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RESEARCH ARTICLE

Head-On Collision Between a DNA Replication Apparatus and RNA Polymerase Transcription Complex

Bin Liu and Bruce M. Alberts*

An in vitro system reconstituted from purified proteins has been used to examine what happens when the DNA replication apparatus of bacteriophage T4 collides with an *Escherichia coli* RNA polymerase ternary transcription complex that is poised to move in the direction opposite to that of the moving replication fork. In the absence of a DNA helicase, the replication fork stalls for many minutes after its encounter with the RNA polymerase. However, when the T4 gene 41 DNA helicase is present, the replication fork passes the RNA polymerase after a pause of a few seconds. This brief pause is longer than the pause observed for a codirectional collision between the same two polymerases, suggesting that there is an inherent disadvantage to having replication and transcription directions oriented head to head. As for a codirectional collision, the RNA polymerase remains competent to resume faithful RNA chain elongation after the DNA replication fork passes; most strikingly, the RNA polymerase has switched from its original template strand to use the newly synthesized daughter DNA strand as the template.

The *Escherichia coli* genome is arranged in a curious way, inasmuch as most of the heavily transcribed genes are oriented in the direction of the leading strand of the DNA replication fork (*1*, *2*). A similar nonrandom gene organization is found in other bacteria (*3*), plasmids, and bacteriophages (*1*). These observations suggest that a codirectional collision between RNA and DNA polymerases is less disadvantageous to an organism than an oppositely oriented (head-on) collision.

Using a highly purified in vitro system, we previously examined the consequences of a collision between a DNA replication fork and codirectionally moving RNA polymerase (*4*, *5*). We found that the replication fork can pass the RNA polymerase ternary complex even in the absence of a DNA helicase; surprisingly, the bypassed RNA polymerase ternary complex remained bound at its original place on the DNA template, and it was fully competent to resume RNA chain elongation.

We have now examined the consequences of an oppositely oriented collision between a replication fork and an RNA polymerase ternary transcription complex. We found that the replication fork stalls for a long time during such a head-on collision with RNA polymerase when no DNA helicase was present. However, when the DNA helicase was added, the replication fork passed the RNA polymerase after a brief pause. We have investigated the consequences of this bypass reaction and found that the RNA polymerase switched its template strand, requiring that its RNA-DNA helix break up and re-form with a new DNA partner.

A head-on collision between a replication fork and RNA polymerase. A singly nicked circular DNA molecule containing an appropriately oriented *E. coli* σ^{70} promoter was used as a DNA template that supports oppositely directed DNA replication and DNA transcription (in this molecule, the nick that primes leading-strand DNA synthesis is located in the DNA strand that serves both as the template for transcription and as the template for lagging-strand DNA synthesis). We began our reaction by adding purified *E. coli* RNA polymerase and ribonucleoside triphosphates (NTPs) to this DNA; because we omitted cytidine triphosphate (CTP), the RNA polymerase began synthesis at the promoter but stopped at the first G nucleotide on the template. This created a stable ternary transcription complex composed of RNA polymerase, an 18-nucleotide (nt) nascent RNA transcript, and the DNA template (*6*). After purifying this ternary complex on Sepharose Cl-2B to remove a few other, less stable ternary complexes and any RNA polymerase molecules bound to DNA without a transcript (*4*), we added the proteins and nucleotides required to start DNA synthesis. Because the σ factor and NTPs were removed by the treatment with Sepharose Cl-2B, new RNA chains could not be initiated during the DNA replication reaction (*4*).

For DNA synthesis, we used an in vitro replication system composed of seven highly purified bacteriophage T4-encoded proteins that catalyze efficient leading-strand DNA synthesis. The proteins were the T4 DNA polymerase holoenzyme (consisting of the products of T4 genes 43, 44, 62, and 45), a helix-destabilizing single-stranded DNA-binding protein (gene 32 protein), the highly processive DNA helicase (gene 41 protein), and the gene 59 protein that greatly facilitates the loading of the gene 41 protein onto DNA at a replication fork (*7*). An eighth protein, the gene 61 protein (DNA primase), interacts with the gene 41 protein to form the primosome that makes primers for lagging-strand (Okazaki fragment) DNA synthesis; in some experiments, this protein was added to complete the T4 replication apparatus that catalyzes coupled leading- and lagging-strand DNA synthesis at a rate comparable to that observed in vivo (*7*).

Using alkaline agarose gel electrophoresis (*8*), we determined the effect of stalled RNA polymerase ternary complexes on the movement of oppositely oriented replication forks by analyzing the rate of increase in DNA strand lengths during replication. We used either naked DNA or purified ternary complexes as the DNA template in side-by-side reactions. In the absence of the gene 41 DNA helicase, the replication fork

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stalled for many minutes during a head-on collision with the ternary transcription complex (Fig. 1A, compare lanes 1 to 3 with lanes 7 to 9).

When DNA helicase was added to the reaction mixture, the replication fork quickly overcame the ternary complex roadblock but advanced at a net rate that was somewhat less than that observed for a template lacking RNA polymerase (compare lane 4 with lane 10 in Fig. 1A), suggesting that the fork paused transiently before passing the ternary complex. From such data, this pause time can be estimated at 1.7 seconds for a head-on collision, which is about twice as long as the estimated pause time observed for colliding polymerases that are oriented in the same direction (*4*).

We next allowed coupled leading- and lagging-strand DNA synthesis to occur by adding gene 61 DNA primase (along with gene 41 protein) to the reaction (*8*). [Adenosine triphosphate (ATP) and CTP were also added because they are required to initiate Okazaki fragment synthesis (*9*), causing the 18-nt RNA to be elongated to a 22-nt RNA as an incidental consequence (*5*)]. The complete replication fork was again able to pass the ternary complex after a pause (estimated also at 1.7 seconds), and the synthesis of Okazaki fragments was unaffected by the presence of the RNA polymerase (Fig. 1B).

The fate of the bypassed ternary complex. The experiment in Fig. 1 shows that, when a DNA helicase was present, the DNA replication fork passed a DNA template-bound RNA polymerase molecule that carried a nascent transcript. Consider the simplest case, where the only DNA synthesis is that on the leading strand. In principle, there are three possible fates for the bypassed RNA polymerase (Fig. 2A): the ternary transcription complex can fall off the DNA template, it can remain attached to its original template strand (the displaced, single-stranded DNA tail in this case), or it can be reestablished on the replicated, duplex DNA (which requires a switch of template strands). To distinguish between these possibilities, we used ternary complexes that were ^{32}P-labeled on their 18-nt RNA as the templates for replication. After extensive DNA replication in the presence of the gene 41 DNA helicase, the DNA products were cut with the restriction enzymes Sma I and Hae III. The amount of RNA-labeled, Sma I–Hae III DNA fragment remaining was then determined by electrophoresis through a neutral polyacrylamide gel under conditions that leave the ternary transcription complex intact (*10*).

This DNA fragment, which carried RNA polymerase and its nascent RNA, was recovered almost completely after replication (quantification of the radioactivity typically showed less than 8 percent difference before and after replication), indicating that the ternary complex remained bound to duplex DNA rather than being displaced into solution or onto a DNA single strand (Fig. 2B).

The above experiment was significant only if most of the DNA molecules bearing ternary complexes had been replicated. To assess the extent of replication, we analyzed the mobility of the RNA label bound to intact, replicated DNA molecules by neutral agarose gel electrophoresis (*11*). The nonreplicated circular DNA templates moved as a defined band during such electrophoresis, but replication converted them to circular molecules with long single-stranded tails that migrated more slowly. Since only RNA was labeled, the changing distribution of radioactive signals in the gel reflects the efficiency of replication on templates bearing ternary complexes (Fig. 2C). Quantitation of radioactivity at the position corresponding to the nonreplicated template revealed that 70 to 75 percent of the templates with a ternary complex had replicated. We conclude that most of our DNA templates had undergone extensive DNA synthesis, and therefore our experiment (Fig. 2B) showed that a ternary transcription complex that has been passed by a replication fork remained bound to duplex DNA.

To provide a more direct demonstration of this crucial point, we used the RNA-labeled ternary complexes as templates for replication with deoxyuridine triphosphate (dUTP) as one of the four dNTP substrates (*12*). DNA containing deoxyuridine monophosphate on one strand is resistant to double-strand cleavage by the restriction enzyme Bgl II. The sensitivity of the RNA-labeled replication products to Bgl II, as analyzed by native polyacrylamide gel electrophoresis, can therefore be used to analyze whether the replication fork has passed a ternary complex without displacing it (*4*) (Fig. 2D). After replication with dUTP, about 80 percent of the DNA molecules resist Bgl II digestion (compare lane 3 with lane 1). Comparing the amount of uncut ternary complexes in the lane 2 control from

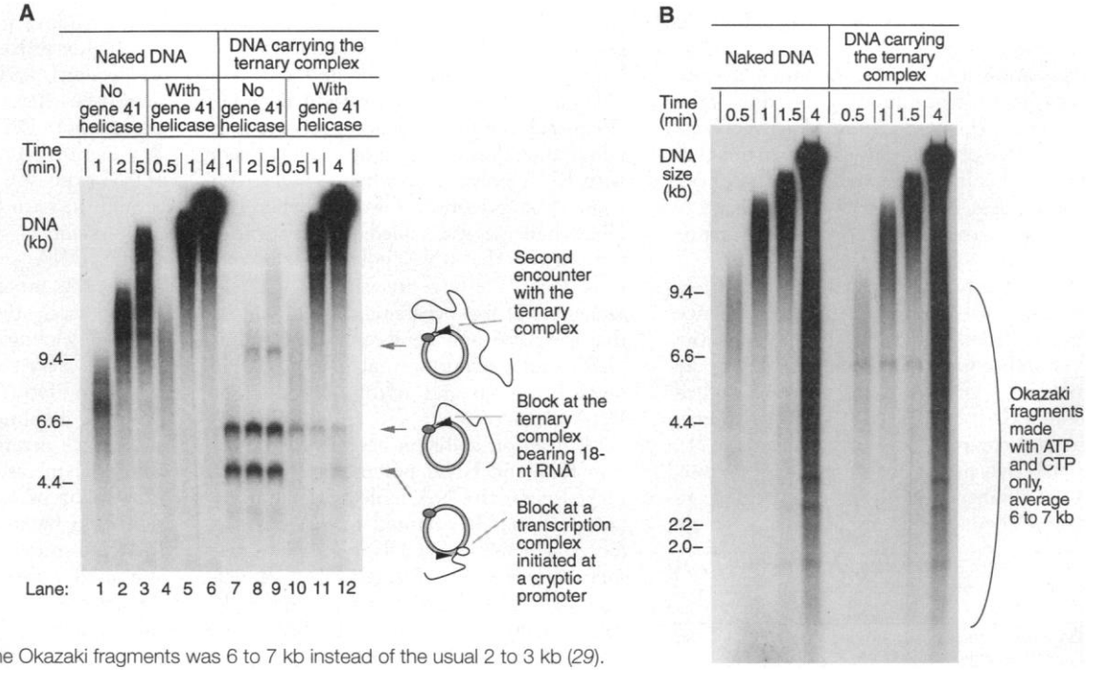

Fig. 1. Effect of the ternary complex on DNA replication. (**A**) Leading-strand DNA synthesis only. The products of in vitro DNA synthesis with or without gene 41 helicase, sampled at the indicated time points with either naked DNA (as control) or column-purified ternary complexes as the template, were analyzed by alkaline agarose gel electrophoresis and autoradiography. Arrows at right indicate band positions caused by the indicated blockages. (**B**) Complete replication fork catalyzing coupled leading- and lagging-strand DNA synthesis. The gene 61 and 41 proteins were both present in these reactions. However, as only ATP and CTP were present as ribonucleoside triphosphates, the priming efficiency is lower than normal and the average size of the Okazaki fragments was 6 to 7 kb instead of the usual 2 to 3 kb (*29*).

the amount in lane 3 reveals that the DNA molecules that have undergone replication have retained their ternary complex.

Maintenance of function by the bypassed RNA polymerase ternary complex. We next assessed the functional competence of the ternary transcription complexes after replication forks have passed through them (*13*). As before, ternary complexes bearing ^{32}P-labeled nascent transcripts were purified through Sepharose Cl-2B. Replication proteins (including gene 41 protein) were added and replication was allowed to proceed until the fork had traveled several times around the circular DNA template. Unlabeled NTPs were then added to permit RNA elongation. If the ternary complexes were inactivated by the passage of the replication fork, the previously labeled, radioactive 18-nt nascent transcripts would not be elongated into full-length RNAs. No new ternary complexes can form under our experimental conditions (no NTPs or σ factor present during replication, and no σ factor present during RNA elongation); moreover, a newly initiated transcript would not be ^{32}P-labeled.

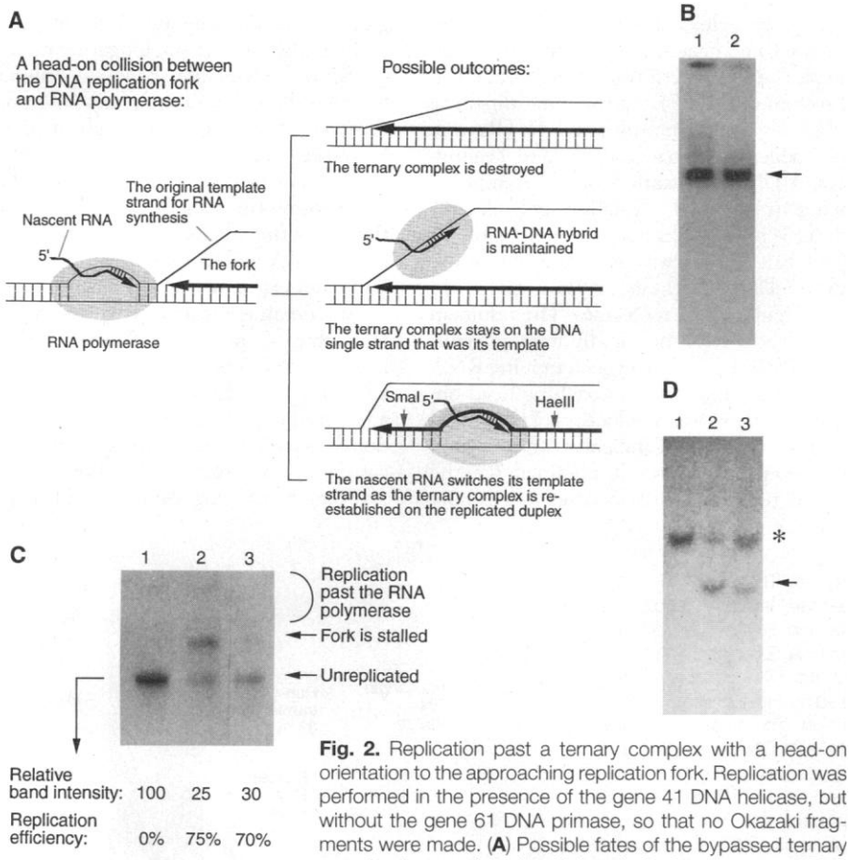

Fig. 2. Replication past a ternary complex with a head-on orientation to the approaching replication fork. Replication was performed in the presence of the gene 41 DNA helicase, but without the gene 61 DNA primase, so that no Okazaki fragments were made. (**A**) Possible fates of the bypassed ternary complex for reactions in which only the leading strand DNA was synthesized. Because the displaced DNA single strand is the original template for RNA synthesis, the RNA polymerase must switch its template to the newly synthesized daughter DNA strand to remain on duplex DNA. The possibilities shown can be distinguished by monitoring the electrophoretic mobility of the Sma I–Hae III fragment carrying the RNA-labeled ternary complex; this DNA fragment remains unchanged after replication only if the bypassed RNA polymerase resides on duplex DNA. (**B**) Gel autoradiograph of a test for retention of the RNA polymerase ternary complex after replication under the Fig. 1A conditions. The DNA was cleaved with Sma I and Hae III after replication for about 30 seconds at 37°C, and the Sma I–Hae III fragment bearing the ternary complex (arrow) was monitored for its mobility change. (Lane 1) Control, electrophoretic mobility before replication; the ternary complex was identified by its radioactive nascent transcript. (Lane 2) Mobility after replication; no significant reduction of the radioactive signal is seen, indicating that the bypassed ternary complex stays on duplex DNA. (**C**) Determination of the replicated fraction of DNA molecules carrying a ternary complex (replication efficiency). In this experiment, all the DNAs were left intact (no restriction nuclease digestion), so that the replicated molecules moved as a heterogeneous smear near the top of the gel. (Lane 1) Nonreplicated molecules carrying the labeled ternary complex. (Lane 2) Replication without the gene 41 DNA helicase for 1 minute at 37°C; the fork stalls, generating a branched DNA structure that appears as a band and moves more slowly than the original DNA band. (Lane 3) Replication with the gene 41 DNA helicase for 30 seconds at 37°C; the replication efficiencies are calculated from the reduction of the ^{32}P-labeled RNA signal (quantified with a PhosphorImager) at the position of the nonreplicated molecules. (**D**) A direct test for retention of the RNA polymerase ternary complex following replication. After the DNA is replicated with dUTP in place of dTTP, an Ava I–Hha I fragment bearing the ^{32}P-labeled ternary complex is tested for its susceptibility to Bgl II restriction nuclease digestion. Lane 1 is a control, showing the amount of ternary complexes before replication. Lane 2 is a second control that reveals the cutting efficiency of Bgl II on nonreplicated molecules (about 70 percent). (Lane 3) The result after replication with dUTP and cleaving with Bgl II. About 80 percent of the DNA in these RNA-labeled complexes survives Bgl II digestion. Normalization against the background in lane 2 shows that about 50 percent of the DNA molecules have resisted Bgl II cutting because of being replicated with dUTP. The asterisk indicates ternary complexes residing on the (uncut) Ava I–Hha I fragment. The arrow indicates ternary complexes residing on the (cut) Bgl II–Hha I fragment.

In the above experiment, as expected, the nascent transcripts on the original column-purified ternary complexes were elongated to 427-nt full-length RNA (Fig. 3A). (Lane 1 shows the expected 18-nt nascent transcript prior to the NTP addition; lane 2 is a control with NTPs added without replication.) The results in lane 3 show that the 18-nt transcripts were nearly completely (less than 5 percent difference with lane 2) converted to full-length transcripts after the replication reaction; thus, the bypassed ternary complexes remained fully functional after a head-on collision with the replication fork.

As a control for the experiment described in Fig. 3A, we repeated it in the presence of excess competitor DNA. For this experiment, template DNA lacking RNA polymerase was cleaved with Hha I and added in excess (10 times more) to the uncut DNA template carrying ternary complex. DNA replication was then begun. If the RNA polymerase were completely displaced from the circular template by the replication fork in a special conformation that allowed it to very rapidly rebind to its homologous DNA site, then it should be trapped by the excess of linear, Hha I–cut DNA template and produce truncated RNAs after addition of NTP. Instead, we recovered more than 95 percent of the full-length transcripts with no truncated RNAs. We conclude that the RNA polymerase remained bound to its original DNA molecule throughout the passing event.

To assess the fidelity of RNA synthesis after replication, we repeated the experiment in Fig. 3A on a DNA template cut with Alu I, where we expected only a 33-nt run-off transcript. Identical yields of run-off transcripts were obtained before and after replication (less than 5 percent difference), demonstrating that there was a precise retention of position by the functional ternary complex (Fig. 3B).

There was no lagging-strand DNA synthesis in the Fig. 2 or Fig. 3, A and B, experiments because the DNA primase

(gene 61 protein) was omitted. When we repeated the experiment in Fig. 3A with the primase present to allow both leading- and lagging-strand DNA synthesis, the original ternary complex bearing an 18-nt RNA was converted into one bearing a 22-nt RNA, because of the presence of the ATP and CTP required for priming (5). This 22-nt RNA remained fully functional and could be elongated into a 427-nt full-length RNA after replication (Fig. 3C). This result extended our observation to a second ternary complex, located 4 nt downstream from the original one.

For all of the Fig. 3 experiments, the replication efficiency was estimated as 70 to 75 percent (the assay used is described in Fig. 2C). The resumed RNA transcript terminated at the normal termination site (Fig. 3, A and C), and its termination occurred prematurely after restriction enzyme digestion of the DNA duplex (Fig. 3B), confirming the observation made in Fig. 2B that the bypassed ternary complex remained on double-stranded DNA even when there was no lagging-strand DNA synthesis.

This outcome was surprising. Because of the 5′ to 3′ direction of polymerization of both DNA and RNA polymerase, two polymerase molecules that were moving in opposite directions on the same DNA duplex were using complementary DNA strands as the template during a head-on collision. At first glance, a head-on collision might be most easily resolved if each respective polymerase molecule held onto its original DNA template strand. In this case, the DNA polymerase would keep moving along the leading-strand template, whereas the RNA polymerase would remain bound to its displaced template strand (the DNA that becomes single-stranded in our experiments and is the potential template for lagging-strand DNA synthesis; see illustrations in Fig. 2A). However, our results indicate that this conceptually simple outcome of a polymerase head-on collision is incorrect. Instead, the RNA polymerase switches to a different template strand when the replication fork passes: the strand that has just been synthesized by the leading-strand DNA polymerase molecule. Because the T4 DNA helicase propels itself along the original template strand for the RNA polymerase (7), the requirement for the helicase to achieve bypass may reflect a central role for it in the template switch.

Replication through moving RNA polymerase molecules. A stalled ternary transcription complex is an incomplete model for the many intermediates in transcription elongation, whose structures are likely to be kinetically determined (14). To determine whether our findings reflect the behavior only of a stalled RNA polymerase molecule, we performed concurrent DNA replication and DNA transcription reactions, as was done previously for a codirectional collision (5).

In order to maintain transcription for a prolonged period, a low concentration of the four NTPs was added to the purified ternary complex, causing the RNA polymerase to elongate at a slow rate that completes the full-length transcript of 427 nt in 2 to 5 minutes (15). At the same time, the DNA replication proteins and dNTPs were also added to allow leading- and lagging-strand DNA replication on the templates being transcribed. Alkaline gel electrophoresis followed by autoradiography of the ^{32}P-labeled DNA was used to examine the rate of DNA synthesis on these transcribing, circular DNA molecules. The results in Fig. 4 show that the rapidly moving replication fork was able to pass a moving RNA polymerase molecule approaching head-on, although there was a reduction of the strand elongation rate that indicates a brief pause, analogous to the result obtained for the stalled ternary complex. After a 1-minute incubation, when the RNA polymerase was in the middle of the transcription unit, the replication fork had traveled three to four times around the circular template, as judged by the length of the DNA product strands (see Fig. 4).

We next examined the fate of the elongating RNA polymerase molecules during DNA replication. If an elongating molecule of RNA polymerase behaves differently from a stalled ternary complex and is unable to survive the passage of a replication fork, no more than 25 to 30 percent of the control amount of full-length ^{32}P-labeled RNA transcripts should be recovered in these experiments, because 70 to 75 percent of the DNA molecules are being replicated under our experimental conditions. However, we obtained nearly complete recovery (less than 5 percent difference) of full-length transcripts (Fig. 5, compare lane 5 with lane 10). This result demonstrates that a transcribing RNA polymerase molecule is not displaced when it collides with a replication fork approaching head-on.

Some surprising outcomes of a poly-

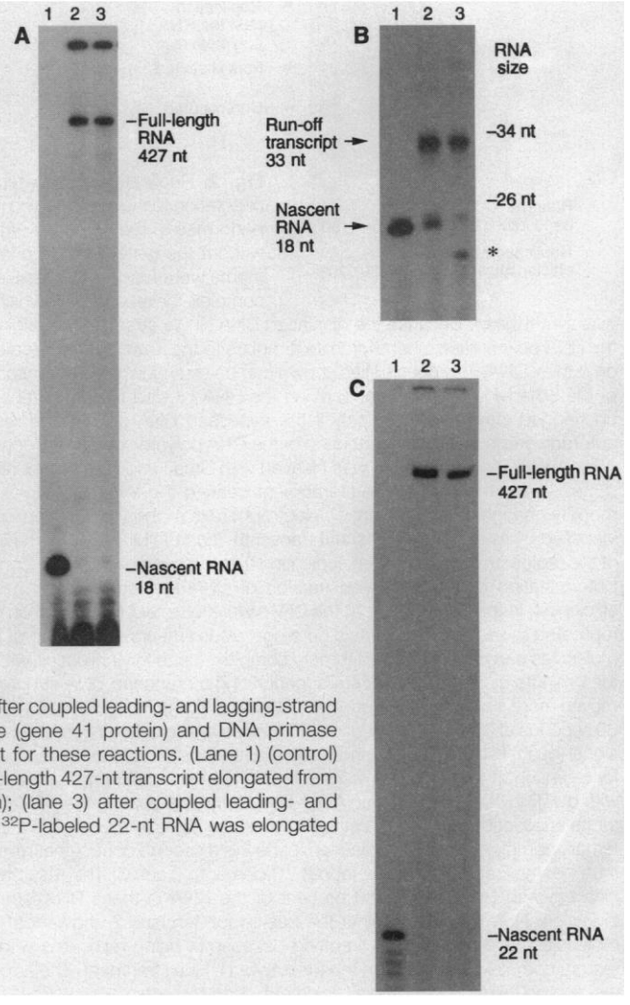

Fig. 3. The ability of a bypassed ternary complex to resume RNA chain elongation. (**A**) Elongation on a full-length DNA template after leading-strand DNA synthesis in the presence of the gene 41 DNA helicase. (Lane 1) (control) nascent ^{32}P-labeled 18-nt RNA; (lane 2) (control with no replication) full-length 427-nt transcript elongated from the 18-nt RNA; (lane 3) after DNA replication, the ^{32}P-labeled 18-nt RNA was elongated with unlabeled NTPs. (**B**) Elongation on a linear, Alu I–cleaved DNA template after leading-strand DNA synthesis. (Lane 1) 18-nt RNA control; (lane 2) control run-off transcript (33-nt RNA); (lane 3) run-off transcript after DNA replication. The asterisk indicates a cleavage product of the 18-nt RNA that is carried by an inactive ("dead-end") ternary complex (30). (**C**) Elongation on a full-length DNA template after coupled leading- and lagging-strand replication. Both DNA helicase (gene 41 protein) and DNA primase (gene 61 protein) were present for these reactions. (Lane 1) (control) 22-nt RNA; (lane 2) (control) full-length 427-nt transcript elongated from the 22-nt RNA (no replication); (lane 3) after coupled leading- and lagging-strand replication, the ^{32}P-labeled 22-nt RNA was elongated with unlabeled NTPs.

merase head-on collision. We have studied the consequences of a head-on collision between the T4 DNA replication machinery and a transcribing *E. coli* RNA polymerase molecule and obtained unexpected results. (i) The replication fork readily passes both an oppositely oriented ternary transcription complex and one that is codirectionally oriented when the T4 DNA helicase is present; however, the helicase is only required for this bypass during a head-on collision. (ii) During a head-on collision, the bypassed RNA polymerase molecule switches to a newly synthesized daughter DNA strand as its template, remaining on the double-stranded DNA where it is competent to resume faithful RNA chain elongation.

The current model for DNA replication involves a large replication machine, formed from coupled leading- and lagging-strand DNA polymerase complexes plus additional proteins such as DNA helicase and DNA primase (*7*). The blockage of any one component in this complex could impede the movement of the entire replication complex (*16*). However, we observed the most interference with fork movement for a minimal replication system that allows only leading-strand DNA synthesis in the absence of a DNA helicase, suggesting that the blockage acts directly on the leading-strand DNA polymerase holoenzyme rather than indirectly on other components. Although other possibilities could be entertained (*17*), we suggest that the increased difficulty we observe in passing the RNA polymerase from its front side reflects the polarity of the RNA polymerase ternary complex itself.

Perhaps our most surprising observation is that the RNA polymerase switches its template during passage of the replication fork. At present, there are two competing models that describe the structure of a transcription intermediate: one invokes a relatively long RNA-DNA hybrid (about 12 bp) that plays a role in stabilizing the ternary complex (*18*); the other suggests that the RNA-DNA hybrid is very short (about 2 to 3 bp) and unlikely to be a major contributor to the stability of the ternary complex (*19*). Our results are most easily explained by the latter model, inasmuch as it would seem to make template switching a less daunting task. At any rate, if a long RNA-DNA hybrid exists, it cannot play a major role in stabilizing the ternary complex. (Unlike the case of a codirectional collision, the RNA-DNA hybrid would not be destined for destruction in a head-on collision if the RNA polymerase remained on its original DNA template strand.)

Switching templates and holding onto DNA in the midst of traffic undoubtedly requires some acrobatic movements by the RNA polymerase. This flexibility may originate in part from the complexity of this enzyme. The *E. coli* RNA polymerase is a large, multiple subunit protein complex, wrapping around at least four turns of double helical DNA during RNA elongation (*20*). Partial detachment of the enzyme from DNA, an almost inevitable step to accommodate a passing DNA polymerase, is presumably tolerated (*4*). In contrast, the members of a family of bacteriophage-encoded RNA polymerases are much simpler; a single polypeptide chain of about 110 kD executes all the functions of promoter recognition and RNA chain elongation (*21*). It would be interesting to determine whether these simpler RNA polymerases are displaced by the passage of the DNA replication machinery.

Relevance to intracellular events. It is worth discussing whether our conclusion is consistent with in vivo observations. The available physiological studies on this subject have yielded controversial results. On the one hand, French has used electron microscopy to examine the fate of a replication fork traveling through an *E. coli* ribosomal RNA operon (*22*). She observed that the movement of the replication fork was hardly affected by codirectionally transcribing RNA polymerase molecules, but was retarded significantly by RNA polymerase molecules approaching from the opposing direction, consistent with our in vitro observations [(*4*, *5*) and this study]. However, French suggested that RNA polymerase is dislodged from the template when the replication fork approaches from either direction. Possible causes of this discrepancy have been discussed (*4*). Gene units other than the ribosomal RNA operon should be studied to help resolve this issue.

Unlike *E. coli*, where DNA replication starts from a single site of origin (OriC), eukaryotes initiate DNA synthesis from numerous discrete sites along their large chromosomes. They also have large genes that measure up to several megabases and require several hours to be completely transcribed (*23*). Aborting such large transcripts during a collision with the DNA replication apparatus is clearly inefficient. When the fate of the large *Drosophila* Ubx gene was studied, it was found that DNA synthesis did not abolish the ongoing transcription, although the origin of replication has not yet been mapped and the orientation of replication fork movement is unknown (*24*). In view of the results of our in vitro

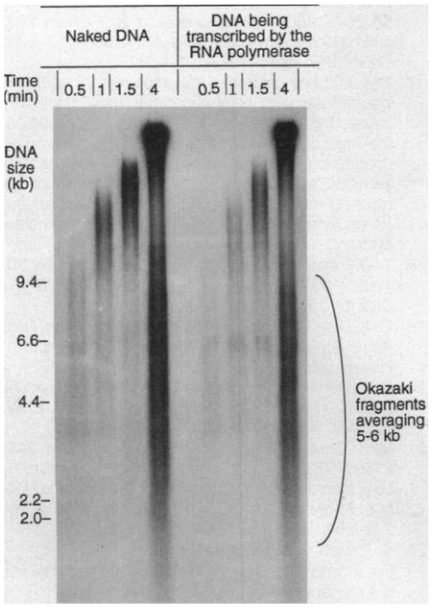

Fig. 4. Increase in DNA strand lengths with time during DNA replication on a DNA template undergoing concurrent transcription (*15*). Low concentrations of NTPs (0.1 mM for ATP and GTP, 0.05 mM for CTP, and 0.02 mM for UTP) were added to a purified ternary complex in order to allow elongation of the RNA polymerase at a slow rate (about 3 nt per second), along with a complete set of DNA replication proteins and dNTPs to start coupled leading- and lagging-strand DNA synthesis. Samples were taken at the indicated time points and DNA strand lengths were analyzed on a 0.6 percent alkaline denaturing gel.

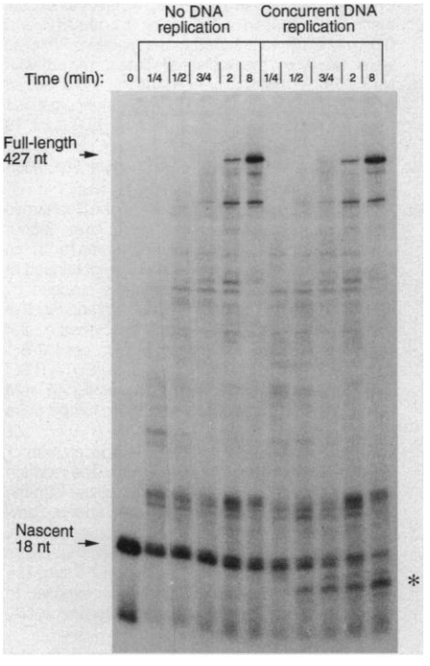

Fig. 5. Increase in RNA size caused by RNA synthesis, with and without concurrent DNA replication. Concurrent replication and transcription reactions were performed as described (*15*). Samples were taken at the indicated time points and analyzed on a 10 percent denaturing polyacrylamide gel containing 8 M urea. The asterisk indicates a cleavage product from the "dead-end" ternary complex described in the legend to Fig. 3B (*30*).

studies showing that the *E. coli* RNA polymerase can stay on a DNA duplex regardless of the orientation of the collision [(*4*, *5*) and this study], and because eukaryotic DNA and RNA polymerases share a common structural organization with their prokaryotic counterparts (*25*), we might predict that the eukaryotic transcription apparatus also survives DNA replication.

So far, our study has revealed only one clear disadvantage for a polymerase head-on collision: a partially obstructed movement of the replication fork. However, this may not be the only problem generated by this type of collision. Our suspicion in this regard arises from examining the ribosomal DNA locus in yeast and other eukaryotic cells (*26*). The yeast ribosomal DNA locus consists of tandemly repeated transcription units (genes) with replication origins situated in the nontranscribed spacers. The two forks initiating at each of these origins experience unequal fates. The fork moving in the direction of transcription proceeds unimpeded through multiple gene repeats, while the oppositely directed fork arrests at a polar barrier just before it encounters the transcription terminator for the adjacent, upstream transcription unit (*27*). The arrest is mediated by proteins binding to specific DNA sequences, regardless of transcription (*28*). Polar replication fork barriers permanently block rather than reduce the rate of movement of the replication fork (*27*, *28*). We speculate that, in addition to retarding the fork movement, a head-on collision may expose the RNA polymerase to other potential problems, whose nature has not been revealed by our experiments.

We do not yet know whether a selective pressure against a head-on collision between RNA and DNA polymerase has been maintained throughout the evolution of all cells. However, we should soon have the information we need to catalog the gene organization around each local replication origin in yeast, allowing us to determine whether the relative directions of DNA replication and transcription are nonrandomly arranged in this eukaryote, as they are in *E. coli*.

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6. The 3.3–kilobase pair (kb) circular plasmid was derived from pRT510–C+18 (*4*) by inversion of a 116-bp Eco RI–Eco RI fragment containing the replication origin of bacteriophage M13, located about 190 bp away from the stalled RNA polymerase. It contains an *E. coli* σ^{70} promoter superimposed with a phage T4 late promoter. In the presence of the dinucleotide UpG, the RNA made from this promoter starts with the 18-nt sequence UGAUAUGAAGAGUUGGAU inasmuch as there is no C nucleotide until position 19. To allow initiation of DNA synthesis on this circular plasmid, the DNA was specifically nicked at the M13 bacteriophage gene 2 protein recognition site. The ternary complex was purified through Sepharose CI-2B as described (*4*); it carries a ^{32}P-labeled 18-nt RNA molecule. Examination by electron microscopy indicated that all the template DNA was occupied by the desired ternary complex. About 50 percent of the DNA molecules also bear an additional ternary complex initiated at a cryptic promoter (located about 2 kb away from the desired ternary complex). The degree of occupancy by the cryptic complex decreased when increased salt concentrations were used for purification of the ternary complex on the CI-2B column; the cryptic complex can be eliminated by treatment with high salt (> 0.5 M NaCl), but this treatment also decreases the recovery of the desired ternary complex.
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10. After replication of the DNA present in the Sepharose CI-2B column-purified ternary complexes (about 0.01 pmol) for 30 seconds, 10 units of Sma I and Hae III were added and the incubation was continued at 37°C for 1 minute. The reaction was stopped by chilling on ice, and heparin (100 μg/ml) and Ficoll (3 percent) were added. Samples were placed on a 4 percent nondenaturing polyacrylamide gel [37.5:1 acrylamide:bisacrylamide in tris-borate EDTA (TBE) (89 mM tris base, 89 mM boric acid, 2.5 mM Na_3EDTA)] for electrophoresis at room temperature for 5 hours at 11 V/cm.
11. After 30 seconds or 1 minute of replication with or without gene 41 helicase, respectively, the reaction was stopped by chilling the sample on ice. Heparin (100 μg/ml) and Ficoll (3 percent) were added. Samples were then placed on an 0.8 percent neutral agarose gel in TBE and subjected to electrophoresis at room temperature for 4 to 5 hours at 7 V/cm. The gel was dried and autoradiographed, or exposed to a PhosphorImager screen (Molecular Dynamics, CA) for quantitative analysis.
12. After replication (with gene 41 helicase) on the Sepharose CI-2B column-purified ternary complexes (about 0.01 pmol) with dUTP, dCTP, dATP, and dGTP (0.2 mM each) for 1.5 minutes, 10 units of Ava I and Hha I were added and the incubation was continued at 37°C for 3 minutes. Where indicated, 5 units of Bgl II were then added for another 4 minutes at 37°C. The reaction was stopped by chilling on ice; Heparin (80 μg/ml) and Ficoll (3 percent) were added. Samples were placed on a 4 percent nondenaturing polyacrylamide gel for electrophoresis at room temperature for about 5 hours at 11 V/cm.
13. For the experiment shown in Fig. 3A, leading-strand replication was carried out for 1 minute, followed by the addition of unlabeled NTPs (0.5 mM ATP, 0.5 mM GTP, 0.2 mM CTP, and 0.2 mM UTP) to elongate the nascent RNA transcript at 37°C for 8 minutes. Samples were then chilled on ice, treated with 2 units of deoxyribonuclease I (with 0.5 mM $CaCl_2$), extracted with phenol-chloroform, and subjected to electrophoresis on a 10 percent denaturing polyacrylamide gel. The experiment shown in Fig. 3B differed only in that the RNA chains were elongated on DNA templates that had been digested with 10 units of Alu I. For the experiment shown in Fig. 3C, coupled leading- and lagging-strand replication was carried out for 1 minute and unlabeled NTPs (0.5 mM ATP, 0.5 mM GTP, 0.2 mM CTP, and 0.3 mM UTP) were added at 37°C for 8 minutes to elongate the nascent transcript.
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15. Concurrent replication and transcription were performed as follows; to the purified ternary complex bearing ^{32}P-labeled 18-nt RNA, NTPs were added at low concentrations (100 μM ATP, 100 μM GTP, 50 μM CTP, and 20 μM UTP), along with the previously described concentrations of DNA replication proteins and dNTPs with (Fig. 4) or without (Fig. 5) 80 μM [α-^{32}P]dCTP (specific activity about 50,000 to 100,000 cpm/pmol) to label the synthesized DNA. At the indicated times, samples were either mixed with Na_3EDTA (20 mM final concentration) and placed on a 0.6 percent agarose alkaline denaturing gel (Fig. 4), or treated with 2 units of DNase I (with 0.5 mM $CaCl_2$), extracted with phenol and chloroform, and subjected to electrophoresis on a 10 percent denaturing polyacrylamide gel containing 8 M urea (Fig. 5).
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30. This reaction differs from the spontaneous transcript cleavage reported in (*31*) because (i) it is specific to the inactive ternary complex and no cleavage products are seen if the ternary complex is fully active (Fig. 3, A and C); (ii) it requires the addition of gene 43 protein. We do not know whether this cleavage is caused directly by the gene 43 protein or by contaminating ribonucleases in the gene 43 protein preparation. In any event, because this reaction concerns only inactive complexes, it has little relevance to this study.
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