Using yeast RNA polymerase III ternary complexes stalled at various positions on the template, we have analyzed the cleavage products that are retained and released by the transcription complexes. The retained 5' products result from cleavage at uridine residues during retraction, whereas the yield of mononucleotides and dinucleotides released indicates that multiple cuts occur near the 3' end. Comparison of the cleavage patterns of uridine-containing and 5-bromouridine-containing transcripts suggests that RNA within an RNA-DNA hybrid duplex is the substrate for the 3'-5' exonuclease. During transcription of the SUP4 tRNA{Sup} gene, RNA polymerase III produces not only full-length pre-tRNA{Sup} but also short oligonucleotides, indicating that exonuclease digestion and transcription are concurrent processes. To explore the possibility that these oligonucleotides are released by the action of the RNA polymerase III nuclease at previously observed uridine-rich pause sites, we tested modified templates lacking the arrest sites present in the SUP4 tRNA{Sup} gene. Comparative studies of cleavage during transcription for these templates show a direct correlation between the number of natural pause sites and the yield of 3' products made. At the natural arrest sites and the terminator, RNA polymerase III carries out multiple cleavage resynthesis steps, producing short oligoribonucleotides with uridine residues at the 3' terminus.

A hydrolytic activity that effectively reverses the course of gene transcription has been found for Escherichia coli RNA polymerase, eukaryotic RNA polymerases I, II, and III, and the vaccinia virus RNA polymerase (1–9). In the first three cases a separate protein co-factor serves to activate the nuclease, whereas for RNA polymerase III (Pol III) and the vaccinia virus RNA polymerase (1–9), separate protein co-factor serves to activate the nuclease, whereas for RNA polymerase III (Pol III) and the vaccinia virus RNA polymerase (1–9) the nuclease activity associated with yeast Pol III elongation complexes that we examined, the cleavage occurs just 5' to an internal uridylate residue. Because the bond between a 3'-terminal uridylate and the penultimate nucleotide is not efficiently cleaved, we infer that in growing RNAs having an oligouridine stretch at the 3' end, the last residue is not base paired to the DNA template. This suggests that at arrest sites, the 3' transcript end becomes detached from the template due to the exceptional instability of (rU{rU}) hybrids (17). Nucleolytic removal of several residues from the RNA 3' terminus can effectively "reset" elongation by restoration of the DNA-RNA heteroduplex.

Although elongation arrest, 3' end misalignment, and retraction with restoration of alignment provide an attractive scenario for polymerase-nuclease function, our data suggest that the system possesses one additional element. The RNase H-like activity associated with yeast Pol III chooses to cut nascent RNA just 5' of unstable RNA-DNA base pairs. A base pair even more unstable than those we have studied would result from base misincorporation. This line of reasoning suggests that Pol III nuclease may have a proofreading function similar to the well known 3'-5' exonuclease of many DNA polymerases (18).

**MATERIALS AND METHODS**

Reagents and Enzymes—[γ-32P]ATP and [α-32P]NTPs were purchased from NEN Life Science Products and Amersham Corp. FPLC-purified ribo- and deoxy-NTPs were obtained from Pharmacia Biotech Inc. RNase A, poly(A), ribo-CpG, ApG, ApU, and UpA were obtained from Sigma; other ribooligonucleotides were obtained from Oligo Etc., Inc. M-280 Streptavidin Dynabeads were purchased from Dynal Inc., and DMT-Biotin-C6-PA was obtained from Cambridge Research Biochemicals. RNase CL3, endonuclease from Neurospora crassa, and DNase and RNase free bovine serum albumin were purchased from Boehringer Mannheim. Calf intestinal phosphatase, Vent DNA Polymerase, and T4 Polynucleotide kinase were obtained from New England Biolabs. RNase T1 and U1 were obtained from U. S. Biochemical Corp. The Klenow fragment in vitro mutagenesis system was obtained from Amer-sham Corp. Microcon 3 microcentrifuges were purchased from Ami-con. RNase free solutions, glassware and plasticware used in all experiments were prepared according to the instructions described in Ref. 19. DNA Templates for Transcription Reactions—M13mp18 plasmid (20).
carrying the 256-bp BamHI-BamHI SUP4 tRNA^{Tyv} fragment (21) was used to produce SUP4-genes triply mutated at positions +15, +16, and +17 by following the procedure of the Amersham Corp. Sculptor kit. To produce template with fewer than the original SUP4 number of pause sites, BamHI-BamHI fragment with mutant -Asn539-SUP4 (22) was inserted into m13mp18 vector to provide a Pol III template devoid of oligoA sequences, prior to the terminator, a chimeric gene was fabricated by placing an artificial terminator between a synthetic A block and the B block of yeast tRNA^{Tyv}-3 gene. SUP4 tRNA^{Tyv} sequences preceded the A block. The resulting ~1300-bp EcoRI-PstI fragment was subcloned into plasmid pUC119.

Resulting circular double-stranded plasmid DNAs were used as templates for polymerase chain reaction amplification of ~400–500 bp linear double-stranded fragments carrying original, mutant SUP4 tRNA^{Tyv} or chimeric Pol III genes. Oligonucleotides complementary to SUP4; devoid of oligoA sequences, prior to the terminator, a chimeric gene was inserted into m13mp18 vector. To provide a Pol III template, we used Microcon 3 microconcentration, which allowed us to get rid of molecules longer than 10 nucleotides in length or larger than ~3000 in molecular weight. Then 3’ products were desalted by G-10 gel filtration using spin columns pre-equilibrated with water. Digestion with cytidine-specific RNAase CL3 was performed by incubation in 20 mM HEPES-KOH (pH 7.9) in the presence of 8 mM urea at 50 °C for 1 h. Pyrimidine-specific reactions with RNAase A and guanosine-specific digestions with RNAase T1 were carried out in 50 mM Tris-HCl (pH 7.5) at 25 °C for 1 h. Adenosine-specific reactions with RNAase U2 were performed in 8 mM sodium citrate buffer (pH 3.5) in the presence of 25 °C at 1 h. Calf intestinal phosphatase was added, when necessary, and dephosphorylation was carried out at 25 °C for 5 min. Reactions were stopped by adding 1.3 v/v% of 98% formamide and heating at 95 °C for 30 min. Samples were loaded on sequencing size 20% polyacrylamide gels with appropriate marker ribooligonucleotides. Electrophoretic separation was carried out at 800 V, and after xylene cyanole had entered the gel, the voltage was being increased to 1200 V. This allowed us to obtain undistorted picture of short mono- and dinucleotide products with mobility that depended on length and sequence composition.

We identified short 3’ products by their mobility relative to the mobility of corresponding ribonucleotide markers and by digestion with sequence-specific RNAases (see Fig. 9). The main product had the mobility of PpyPy (lane 5) and was complementary to pG*pU and pA*pU, with minor ones *pUpG/*pPypPypPy and *pUpA (compare lanes 5 with lanes 9 and 11). Quantitative PhosphorImager analysis of various RNAase digests of the 3’ products, with or without dephosphorylation, allowed us to evaluate the ratios between different PpyPy products that could not be resolved electrophoretically. Because two lower bands of dephosphorylated products disappeared when they were subjected to RNase U2 digestion, we assumed that the intensity of the upper band corresponds to pU*pU and C*pU products, which are present in equal amounts. According to the number of radioactive phosphate groups, we assumed that the intensity of pU*pU product should be two times higher than that of pC*pU. Because we observed a ~10% difference between the total 32P content in all PpyPy dinucleotide and the intensities of pU*pU and pC*pU calculated for the amounts of U*pU and C*pU, we concluded that ~45% of PpyPy product consists of pU*pU, ~45% consists of pC*pU, and ~10% consists of pUpC. We did not observe any noticeable digestion with the cytidine-specific RNAase CL3 (see Fig. 9, compare lanes 6 and 8). We attributed this to the inability of this enzyme to work efficiently with substrates as short as dinucleotides. Dephosphorylation in the presence of guanosine-specific RNAase T1 and adenosine-specific RNAase U2 revealed that the upper dephosphorylated band corresponds to G*pU and A*pU products (see Fig. 9, compare lane 6 with lanes 10 and 12). We explain the appearance of an additional unidentified band during dephosphorylation in the presence of RNAase U2 (see Fig. 9, lane 12, band marked with an asterisk) by the known ability of this enzyme to catalyze oligonucleotide synthesis (25).

RESULTS

In their studies of Pol III elongation complexes made with highly purified Pol III and a 3’ extended template, Whitehall et al. (8) showed that stalled polymerase can efficiently degrade the intact transcript. From their experiment, the mRNA complex having UUCU at the 3’ end, these authors concluded that the hydrolytic activity cuts primarily in dinucleotide increments. They noticed that mononucleotides were also produced but at a lower frequency. To examine this process in detail, we studied cleavage reactions in Pol III transcription complexes stalled within many different sequence contexts. All
complexes were formed in a promoter-dependent system that required Pol III factors for initiation. The transcripts were elongated up to the first G of the SUP4 gene (see Fig. 1), and then the 17-mer ternary complexes were isolated magnetically, extensively washed, and “walked” to other positions of interest on the template. To generate additional types of 3'-terminal sequences, we mutagenized the SUP4 gene triply at positions +15, +16, and +17, thereby changing the TCT sequence in SUP4 to AAA in mutant template SUP4-AAA and to TTT in mutant SUP4-TTT (see Fig. 1).

Cleavage in the 3'-5' Direction in Stalled Ternary Complexes Occurs in a Sequence-specific Manner—To detect the cleavage products that remain in ternary complex (5' products) we labeled the 5'-proximal 17-mer with 32P. When the washed 17-mer complex was incubated with 7 mM Mg2+, both the rate and the pattern of cleavage were similar to those reported previously (8) (Fig. 2A). In this experiment the sequence of the transcript 3' end was the same as that made and cleaved by Whitehall et al. (8). Fig. 2B shows the cleavage of 17-mer formed on the SUP4-AAA template. The observed pattern of 5' products indicates cleavage at positions +16 and +14, with +14 as the preferred cut site. At first glance, this would appear to confirm the previously proposed dinucleotide mode of cleavage. However, the patterns of nuclease cleavage of 19- and 22-mer arrested products made on the SUP4 and SUP4-AAA templates (Figs. 3, A and B, and 4, A and B) suggest that preferential cleavage occurs at certain sites. Comparison of the gel patterns with the SUP4 and SUP4-AAA sequences shows that selective cleavage occurs at the 5'-phosphate groups of uridine residues, beginning with the uridine nearest the 3' end and then proceeding processively in the 3'-5' direction. When a cluster of at least three consecutive A residues is present, Pol III can also cut 5' to the central adenosine residue.

RNA in a Duplex with Template DNA Is a Substrate for the RNA Polymerase III Nuclease Activity—We used the SUP4-TTT template to examine further the basis for the preferential cleavage at uridine residues. SUP4-TTT transcripts limited to length 17 were made either with UTP, CTP, and ATP or with BUTP, CTP, and ATP. The mode of exonuclease degradation for the two stalled ternary complexes is shown in Fig. 5 (A and B). Cleavage of the bromouridine transcript occurs processively beginning next to the 3'-terminal bromouridine residue (position +17), whereas for uridine-containing RNA it occurs mainly...
from that nearest the 3'-19-mer, RNA cleavage occurs at each uridine residue starting
were formed in the presence of ATP, CTP, and [a-32P]UTP and after purification were chased to positions +19 and +22 with GTP or (UTP + GTP + ATP), respectively. Cleavage was performed with purified ternary complexes in the presence of 7 mM MgCl2 for the times indicated. Sites of cleavage are shown with arrows.

After 5 min of incubation with Mg2+, all bromouridine-containing ternary complexes were chased quantitatively into full-length RNA. In the case of uridine-containing 19-mer complexes, only products resulting from elongation (24- and 22-mer ternary complexes) were elongated. In these the uridine residues near-terminus of nascent RNA we attribute this difference in nuclease action between uridine and bromouridine has the effect opposite to that noted above. The results of cleavage of uridine- and bromouridine-containing 19-mer RNAs formed on SUP4-TTT are shown in Fig. 5 (C and D). In the case of uridine-containing 19-mer, RNA cleavage occurs at each uridine residue starting from that nearest the 3'-end and proceeds sequentially (Fig. 5C). The major cleavage sites observed were at positions +17, +16, and +15. In the case of bromouridine-containing 19-mer, similar sequential cleavage at positions +17, +16, and +15 was observed but to a significantly lesser extent at the last two positions than for the uridine-containing transcript (Fig. 5D). The major cleavage site was at position +17.

Hence, when a UUUU sequence was located at the very 3'-end of transcript (17-mer complex), it was not cleaved effectively, whereas for the 19-mer complex that has two guanosine residues at the 3'-end to stabilize the heteroduplex structure, this same oligouridine stretch was recognized as a nuclease substrate and cleaved at each uridylicate. The opposite effect was observed for the (BU)4 stretch. When located at the 3'-end, it was readily cleaved, whereas when it is followed by two guanosines (in the 19-mer) the nuclease cut only at the 3'-most bromouridylate (position +17). These data imply that the nuclease activity is sensitive to the secondary structure of RNA-DNA heteroduplex rather than to the chemical structure of the ribonucleotide bases in selecting cleavage sites.

Identification of the 3' Cleavage Products—To analyze the small products cleaved from the 3'-terminus of nascent RNA we made 19-, 22-, 24-, and 27-mer ternary complexes in which RNA was labeled with [a-32P]GTP. If the 5' products of nuclease action upon 19- and 22-mer stalled transcripts were generated by a single nuclease cut, we would have expected to observe pUpC*pG*pG as the 3' product of 19-mer cleavage and pUpA*pG and pUpC*pG as products of 22-mer degradation. Instead of these, significantly shorter 3' products were formed, suggesting cutting at additional positions (Fig. 6). To further analyze the 3' products formed concurrently with the 5' products, we performed cleavage experiments with 24- and 27-mer ternary complexes. In these the uridine residues near-
est the 3’ terminus were five and eight nucleotides away, respectively. Results on the cleavage of 24- and 27-mer ternary complexes are shown in Fig. 6 (C and D). Although a 5’ product was observed that corresponds to the expected cleavage at U20, 3’ products longer than two nucleotides were not detected. Hence, for all ternary complexes investigated we observed 5’ products corresponding to uridine-specific cleavage but multiple 3’ products that were shorter than expected.

The data on 3’ products formed during cleavage of 19- and 22-mer are summarized in Table I. According to their mobility in the 20% polyacrylamide gel the 3’ products formed by 19-mer cleavage are identified as *pG, pC*pG, and *pG*pG (Fig. 7A, lane 2). In the case of 22-mer cleavage *pG, pC*pG, pA*pG, and *pG*pG 3’ products were produced (panel A, lane 7). When transcripts were synthesized in the presence of [α-32P]UTP *pU, *pUpC, and *pUpA 3’ cleavage products were observed in the case of 19-mer (panel B, lane 2) and *pU, *pUpC, *pUpA, trace amounts of *pUpApG, *pUpC*pU, and/or pC*pUpC 3’ products were formed during 22-mer cleavage (panel B, lane 6).

To examine the possibility that the apparent contradiction between observed 5’ and 3’ products results from the action of an exogenous contaminating RNase activity, we analyzed the effect of two potential nuclease inhibitors. These were UpA and an exogenous contaminating RNase activity, we analyzed the between observed 5’ products formed by 19-mer ternary complexes, 17-mer complexes were chased to position +19 with GTP. Cleavage was performed with purified ternary complexes in the presence of 7 mM MgCl₂ for the times indicated. Sites of cleavage are shown with arrows.

RNA to short products occurs only within the ternary complex.

While Transcribing the SUP4 tRNA<sup>Tyr</sup> Gene RNA Polymerase III Produces Both Pre-tRNAs and Short Oligoribonucleotides—To test for a possible role of the nuclease function in overall Pol III enzymatic activity we carried out experiments to detect all products formed during single-round transcription of the SUP4 tRNA<sup>Tyr</sup> gene.

After the initiation of transcription and formation of arrested 17-mer complexes, all dissociable proteins as well as possible abortive initiation transcription products were removed by extensive washing of the bead-bound ternary complexes. Elongation was then restarted in the presence of heparin to prevent reinitiation. After transcription had gone on for various times, reactions were stopped and samples were loaded directly onto a 20% polyacrylamide gel. If during elongation without interruption cleavage events occur as they do for artificially arrested complexes, we would have expected to see short oligoribonucleotide products formed. In fact (Fig. 8A, lanes 9–12), large amounts of dinucleotides were formed concurrently with pre-tRNA molecules during transcription of the SUP4 tRNA<sup>Tyr</sup> gene. These consisted mainly of pUpU, pCpU, pApU, and pGpU (see “RNase Analysis of 3’ Cleavage Products” under “Materials and Methods” and Fig. 9). In addition, small amounts of pUpC, pUpA, and pUpG were formed. Each product possessed a 5’-phosphate group removable by phosphatase treatment. Because these products accumulate progressively with time and have the same structure as the cleavage products made by arrested complexes, we conclude that these oligoribonucleotides are produced by RNA polymerase III molecules traversing the gene.

RNA Polymerase III Carries out Multiple Cleavage and Re-synthesis Steps At Intrinsic Arrest Sites and Terminators—To test for a possible dependence of 3’ nucleolytic cleavage upon
the arrest of ternary transcription complexes at natural pausing sites, we made qualitative and quantitative comparisons of the 3' products formed on three different templates varying in the number of pause sites. These were a chimeric Pol III template (Table II), containing its only uridine residue at position +20; the -A36A37-SUP4 deletion mutant (22), which has only one UUU sequence before its terminator; and the SUP4 tRNA-Tyr gene, which has three UUU sequences within the transcribed region. When transcribed these three genes exhibit, as expected, no, one, and three strong pause sites, respectively. The sequences of these templates are listed in Table II.

Because the -A36A37-SUP4 template terminates transcription at the T6 sequence (positions +47–52) created by deletion (Fig. 8, lanes 5–8), there remains only one pause site, which gives the doublet bands at sites +33 and +34. As expected, the chimeric template gives no paused products, only terminated

the number of pause sites. These were a chimeric Pol III template (Table II), containing its only uridine residue at position +20; the -A36A37-SUP4 deletion mutant (22), which has only one UUU sequence before its terminator; and the SUP4 tRNA-Tyr gene, which has three UUU sequences within the transcribed region. When transcribed these three genes exhibit, as expected, no, one, and three strong pause sites, respectively. The sequences of these templates are listed in Table II.

Because the -A36A37-SUP4 template terminates transcription at the T6 sequence (positions +47–52) created by deletion (Fig. 8A, lanes 5–8), there remains only one pause site, which gives the doublet bands at sites +33 and +34. As expected, the chimeric template gives no paused products, only terminated
FIG. 9. Identification of 3′ products formed during transcription of SUP4 tRNA^Tyr gene. Lane 1, untreated 3′ products; lane 2, dephosphorylated with calf intestinal phosphatase 3′ products; lane 3, digestion with RNase CL3; lane 4, digestion with RNase CL3 followed by dephosphorylation; lane 5, untreated 3′ products; lane 6, dephosphorylated 3′ products; lane 7, digestion with RNase A; lane 8, digestion with RNase A followed by dephosphorylation; lane 9, digestion with RNase T1; lane 10, digestion with RNase T1 followed by dephosphorylation; lane 11, digestion with RNase U2; lane 12, digestion with RNase U2 followed by dephosphorylation.

Table II

| Chimeric RNA pol III template | +1 | +17 | +40 | +46 |
|------------------------------|----|-----|-----|-----|
| AACAATTAAATACTCTCGGTAGCCAGCGGAGGGCAGGTTTTTTTGTAACTG |

| −A36A37-SUP4 template | +1 | +17 | +31 | +47 | +52 |
|-----------------------|----|-----|-----|-----|-----|
| AACAATTAAATACTCTCGGTAGCCAGGAGGGCAGGTTTTTTTATCA |

| SUP4 tRNA^Tyr template | +1 | +17 | +31 | +47 | +52 | +69 |
|-----------------------|----|-----|-----|-----|-----|-----|
| AACAATTAAATACTCTCGGTAGCCAGGAGGGCAGGTTTTTTTATCAACAATTTGTTTACGGAATTTCTCATAGGTGAGATCGG |

Discussion

The RNA polymerase-related nucleases previously described in detail exhibit a dual nature (1–8, 28). The ribonuclease activities associated with Escherichia coli RNA polymerase and with eukaryotic Pol II both degrade nascent RNA sequentially from the 3′ terminus, releasing, in many instances, mono-, di-, and trinucleotide split products (1, 4, 5, 28). In that sense, their associated nuclease activities behave as processive 3′-5′ exonucleases. However, depending both upon the type of elongation block imposed and the elongation factor that is present, both nucleases (1, 4, 6) can produce longer (7–14 nucleotides) 3′ products.

For retraction by RNA polymerases, the processive cleavage that occurs starting from the 3′ terminus is indicative of exonucleolytic degradation, whereas the ability to generate long 3′ products under other conditions implies an endonucleolytic mode of action.

The Substrate for Ribonuclease Activity—Our study of the Pol III nuclease has concentrated mainly upon the roles that RNA sequence and RNA-DNA secondary structure play in determining cleavage site selectivity. At each arrest site we studied, newly formed shortened transcripts were successfully elongated up to the full-length product, independently of the length of RNA digested away. The resumption of transcription by the site of cleavage is held in an RNA-DNA heteroduplex. This raises a question as to the structure of the nuclease substrate. To answer that question we compared the pattern of cleavage for a 17-mer transcript having four uridine residues at the 3′ end with that of bromouridine-containing 17-mer transcript. Although the bromouridine transcript was cleaved processively starting at the 3′-most bromouridine, cleavage of the uridine-containing transcript oc-
curled mainly 5’ to the penultimate uridine and to a lower overall extent. The exceptionally weak heteroduplex formed by rUdA base pairs (17) makes it likely that the 3’-terminal uridine residue of uridine-containing 17-mer exists mostly in an unpaired state. Because the terminal uridylicate is not recognized as a substrate for cleavage, we conclude that the nuclease cleaves only RNA that is heteroduplexed to template DNA. In bromouridine-containing transcripts, stronger dA-rBU H-bonding ensures that the 3’-terminal residue will be heteroduplexed to DNA. Consequently, in these complexes it is recognized as a cleavage substrate. The possibility that the observed difference is merely due to different rates of cleavage at uridine and bromouridine residues is excluded by the fact that in the degradation of 19-mers having internal U₄ or (BU)₄ sequences followed by two guanosines that stabilize the secondary structure, uridine to bromouridine substitution has the opposite effect. In the case of the uridine-containing 19-mer, the nuclease scours backwards from two well paired 3’-terminal guanosines to the neighboring (base paired) U17 residue, initially cutting just 5’ of this uridine and then effectively cutting at each residue in the uridine stretch (at positions +16, +15, and +14). At the same time, the efficiency of cleavage of bromouridine-containing 19-mer is considerably lower, and propagation in the reverse direction is limited to the closest bromouridine-containing 19-mer is considerably lower, and propagation in the reverse direction is limited to the closest bromouridine-containing transcript terminus to the polymerization site of the enzyme. A concerted reattachment of the newly formed 3’ end is caused by noncomplementarity of the RNA and DNA 9 base pairs (17) makes it likely that the 3’-terminal fragment is still in a hybrid with DNA undergoes further cutting, requiring 5’-3’ translocation of the nuclease active center with a concerted reattachment of the newly formed 3’ end to DNA to

The Relation of 5’ Products to 3’ Products—For most of the ternary complexes we investigated, the 5’ products correspond to uridine-specific cleavage only, whereas the 3’ products indicate that multiple cuts occur within 3’-terminal sequences that lack uridylicates. Several possible explanations for this come to mind. The first involves a single mode of nuclease action, whereby rapid cleavage occurs in sequential short steps. To explain our data by this mechanism requires that there be a means whereby sequential nuclease action on the remaining 5’ fragments is drastically slowed immediately after the removal of a mono- or dinucleotide that bears 5’-UMP. Alternatively, the apparent contradiction between the observed patterns of released 3’ products and retained 5’ products might be explained by bidirectional movement of the nuclease along its RNA-DNA hybrid substrate. Initially, the nuclease active site translocates in a 3’ to 5’ sense, scanning for the first internal uridine residue. Once a cut has been made 5’ to uridine, the distal RNA fragment that is still in a hybrid with DNA undergoes further cutting, requiring 5’-3’ translocation of the nuclease active center with a concerted reattachment of the newly formed transcript terminus to the polymerization site of the enzyme.

Our attempts to trap hypothetical 5’ and 3’ intermediates by varying the temperature from 0 to 30 °C and by sampling the reaction at times as short as 1 s were not successful. To resolve between the possibilities mentioned above, new methods of analysis will be needed.

Oligonucleotides Made at the 3’ Terminus during Elongation—We have observed that RNA polymerase III carries on multiple cleavage resynthesis steps at the arrest sites and terminator, producing a large amount of short cleavage products, predominantly with uridines at their 5’ ends (pNpU). The direct correlation of the amount and composition of 3’ products with the number and the sequences of the arrest sites further supports our conclusion that cleavage events are mostly associated with slow progression of RNA polymerase III through oligouridine tracts. When elongation is impeded, the nuclease activity of RNA Pol III removes the frayed 3’ end of RNA to restore proper alignment of the transcript 3’ terminus so that elongation may resume. Analogous to this situation is the one that exists when a misincorporated base is present at the end of a growing transcript. In that case, misalignment of the RNA 3’ end is caused by noncomplementarity of the RNA and DNA bases that are in opposition. By resetting such mispaired termini, the Pol III nuclease can act as a part of proofreading apparatus.

Similar suggestions for human RNA Polymerase II were made previously by Reines and co-workers (2, 10–12), who observed that an increase of transcription efficiency through natural pause sites and DNA-bound protein roadblocks was caused by transcription factor IIIS activation of multi-round cleavage and resynthesis. In a recent study of the possible editing role of the nuclease activity of RNA Polymerases, Erie et al. (29) showed that the presence of GreA elongation factor reduces the misincorporation of UTP by E. coli RNA polymerase (>50%) by preferential rapid cleavage of terminally misincorporated transcripts. This and several other recent observations (28, 30) point to possible proofreading by RNA polymerases led to a reconsideration of the traditional viewpoint (31) that transcription is an unedited process.

Acknowledgments—We thank Drs. Stuart Clarkson, Natalie Lin-Marq, and David Price for very helpful suggestions about magnetic bead immobilization of ternary transcription complexes. We also thank Dr. G. N. Zecherlie for providing the m15mpl8 plasmid carrying the SUP4 gene and D. M. Chudzik for excellent assistance in construction of the chimeric tDNA template.

REFERENCES

1. Surrat, C. K., Milan, S. C., and Chamberlin, M. J. (1991) Proc. Natl. Acad. Sci. U. S. A. 88, 7983–7987
2. Reines, D. (1992) J. Biol. Chem. 267, 3795–3800
3. Borukhov, S., Poljakov, A., Nikiforov, V., and Goldfarb, A. (1992) Proc. Natl. Acad. Sci. U. S. A. 89, 8899–8902
4. Borukhov, S., Sagitov, V., and Goldfarb, A. (1993) Cell 72, 459–466
5. Isban, M. J., and Luse, D. S. (1993) J. Biol. Chem. 268, 12864–12873
6. Isban, M. J., and Luse, D. S. (1993) J. Biol. Chem. 268, 12874–12885
7. Hagler, J., and Shuman, S. (1993) J. Biol. Chem. 268, 2166–2173
8. Whitehall, S. K., Bardeleben, C., and Kasavneti, G. A. (1994) J. Biol. Chem. 269, 2299–2306
9. Tschonner, H. (1996) Proc. Natl. Acad. Sci. U. S. A. 93, 12914–12919
10. Reines, D., Ghanouni, P., Li, Q., and Mote, J., Jr. (1992) J. Biol. Chem. 267, 15516–15522
11. Reines, D., and Mote, J., Jr. (1993) Proc. Natl. Acad. Sci. U. S. A. 90, 1917–1921
12. Mote, J., Jr., Ghanouni, P., and Reines, D. (1994) J. Mol. Biol. 236, 725–737
13. Platt, T. (1986) Annu. Rev. Biochem. 55, 339–372
14. Reines, D., Wells, D., Chamberlin, M. J., and Kane, C. M. (1987) J. Mol. Biol. 196, 399–412
15. Geiduschek, E. P., and Tocchini-Valentini, G. P. (1988) Annu. Rev. Biochem. 57, 873–914
16. Kerppola, T. K., and Kane, C. M. (1991) FASEB J. 5, 2833–2842
17. Martin, F. H., and Tinoco, I., Jr. (1988) Nucleic Acids Res. 8, 2295–2299
18. Kornberg, A. (1980) DNA Replication, pp. 127–130, W. H. Freeman and Co., San Francisco
19. Sambrook, J., Fritsch, E. F., and Maniatis, T. (1989) Molecular Cloning: A Laboratory Manual, 1st ed., Vol. 1, Sections 7.3–7.5, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
20. Messing, J., Gonenborn, B., Muller-Hill, B., and Hans-Hoschneider, P. (1977) Proc. Natl. Acad. Sci. U. S. A. 74, 3642–3646
21. Baker, R. E., Gabrielson, O., and Hall, B. D. (1986) J. Biol. Chem. 261, 5275–5292
22. Kurjan, J., and Hall, B. D. (1982) Mol. Cell. Biol. 2, 1501–1513
23. Allison, D. S., Gol., S. H., and Hall, B. D. (1983) Cell 34, 655–664
24. Cameron, V., and Uhlenebeck, O. C. (1977) Biochemistry 16, 5120–5126
25. Uchida, T., Arima, T., and Egami, F. (1970) J. Biochem. 67, 91–102
26. Holenberg, N., Guerin, D., Cleveland, D., and Truchet, H. (1981) Cell 27, 563–572
27. Jacobs, B. L., Mijamoto, N. G., and Samuel, C. E. (1988) J. Interferon Res. 8, 617–631
28. Wang, D., and Hawley, D. K. (1993) Proc. Natl. Acad. Sci. U. S. A. 90, 843–847
29. Ernie, D. A., Huisjiejadivadi, O., Young, M. C., and von Hoppel, P. H. (1993) Science 262, 867–873
30. Oriova, M., Newlands, J., Das, A., Goldfarb, A., and Borukhov, S. (1995) Proc. Natl. Acad. Sci. U. S. A. 92, 4566–4600
31. Kornberg, A. (1980) DNA Replication, pp. 232–233, W. H. Freeman and Co., San Francisco