* *E. coli* and *S. marcescens trp* pause RNAs can be isolated from growing cultures of appropriate *E. coli* strains. The *S. marcescens* and *E. coli trp* RNAs were characterized by S1 nuclease mapping and oligonucleotide fingerprinting, respectively. Each method revealed that the *in vivo trp* pause RNAs

contain the same 3′ termini as the major *in vitro* pause species. Although the presence of contaminating RNAs that comigrated with the pause RNA precluded kinetic analysis of the *E. coli* pause RNA, a pulse–chase experiment with the *S. marcescens* pause RNA species showed that they behaved as kinetic intermediates rather than degradation products. Also, using the *S. marcescens* template, we found that the wild-type *trp* pause RNA is detectable in a strain with wild-type RNA polymerase and RNase activities. These results establish that transcription pausing is detectable *in vivo*.

Inhibition of Leader Peptide Synthesis Did Not Enhance Pausing *in Vivo*. The relative amount of pause RNA detected with the Δ*trp307* template was only 2-fold greater than that from the wild-type *S. marcescens* template. The same deletion was found to enhance pausing 10-fold in the coupled transcription/translation system (18). One possible explanation for this finding is that the deletion RNA was degraded more rapidly because it lacked the 5′-most RNA hairpin (Fig. 1*A*). It also is possible that degradation of the untranslated deletion RNA is more rapid *in vivo* than in the S-30 *in vitro* system, which was designed to minimize mRNA degradation (30). More rapid degradation of untranslated RNA also may explain why the *E. coli trpL29 in vivo* pause RNA was so heavily contaminated with degradation products (see Fig. 2*B*, lane 1).

Detection of the *trp* Leader Pause RNA *in Vivo* Supports a Role for Pausing in Coupling Transcription of the Leader Region to Synthesis of the Leader Peptide. It has been proposed that transcription pausing and ribosome release of the pause complex are the key events responsible for synchronization of transcription and translation in the *trp* leader region that is essential to attenuation (5, 13, 18). Detection of the *trp* leader pause RNA *in vivo* strongly supports this view. However, final proof of this model requires the demonstration that impaired pausing *in vivo* affects the attenuation mechanism.

Transcription Pausing *in Vivo* May Couple Protein Synthesis to Transcription and Thereby Prevent Translational Polarity. The *in vivo* role of transcription pausing may extend beyond coupling leader peptide synthesis to transcription in the regulation of operons controlled by attenuation. It has been well established in multicistronic operons that the inability to complete translation of message segments specified by protein-coding genes causes transcription termination (31, 32). This termination is manifested by a reduction in expression of genes downstream of the gene with the translational block (31, 32). This phenomenon, termed translational polarity, is widespread in enteric bacteria (31, 32). Polarity-associated termination is thought to be caused by the binding of the termination factor, ρ, to untranslated, unstructured RNA (32). How does the bacterium prevent RNA polymerase from producing transcripts faster than ribosomes can translate them, thereby triggering ρ-dependent termination? We suggest that an important role of *in vivo* transcription pausing is to prevent ρ-mediated termination by making the rate of transcription largely dependent on ribosome movement. Most mRNA segments can fold into multiple, sequential secondary structures (33). If RNA polymerase were to pause at such structures near the beginning of a coding region, this would permit the initial translating ribosome to catch up with the transcribing polymerase and disrupt the RNA secondary structure, thereby releasing the paused polymerase. Thereafter polymerase and ribosome would move together and the ribosome would block ρ entry sites on the transcript. This form of coupling would be essential for messages with inefficient translation initiation sites. Thus, transcription pausing may play a general role in controlling gene expression by allowing transcription to proceed to completion except under the extraordinary circumstances where amino acid deprivation prevents mRNA translation.

This work was supported by Public Health Service Grant GM-09738 and by Grant 69-015 from the American Heart Association. R.L. and J.C. were postdoctoral fellows supported by National Research Service Awards from the Public Health Service during the period this work was performed. C.Y. is a Career Investigator of the American Heart Association.

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