GreA- and GreB-induced transcript cleavage drives reverse translocation of *Escherichia coli* RNA polymerase on a DNA template in the absence of NTPs (Feng, G.-H., Lee, D. N., Wang, D., Chan, C. L., and Landick, R. (1994) *J. Biol. Chem.* 269, 22282-22294, accompanying report). During transcript elongation, the sizes of the DNA footprint and the single-stranded transcription bubble vary markedly among transcription complexes halted at different template positions. To test whether transcription complex intermediates formed during transcript cleavage-induced reverse translocation also display heterogeneous conformations at different template positions, we examined the structures of two different transcription complexes before and after GreA treatment. Transcription complexes halted at position +16 after initiation at the T7A1 promoter or paused at the trpL pause site exhibited strong blocks to transcript cleavage after removal of 6 to 10 nucleotides. In both cases, the downstream contact between RNA polymerase and DNA moved little during transcript cleavage, thereby increasing its distance from the active site, whereas the upstream DNA contact and the borders of the transcription bubble moved in approximate register with the transcript 3'-end. The backward movements of halted *E. coli* RNA polymerase are similar to a recently postulated model for discontinuous translocation during transcription, but differ from those reported for arrested RNA polymerase II transcription complexes.

Both bacteria and eukaryotes contain proteins that can stimulate cleavage near the 3'-end of the nascent transcript in transcription complexes halted *in vitro* in the absence of NTPs* (Surratt, 1991; Borukhov et al., 1992, 1993; Izbán and Luse, 1992a, 1992b, 1993a, 1993b; Kassavetis and Geiduschek, 1993; Reines, 1992; Wang and Hawley, 1993; Gu, 1993; Guo, 1993). Two modes of cleavage have been described. One yields one to three nt fragments and the other produces larger fragments, up to at least 12 nt in size. In bacteria, the GreA protein induces only short fragment cleavage, whereas GreB induces both modes of cleavage (Borukhov et al., 1993). In eukaryotes, the TFIIIS elongation factor (S-II) stimulates either small or large fragment cleavage, depending on reaction conditions and the particular complex examined (Izbán and Luse, 1993b; Wang and Hawley, 1993). Although the biological function of transcript cleavage is not known, *in vitro* it can both increase transcriptional fidelity (Erie et al., 1993) and allow RNA polymerase to transcribe through strong blocks to elongation that can otherwise arrest the enzyme on the DNA (Borukhov et al., 1993; Izbán and Luse, 1992b; Reines, 1992; Wang and Hawley, 1993).

When NTPs are absent, TFIIIS, GreA, and GreB can stimulate multiple rounds of transcript cleavage leading to extensive backward translocation of RNA polymerase on a DNA template (Wang and Hawley, 1993; Feng et al., 1994). The backward moving RNA polymerase maintains faithful register on the DNA template and resumes transcription accurately upon addition of NTPs. *Both Escherichia coli* RNA polymerase and human pol II pause during backward translocation. The rate of backward translocation and sites of pausing are influenced both by secondary structures in the nascent transcript and the structure of the DNA template (Feng et al., 1994).

Backward translocation of RNA polymerase is particularly interesting in light of recent findings that suggest forward movement of RNA polymerase during transcription occurs in steps greater than single nucleotide addition. Until recently, the transcription complex was considered a relatively static structure which maintains a DNA footprint and single-stranded transcription bubble of fixed dimensions by sliding forward one bp along the DNA upon each nucleotide addition, melting one bp at the leading edge of the transcription bubble and reannealing another at the lagging edge.

However, recent observations suggest chain elongation may involve multiple conformations and movement of the transcription complex or its constituent parts in cycles greater than single nucleotide addition (Krummel, 1990; Krummel and Chamberlin, 1992; Lee and Landick, 1992; Das, 1993; Mustaev et al., 1993; Chamberlin, 1994; Chan and Landick, 1994). RNA polymerase may add up to 10 nt to the transcript while changing its upstream, but not downstream, DNA contact and then undergo an inchworm-like forward movement to begin a new cycle of NTP addition (Krummel and Chamberlin, 1992; Das, 1993; Chamberlin, 1994; see "Discussion").

Given these observations, we wished to determine if different conformations of the transcription complex form at different template positions during reverse translocation. By using exonuclease III to probe the boundaries of contact between RNA polymerase and DNA and KMnO$_4$ to probe the single-stranded transcription bubble, we found changes in the structure of the transcription complex during reverse translocation which are consistent with the "inchworm" model for RNA polymerase movement. Under "Discussion," we compare these findings to the lack of footprint change observed for transcript cleavage in arrested pol II transcription complexes (Gu et al., 1993) and

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The abbreviations used are: NTPs, nucleoside triphosphates; bps, base pair(s); DEPC, diethylpyrocarbonate; HPLC, high-pressure liquid chromatography; nt, nucleotide(s); PCR, polymerase chain reaction; pol II, RNA polymerase II.
suggest the difference reveals important information about the mechanism of transcriptional arrest.

**MATERIALS AND METHODS**

**Reagents and Enzymes—**Km\textsubscript{N0}, dimethyl sulfate, piperdine, hydrazine, acrydine orange (sodium salt), NTPs, adenosine triphosphate (ApU dinucleotide), Seaphorase CL-6B, and RNase A were purchased from Sigma. Polynucleotide kinase was purchased from New England Biolabs. 3'-deoxynucleotides from Boehringer Mannheim, HPLC-puriﬁed NTPs from Pharmacia LKB Biotechnology Inc., [\textalpha\textsuperscript{32}P]GTP, [\textalpha\textsuperscript{32}P]ATP, and [\textgamma\textsuperscript{32}P]ATP from Amersham, and exonuclease III from New England Biolabs. RNA polymerase and GreA were puriﬁed as described by Feng et al. (1994).

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FIG. 1. DNA template and RNA transcripts in G16 and trp paused transcription complexes before and after GreA treatment. A, DNA template used in the study and scheme for amplification from pDNL279 by PCR (see "Materials and Methods"). The sequence of the transcript present in trp paused transcription complexes is shown above the template schematic. The bases in bold are present in the G16 complexes. The trp pause RNA hairpin is drawn based on analogy to base substitution mapping of the his pause RNA hairpin (Chan and Landick, 1993). B, autoradiogram of 32P-labeled RNA transcripts present in G16 (lanes 1 and 3) and trp paused transcription complexes (lanes 5 and 7) before and after GreA treatment (see "Materials and Methods"). Portions of each sample were incubated with 500 μM NTPs for 2 min (lanes 2, 4, 6, and 8) to assess the ability of transcription complexes to resume transcription before and after GreA treatment.

T1 and T11 on the template strand became reactive (Fig. 2). These results suggest the downstream edge of the transcription bubble moved five to seven bp upstream upon transcript cleavage, in approximate register with the new transcript 3’-end. The upstream edge of the bubble moved at least one, but no more than four nt, since T1, but not T(-4), on the template strand became reactive.

The transcription bubble of trp paused complexes also moved in approximate register with the new 3’-end of the nascent transcript (G71). KMnO₄ modified T70, T72, T74, T75, and T81 in the non-transcribed strand of trp paused transcription complexes (Fig. 3, A and C). Reaction at T81 is weak and not easily seen in Fig. 3. It is apparent on the original autoradiogram and clearly seen in Fig. 4 of Lee and Landick (1992). In that work, we also found that DEPC modifies A73, A77, and A80 but not A68 or A86. Together, the DEPC and KMnO₄ modifications suggest that the single-stranded region in trp paused transcription complexes is 13-16 nt, from G69 or T70 to between G82 (the position of nucleotide addition) and G84. After GreA-induced cleavage of the transcript back to G71, T61, and T62 in the non-transcribed DNA strand became susceptible to KMnO₄ modification, the reactivity of T74 decreased significantly and T75 and T81 became unreactive (Figs. 3, B and C). Little or no change in KMnO₄ modification of the template strand occurred, but this may reflect relocation of the eight-nt protected region (see below). These data suggest the single-stranded region in the GreA-G71 complexes was 13 nt, from T61 to A73. Thus, upon removal of 10 nt of transcript from the trp paused transcription complex by GreA-induced cleavage, the upstream edge of the transcription bubble moved back eight or nine bp and the downstream edge moved back 10-12 bp.

An Eight-nt Region Upstream of the Nucleotide Addition Site on the Template Strand Was Protected from Modification—The eight-nt protected region detected upstream of the nucleotide addition site on the template strand in the 12 transcription complexes we studied previously was attributed either to the presence of an 8-10-bp RNA-DNA hybrid or to contacts between the template strand and RNA polymerase (Lee and Landick, 1992). We were interested in how this protected region changed after GreA-induced transcript cleavage, since removal of the RNA from an RNA-DNA hybrid might expose the DNA to modification by KMnO₄. However, KMnO₄ modification of the GreA-treated complexes was consistent with relocation of...
the protected region upstream of the new 3' -end of the nascent transcript.

After GreA-induced nascent transcript cleavage in G16 complexes, T7 and T5 were no longer reactive, consistent with the presence of a relocated eight-nt protected region (Figs. 2, B and C). On the template strand of trp paused complexes, T73 was modified both before and after GreA treatment (Figs. 3, B and C). In the trp paused complexes, T73 would be upstream of the eight-nt protected region, whereas T77 and T80, both of which did not react with K_MnO_4, would be within it. In the GreA-G71 complexes, T73 corresponds to a position two nt downstream of the transcript 3'-end and the eight-nt protected region is reflected by protection of T68. Although the locations of protected bases in the template strands of the GreA-G10 and GreA-G71 complexes did not establish that the region of protection was still eight nt, some shift in the protected region must have occurred to make these bases unreactive. Thus, our findings are consistent with the repositioning of an invariant eight-nt protected region during reverse translocation. If protection is conferred by pairing with the DNA template strand, apparently the RNA-DNA hybrid must reform upstream as nucleotides are cleaved from the 3'-end of the transcript.

We conclude that GreA-induced transcript cleavage in two different transcription complexes, one halted by nucleotide deprivation and one stopped at a natural pause site, is accompanied by an upstream shift of the transcription bubble. Both edges of the single-stranded region, as well as the intervening eight-nt protected region on the template strand, moved upstream in approximate register with the transcript 3'-end, although the size of the bubble was reduced after transcript cleavage in the G16 complex.

**Upon GreA-induced Transcript Cleavage, the Upstream Edge of the Transcription Complex Relocated in Approximate Register with the Transcribed 3'-End, but the Downstream Edge Moved Only Slightly**—To assess how contacts between RNA polymerase and DNA change during GreA-mediated transcript cleavage, we determined the positions on the DNA at which E. coli exonuclease III, a processive 3'-5' exonuclease, stopped when it encountered RNA polymerase in the four different transcription complexes. This technique has been used previously to locate the edges of elongating RNA polymerase on DNA (Landick, 1987; Metzger et al., 1989; Gu et al., 1993).

In the G16 complexes, the downstream edge of the RNA polymerase footprint, as detected by exonuclease III digestion of the nontranscribed strand, was A29 (Figs. 4, A and C). In GreA-G10 complexes, formed by GreA-induced transcript cleavage of six to seven nt from the G16 complexes, this boundary shifted to A27, or only two bp in the first barrier to exonuclease digestion. The upstream edge of the RNA polymerase footprint in the G16 complexes, as detected by exonuclease III
digested of the template strand, was T1, with a weak stop evident at C(-3) (Fig. 4, B and C). In GreA-G10 complexes, this boundary shifted upstream by five bp, as indicated by a shift in the first strong barrier to exonuclease digestion to G(-5), with a weak stop at G(-9). Thus, both the upstream and downstream contacts between RNA polymerase and DNA changed when six to seven nt were removed from the nascent transcript of G16 complexes by GreA-induced cleavage. Although neither shifts by six to seven bp, the upstream contact shifted more (five bp) than the downstream contact (two bp).

In addition to the edges of the transcription complexes, which were clearly identifiable on both the template and non-transcribed strands by the shift in the first barrier to exonuclease III upon GreA treatment, we also observed some positions of exonuclease III stopping that were unchanged by transcript cleavage. These constant bands, for instance those at G15 and A13 in the non-transcribed strand (Fig. 4A), corresponded to positions where prominent pausing was observed in control exonuclease III digestions of labeled DNA in the absence of RNA polymerase (data not shown), and probably reflect pausing on contaminating free DNA or after exonuclease-induced displacement of the transcription complex.

We also observed biased shifting of the upstream contact between RNA polymerase and DNA in the trp paused transcription complexes and GreA-G71 complexes. Here the downstream edge of the footprint, as indicated by the first strong exonuclease III barrier on the non-transcribed strand, shifted three bp, from A92 to G89, after removal of 10 nt from the nascent transcript upon incubation with GreA (Fig. 5, A and C). Weaker protection to T98 was evident in the paused complex, as reported previously (Landick and Yanofsky, 1987; see "Discussion"), which would give a shift of nine bp. The upstream edge of the transcription complex on the template strand shifted from T68 to between C58 and T60 upon GreA treatment (Figs. 5, B and C; note that 32P-labeled U81 or G71 RNAs are present in these samples). Thus, GreA-induced removal of 10 nt from the nascent transcript in trp paused transcription complexes appeared to be accompanied by relocation of the upstream RNA polymerase-DNA contact in approximate register with the new transcript 3'-end, whereas the strong downstream contact remained nearly fixed.

**DISCUSSION**

In this report we have presented evidence for conformational rearrangement of the ternary transcription complex upon GreA-stimulated cleavage of the nascent transcript. Two different transcription complexes positioned on a DNA template that contained a T7 A1 promoter-trp operon fusion, one halted by NTP deprivation at G16 and another paused at the trp leader pause site, displayed changes in location and dimension of both
Fig. 4. Exonuclease III digestion of G16 and GreA-G10 complexes. A, digestion with exonuclease III of the 32P-end-labeled, nontranscribed strand in transcription complexes (see “Materials and Methods”). Lanes marked G, AG, and CT correspond to DNA sequencing reactions. Lanes marked G16 and GreA-G10 correspond to G16 and GreA-G10 complexes, respectively. Note that exonuclease-digested DNA strands migrate one nucleotide slower than the corresponding bands in the sequencing ladders because the sequencing reactions remove the bases whereas exonuclease does not. B, same as A except the template strand was 32P-end-labeled. C, sequence of the DNA template. Arrows mark the bases present at the ends of DNAs that resisted exonuclease III digestion in the transcription complexes.

the DNA footprint and single-stranded transcription bubble when 6–10 nt were hydrolyzed off the transcript 3′-end. These different conformations appear to be intermediates in the reverse translocation reaction.

*Forward Translocation Can Occur Discontinuously during Transcription*—To understand reverse translocation, we must first examine the current understanding of forward translocation during RNA chain elongation. Data from several different studies suggest this can be discontinuous, both in movement of RNA polymerase on the DNA template and in the size of the transcription bubble (see Das, 1993; Chamberlin, 1994; Chan and Landick, 1994). Transcription complexes halted at successive positions within the initially transcribed 35 bp of two different transcriptional units exhibit discontinuous shifts in the RNA polymerase footprint on DNA (Krummel and Chamberlin, 1992). During addition of up to eight nucleotides, the downstream edge of the footprint remains relatively constant, while the upstream edge of the footprint moves in approximate synchrony with the location of the transcript 3′-end. Further elongation causes a ~10-bp shift in the downstream edge of the footprint.

Likewise, the susceptibility of DNA bases to modification by single strand-specific reagents suggests that the transcription bubble is not constant in different halted elongation complexes, but varies between 12 and 20 nt (Krummel, 1990; Lee and Landick, 1992). Although only a limited set of complexes have been studied, most near the promoter, even complexes up to 100 bp from a promoter exhibit changes from 14 to 18 nt in the single-stranded region when “walked” forward on a DNA template (Lee and Landick, 1992).

To explain these discontinuous changes in DNA footprint and transcription bubble, Chamberlin has proposed an inchworm model for RNA polymerase movement (Chamberlin, 1994; Chan and Landick, 1994; Das, 1993). The model supposes that RNA polymerase is a flexible hinge whose upstream and downstream DNA contacts can separately lock and slide. The active site is anchored to the portion of the enzyme that makes the upstream contact to DNA. The RNA transcript is threaded through an upstream site that also can lock and slide and a 3′-proximal site that can be filled to varying extent. One translocation cycle of RNA polymerase consists of filling the 8-10-nt, 3′-proximal RNA site, concomitant with progressive advance of the upstream, but not downstream, DNA contact. Movement of the upstream DNA contact appears to be accompanied by enlargement of the transcription bubble, until a small footprint, large bubble conformation is reached when the 3′-proximal RNA site is full (Chamberlin, 1994). At this point, discontinuous forward movement of the downstream edge of the footprint and internal rearrangement in the complex reestablishes the starting conformation.

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2 M. J. Chamberlin, personal communication.
GreA-induced Reverse Translocation

Fig. 5. Exonuclease digestion of trp paused and GreA-G71 complexes. A, digestion with exonuclease III of the 32P-end-labeled, non-transcribed strand in transcription complexes (see “Materials and Methods”). Lanes marked G, A > G, and CT correspond to DNA sequencing reactions. Lanes marked paused and GreA-G71 correspond to trp paused and GreA-G71 complexes, respectively. B, same as A except the template strand was 32P-end-labeled. RNA transcripts present in the complexes migrate in the region of interest in these gels and are marked on the right as RNA-U81 and RNA-G71. C, sequence of the DNA template. Arrows mark the bases present at the ends of DNAs that resisted exonuclease III digestion in the transcription complexes.

GreA-induced Transcript Cleavage Causes Rearrangement of the Transcription Complex—Both the upstream and downstream edges of G16 and trp paused transcription complexes shift upon transcript cleavage (Fig. 6). For both complexes the shift in the upstream edge more closely matches the amount of RNA removed by cleavage. For the paused complex, however, the downstream shift is either three or nine bp, depending on the edge assigned for the complex before cleavage (Fig. 6). This ambiguity in position of exonuclease stopping also was evident to lesser extents in the other complexes and may reflect either heterogeneity in the contacts among different complexes in the population (lack of complete phasing among complexes, see Chan and Landick (1994)) or stuttering by exonuclease III as it encounters RNA polymerase. In either case, the dual edge of the paused complex is interesting given its reduced rate of transcript elongation. It is tempting to speculate that the two contacts represent complexes in either paused or elongating conformations, since the stop at T92 was much more pronounced in the presence of NTPs (see Fig. 2 of Landick and Yanofsky (1987); T92 corresponds to T109 on the wild-type trp template).

Given the apparently discontinuous changes in the edges of the complexes upon transcript cleavage, it is possible that inchworm-like movements of RNA polymerase occur in both the forward and reverse directions. Our measurements of the sizes of the transcription bubbles in the different complexes also is consistent with a slight contraction of the transcription bubble upon transcript cleavage (Fig. 6).

Upstream Movement upon Transcript Cleavage in Halted E. coli Transcription Complexes Contrasts with the Lack of Movement Reported upon Transcript Cleavage in Arrested Complexes Containing RNA Polymerase II—In contrast to our conclusion, Gu et al. (1993) report that upstream movement of RNA polymerase does not occur upon removal of seven to nine nt from the transcript in pol II transcription complexes arrested at site Ia in the human histone H3.3 gene. This discrepancy can be explained by a potential difference in the conformation of halted versus arrested transcription complexes first noted by Krummel and Chamberlin (1992). They observed that the upstream DNA contact of an arrested E. coli transcription complex was shifted upstream relative to an active transcription complex positioned at the same template position. Based on this observation and the features of the inchworm model, Chamberlin (1994) proposes that transcriptional arrest may occur when the upstream contact between RNA polymerase and DNA slips backward and displaces the active site from the
transcript 3'-end, perhaps repositioning it over an internal phosphodiester bond in the transcript (see also Feng et al. (1994); Rudd et al. (1994)).

Both complexes we tested were able to resume transcription spontaneously upon addition of NTPs, whereas the arrested pol II complex examined by Gu et al. (1993) cannot (also true for arrested E. coli transcription complexes; see Krummel, 1990; Arndt and Chamberlin, 1990; Krummel and Chamberlin, 1992a, 1992b; Borukhov, 1992; Chamberlin, 1994). Thus, comparison of our results to those of Gu et al. (1993) is consistent with Chamberlin's proposal. Transcriptional arrest may occur when a portion of RNA polymerase "looses its grip" on the DNA and slides backward and out of register with the transcript 3'-end. After arrest, optimal alignment of the catalytic residues around the phosphodiester bond may be mediated by cleavage factor binding (see Feng et al. (1994)), which would explain the requirement for TFIIS or GreB to effect cleavage and reactivation of arrested complexes.

The 3'-Proximal Region of DNA Template Is Protected from KMnO4 after Transcript Cleavage—Previously, we observed that thymidines within an eight-nt region immediately upstream from the nucleotide addition site in 12 different transcription complexes were protected against KMnO4 modification. Similar protection has been observed for transcription complexes paused at the +16 lambda late operon pause site in vitro (Kainz and Roberts, 1992) and, against phenanthroline copper, for halted pol II elongation complexes (Linn and Luse, 1991). Protection could result from hydrogen bonding between the RNA transcript and DNA template in this region, since per-manganese must approach thymine orthogonal to the plane of the ring to react and is sterically excluded by adjacent stacked bases. However, some cases of KMnO4 sensitivity within the eight-nt region have been reported (Chamberlin, 1994; Krummel, 1990) and DEPC, another single-strand-specific modification reagent, was observed to react within this region of the trp paused transcription complex (Lee and Landick, 1992). These results may indicate the eight-nt protected region is an unusual RNA-DNA structure (Lee and Landick, 1992) or conferred by protein contacts to the DNA template (Chamberlin, 1994).

In this light, protection of the eight-nt region of template in the GreA-trp G71 and GreA-G10 complexes is important. Apparently, whatever inhibits base modification within the eight-nt 3'-proximal region of DNA template shifts upstream upon transcript cleavage. If, as we have argued elsewhere, the trp pause RNA hairpin extends to the C50-G71 bp and contributes to the block to further transcript hydrolysis at G71, no RNA bases would be available to pair with the DNA strands, at least in this complex. Further studies using templates on which the pause hairpin is altered may help resolve this question.

The Transcription Bubble Probably Reanneals Outside the Transcription Complex—When we first examined the KMnO4 reactivity of bases in the nontranscribed strand of trp paused transcription complexes, we noted that T70, the furthest upstream reactive base, is also the position at which A-exonuclease stops when it digests the strand 5' to 3' (Landick and Yanofsky, 1987; Lee and Landick, 1992). This suggested to us that the single-stranded bubble reanneals as the strands emerge from the transcription complex. Although we did not use A-exonuclease in the present study, the furthest upstream reactive thymidine and the position at which exonuclease III stops when it encounters the transcription complex are within at most a few nucleotides in all four complexes we examined (Fig. 6). Given the random placement of bases susceptible to modification, this coincidence argues strongly for reannealing of the two DNA strands after they emerge from the upstream side of the transcription complex. Both A-exonuclease and exonuclease III are double strand-specific and may stop digestion at the upstream edge of the transcription complex not because they contact the enzyme, but because the DNA strands become separated.

Conclusion—We have reported here that reverse translocation, like transcription, involves passage of RNA polymerase through different conformations at different template positions. The changes we observed can be accommodated by reversal of the inchworm model for RNA chain elongation, although no contacts were unchanged after transcript cleavage. When compared with the lack of change observed upon transcript cleavage in arrested pol II transcription complexes, our results support the idea that arrest occurs by inappropriate upstream slippage of the upstream DNA contact and the active site.

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Fig. 6. Summary of changes in RNA polymerase-DNA contacts upon transcript cleavage. Sequences for the DNA and RNA (in italics) strands are shown for all four complexes studied. The darkened regions represent the portions of DNA protected from exonuclease III digestion by RNA polymerase. The white regions between the strands are shown for all four complexes studied. The darkened RNA bases would be available to pair with the eight-nt region of the trp RNA transcript and DNA template in this region, since per-manganese must approach thymine orthogonal to the plane of the ring to react and is sterically excluded by adjacent stacked bases. However, some cases of KMnO4 sensitivity within the eight-nt region have been reported (Chamberlin, 1994; Krummel, 1990) and DEPC, another single-strand-specific modification reagent, was observed to react within this region of the trp paused transcription complex (Lee and Landick, 1992). These results may indicate the eight-nt protected region is an unusual RNA-DNA structure (Lee and Landick, 1992) or conferred by protein contacts to the DNA template (Chamberlin, 1994).

In this light, protection of the eight-nt region of template in the GreA-trp G71 and GreA-G10 complexes is important. Apparently, whatever inhibits base modification within the eight-nt 3'-proximal region of DNA template shifts upstream upon transcript cleavage. If, as we have argued elsewhere, the trp pause RNA hairpin extends to the C50-G71 bp and contributes to the block to further transcript hydrolysis at G71, no RNA bases would be available to pair with the DNA strands, at least in this complex. Further studies using templates on which the pause hairpin is altered may help resolve this question.

The Transcription Bubble Probably Reanneals Outside the Transcription Complex—When we first examined the KMnO4 reactivity of bases in the nontranscribed strand of trp paused transcription complexes, we noted that T70, the furthest upstream reactive base, is also the position at which A-exonuclease stops when it digests the strand 5' to 3' (Landick and Yanofsky, 1987; Lee and Landick, 1992). This suggested to us that the single-stranded bubble reanneals as the strands emerge from the transcription complex. Although we did not use A-exonuclease in the present study, the furthest upstream reactive thymidine and the position at which exonuclease III stops when it encounters the transcription complex are within at most a few nucleotides in all four complexes we examined (Fig. 6). Given the random placement of bases susceptible to modification, this coincidence argues strongly for reannealing of the two DNA strands after they emerge from the upstream side of the transcription complex. Both A-exonuclease and exonuclease III are double strand-specific and may stop digestion at the upstream edge of the transcription complex not because they contact the enzyme, but because the DNA strands become separated.

Conclusion—We have reported here that reverse translocation, like transcription, involves passage of RNA polymerase through different conformations at different template positions. The changes we observed can be accommodated by reversal of the inchworm model for RNA chain elongation, although no contacts were unchanged after transcript cleavage. When compared with the lack of change observed upon transcript cleavage in arrested pol II transcription complexes, our results support the idea that arrest occurs by inappropriate upstream slippage of the upstream DNA contact and the active site.

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