The DNA replication fork can pass RNA polymerase without displacing the nascent transcript

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Replication proteins encoded by bacteriophage T4 generate DNA replication forks that can pass a molecule of *Escherichia coli* RNA polymerase moving in the same direction as the fork *in vitro*. The RNA polymerase ternary transcription complex remains bound to the DNA and retains a transcription bubble after the fork passes. The by-passed ternary complex can resume faithful RNA synthesis, suggesting that the multisubunit RNA polymerase of *E. coli* has evolved to retain its transcript after DNA replication, allowing partially completed transcripts to be elongated into full-length RNA molecules.

No known mechanism prevents DNA replication and transcription from taking place on a DNA molecule concurrently; when they move in the same direction, the respective polymerases must use the same DNA single strand as template. In *E. coli*, the rate of replication is 10–15 times faster than the rate of transcription[1,2], so that collisions between the two types of polymerase are inevitable, even when they move in the same direction. Collisions could be resolved in one of three ways: a replication fork could knock an RNA polymerase molecule and its nascent transcript out of its way; a replication fork could slow down and passively follow behind a transcription complex; or a replication fork could pass a transcribing RNA polymerase molecule without displacing it from the template.

Preservation of the nascent transcript when a replication fork passes would be advantageous because RNA chains are generated by an energy-consuming multistep process[3–5]. Is this possible chemically? To answer this question, we examined the consequences of a collision between a replication fork and co-directionally transcribing RNA polymerase. We used the highly purified *in vitro* T4 bacteriophage DNA replication system to replicate through a precisely placed *E. coli* RNA polymerase transcription complex. In this completely defined system, the fate of the nascent transcript after replication can be determined unambiguously. Surprisingly, a replication fork can pass through a transcription complex without displacing it, leaving intact its ability to resume RNA chain elongation.

A template for investigating the collision

A uniquely nicked circular DNA molecule containing an appropriately oriented strong T4 late promoter[6–8] was used as a DNA template that supports co-directional replication and transcription (Fig. 1*a*, left side; note that the template strand for transcription is also the template for leading-strand DNA synthesis). By withholding rCTP, we stalled the RNA polymerase at a specific downstream site, creating a stable ternary transcription complex composed of RNA polymerase, an 18-nucleotide (nt) nascent RNA transcript, and the DNA template. The core RNA polymerase was from *E. coli*; the T4 gene 55 σ-family protein enables it to recognize the T4 late promoter[9].

To reduce the binding of RNA polymerase to the nick[10] and to weak variant T4 late promoters on the plasmid, we either used a low molar ratio of RNA polymerase to template DNA (for example, 4 : 1), or purified the ternary complex on Sepharose CL-2B after exposure to high salt (0.5 M NaCl), as specified in each experiment. The Sepharose CL-2B column excludes the ternary complex, but includes free core RNA polymerase, gene 55 protein and nucleotides. Promoter-bound RNA polymerase and other, less stable ternary complexes dissociate from DNA in 0.5 M NaCl (ref. 11). Thus, the desired ternary complex is highly enriched after passage through the CL-2B column. Moreover, because the gene 55 protein and ribonucleoside triphosphates (rNTPs) have been removed, there is no reinitiation by RNA polymerase during the subsequent DNA replication reaction.

The replication fork passes the ternary complex

Seven highly purified bacteriophage T4-encoded proteins reconstitute an *in vitro* replication system that catalyses efficient leading strand DNA synthesis[12–15]. The proteins involved are 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 (J. Barry and B.M.A., manuscript in preparation).

We analysed the effect of stalled RNA polymerase ternary complexes on the movement of replication forks by alkaline agarose gel electrophoresis. As the DNA template, we used either mock-treated DNA, or CL-2B-purified ternary complexes. Even though about 70–80% of the DNA molecules bear a bound ternary complex (determined by a gel shift assay), there is no strong blockage of DNA synthesis, with or without DNA helicase (gene 41 protein) (Fig. 1*b*). Thus, the ability to pass the RNA polymerase ternary complex is intrinsic to the DNA polymerase holoenzyme (DNA polymerase plus accessory proteins). When helicase is included in the reaction, the replication fork speeds up, and it advances at a slightly reduced rate on templates bearing the ternary complex (compare lanes 7, 8 with lanes 11, 12), suggesting that the fork pauses transiently before passing stalled RNA polymerase. Without a helicase, the fork pauses at many sites, making it difficult to detect any additional pausing

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caused by the RNA polymerase ternary complex (compare lanes 1–3 with lanes 4–6).

The complex stays bound to DNA

The experiment in Fig. 1*b* shows the DNA replication fork readily passing a DNA template-bound RNA polymerase molecule that carries a transcript. To distinguish between the possible fates of this RNA polymerase (Fig. 1*a*), we designed the experiment illustrated in Fig. 2*a*. We used RNA-labelled ternary complexes as templates for replication with dUTP as one of the four dNTP substrates. DNA containing dUMP on one strand is resistant to double-strand cleavage by the restriction enzyme *Dra*I, which recognizes the sequence TTTAAA. The sensitivity of the RNA-labelled replication products to *Dra*I, as analysed by non-denaturing polyacrylamide gel electrophoresis, can therefore be used to analyse whether the replication fork has passed the ternary complex without displacing it (see Fig. 2*a*).

The analysis of such an experiment is shown in Fig. 2*b* (replication with DNA helicase) and Fig. 2*c* (replication without DNA helicase). Because the same amount of RNA-labelled ternary complex is seen in lane 1 (no replication) and lane 2 (after replication), it is evident that the ternary complex is not displaced from the template by the replication fork (quantification of the radioactivity typically shows <5% difference). The slowly migrating, branched structures that would be expected for replication forks stalled behind the ternary complex are not seen. Lane 4 shows the *Dra*I-resistant products, proving that the replication fork has passed through the ternary transcription complex. About 30–40% of the DNA in these RNA-labelled complexes is cut by *Dra*I, in agreement with other results indicating that 60–70% of the DNA template molecules replicate in this experiment. Lanes 3 in Fig. 2*b* and *c* show that *Dra*I digestion goes to completion when DNA is replicated with dTTP. Note that the same results are obtained with or without DNA helicase present.

Retention of a transcription bubble

The experiment outlined in Fig. 3*a* examines whether the ternary complex retains its original position after passage of a replication fork by separately marking DNA for the presence of a ternary complex and for downstream penetration of the replication fork. Enhanced reactivity of the DNA with $KMnO_4$ (a footprint)

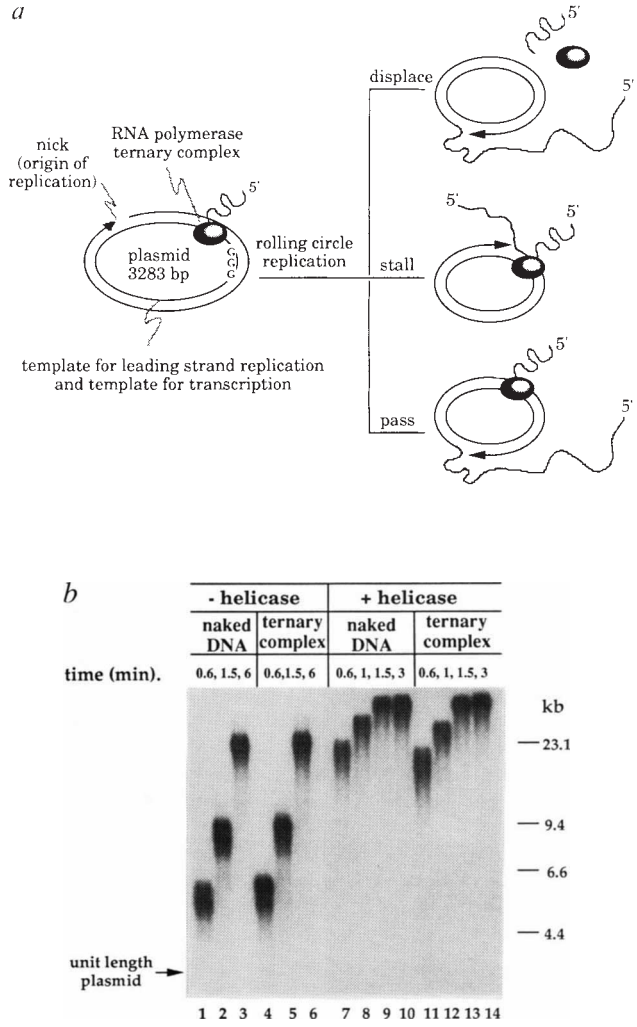


FIG. 1 *a*, The experimental system. The template for *in vitro* replication by the bacteriophage T4 replication proteins is a 3.3-kilobase-pair (kb) circular plasmid containing the replication origin of bacteriophage M13, located ~170 nt behind the stalled RNA polymerase. Nicking this origin with the filamentous bacteriophage gene 2 endonuclease provides a unique DNA 3′ end that serves as a starting site for initiation of rolling circle DNA synthesis *in vitro*[30]. Three consecutive G nucleotides were placed on the template strand 17, 18 and 19 base pairs (bp) downstream of the transcription initiation site. Using the dinucleotide UpG to initiate transcription at bp −1 (that is, one bp upstream of the normally initiating G) in the presence of rATP, rGTP and rUTP and withholding rCTP, we stalled the RNA polymerase at the triple G site with an 18-nt nascent transcript. Because rCTP is withheld, lagging strand DNA synthesis is very inefficient, and we have generally omitted the DNA primase (gene 61 protein)[36,37] from replication reactions, leaving the template for lagging strand DNA synthesis as a displaced single strand. *b*, Effect of the ternary complex on movement of the replication fork. The products of *in vitro* DNA synthesis, using either naked DNA (as control) or column-purified ternary complexes as the DNA template, were analysed by alkaline agarose gel electrophoresis, followed by autoradiography. METHODS. *a*, The plasmid pRT510-C+18, which is derived from pDH310 (ref. 38) through two rounds of mutagenesis[39], contains a −35 σ^{70} consensus sequence placed upstream of the −10 T4 late promoter consensus sequence of gene 23. The resulting promoter, P′23, is efficiently used *in vitro* by both σ^{70}-containing and gene 55 protein-containing RNA polymerases. The P′23 sequence was further changed to −1GATATGAAGAGTTGGATCCC, where +1 designates the start site of transcription (non-template strand; the entire sequence of plasmid pRT510-C+18 is available on request). To initiate DNA synthesis on circular pRT510-C+18, the DNA was specifically nicked at the M13 bacteriophage gene 2 protein recognition site, as described[40]. To prepare the ternary complex, 0.2 pmol nicked DNA was incubated with the following reagents in 40 μl for 30 min at 37 °C: 6 pmol *E. coli* RNA polymerase core, 30 pmol gene 55 protein, 9 pmol gene 33 protein, 27 pmol gene 44/62 protein, 98 pmol gene 45 protein, 1 mM dATP; 100 μM UpG, 4 μM rATP, 4 μM rGTP, 4 μM [α-^{32}P]rUTP (specific activity ~50,000–100,000 c.p.m. pmol^{-1}), 33 mM Tris–acetate (pH 7.8), 250 mM potassium acetate, 10 mM magnesium acetate, 0.5 mM dithiothreitol (DTT), 100 μg ml^{-1} nuclease-free BSA as protein carrier. The reaction was stopped by chilling the sample on ice, followed by the addition of NaCl to 0.5 M and gel filtration through a 1 ml CL-2B column with a 200-μl 0.5 M NaCl loading zone, and elution with replication buffer (33 mM Tris–acetate (pH 7.8), 66 mM potassium acetate, 10 mM magnesium acetate, 100 μg ml^{-1} BSA and 0.5 mM DTT) in the presence of 3–5% glycerol. Radioactive fractions were pooled for the subsequent replication reactions. Typically 70–80% of the DNA templates were occupied by a ternary complex (determined by gel shift assay). *b*, Replication in 40 μl of the replication buffer with 0.02 pmol of the column-purified ternary complex or control naked DNA, 3 μg ml^{-1} gene 43 protein, 80 μg ml^{-1} gene 32 protein, 40 μg ml^{-1} gene 44/62 protein, 20 μg ml^{-1} gene 45 protein, 20 μg ml^{-1} gene 41 protein and 1.2 μg ml^{-1} gene 59 protein (whenever the gene 41 protein was omitted, so was the gene 59 protein), 25–50 μg ml^{-1} rifampicin, 0.5 mM dATP, 0.5 mM dGTP, 0.2 mM dCTP, and 0.08 mM [α-^{32}P]dTTP (~25,000 c.p.m. pmol^{-1}). After 30 s at 37 °C, non-radioactive dTTP was added to 1 mM to stop the labelling. Aliquots were taken at the indicated times, mixed with Na_3-EDTA (20 mM final concentration), loaded on a 0.6% agarose alkaline denaturing gel, and run in 30 mM NaOH, 1 mM Na_3-EDTA for 18 h at 2 V cm^{-1}.

marks the transcription bubble of the ternary complex[5,16]; incorporating 5′-methyl dCMP (dmCMP) in place of dCMP into the newly synthesized DNA generates resistance to cutting by the restriction enzyme *Alu*I. Any *Alu*I-resistant DNA that retains the $KMnO_4$ footprint of the ternary complex can only be generated by replication forks that have replicated past the ternary complex without permanently displacing this complex.

An analysis of the ternary complex footprint by primer extension is shown in Fig. 3*b*. A comparison of lanes 2 and 5 reveals no significant reduction of the footprint signal after replication (typically <5% difference). Proof that the ternary complex is not displaced from the template after replication comes from the demonstration (lane 4) that 40–50% of the molecules that carry a ternary complex also resist *Alu*I cutting (and therefore must have replicated). When dCMP instead of dmCMP is incorporated into DNA, *Alu*I is fully active and the footprint disappears as expected (lane 3). Because the position of the footprint is unchanged after replication (lanes 2, 4 and 5), the by-passed ternary complex retains its place on DNA and its transcription bubble.

A by-passed complex remains fully functional

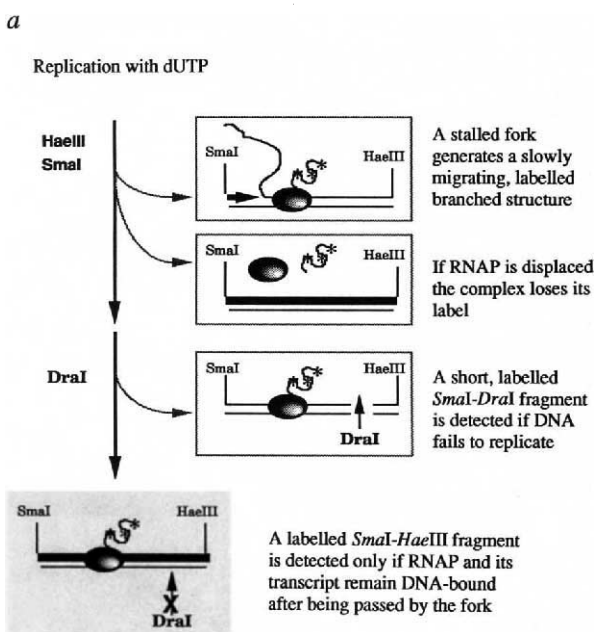
We next assessed the functional competence of ternary complexes after replication forks have passed through them. Ternary complexes bearing nascent transcripts labelled with [α-^{32}P]rUTP were purified through CL-2B. Replication proteins were added and replication was allowed to proceed until the fork had travelled several times around the circular DNA template. Non-radioactive rNTPs were then added to permit the elongation of any nascent transcripts. If the ternary complexes are inactivated by the passage of the replication fork, the pre-labelled, 18-nt nascent transcripts should not be elongated into full-length RNA. No new ternary complexes should form under our experimental conditions (no rNTPs or gene 55 protein present during replication; no gene 55 protein present during the chase); moreover, any newly initiated transcripts would not be radioactively labelled.

The results of the above experiments are shown in Fig. 4*a*. Lane 1 shows the expected 18-nt nascent transcript before a chase. Lane 2 shows that, as expected, the nascent transcripts on column-purified ternary complexes chase into 427-nt full-length RNA in the absence of DNA replication. The important result is that the 18-nt transcripts are also nearly completely converted to full-length transcripts following replication without or with DNA helicase (lanes 4 and 6, respectively). When no rNTPs are added after replication, a 'mock' 6–8 min incubation leaves the nascent 18-nt transcript unchanged (lanes 3 and 5).

To assess the fidelity of RNA synthesis after replication, we repeated the chase experiment on a DNA template cut with *Alu*I to generate only a ~33-nt run-off transcript. Identical run-off transcripts were observed before and after replication (Fig. 4*b*), demonstrating the precise retention of position by the functional ternary complex.

To test whether a nascent transcript that has been released into solution can reassociate with DNA to be further elongated, we added purified 18-nt ^{32}P-labelled RNA to a reaction mixture containing the components of the chase experiment shown in Fig. 4*a*. When incubated with RNA polymerase core (with or without gene 55 protein) and cold rNTPs (either alone or with DNA replication proteins and dNTPs), no 18-nt RNA was elongated, and most of this RNA remained detectable as a radioactive band in the 18-nt position (data not shown).

The above chase experiments are significant if a major fraction of the DNA molecules bearing ternary complexes have been



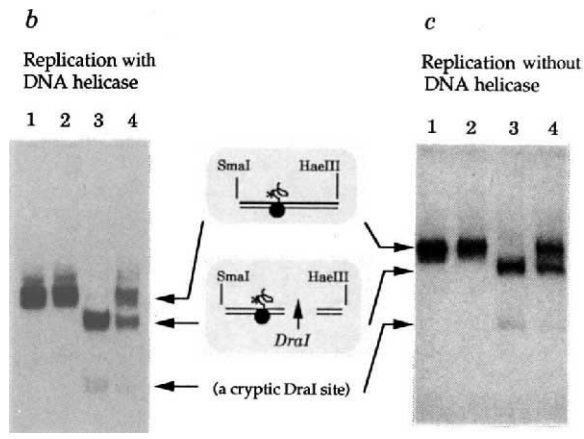


FIG. 2 A test for retention of the RNA polymerase (RNAP) ternary complex, identified by its radioactive nascent transcript, after replication. *a*, Outline of the experiment. After DNA is replicated with dUTP in place of dTTP, a *Sma*I–*Hae*III fragment bearing the ternary complex is tested for its susceptibility to *Dra*I. *b*, Gel autoradiograph after replication through RNAP ternary complexes with DNA helicase (gene 41 protein) present. Lane 1, control ternary complex on the *Sma*I–*Hae*III fragment (no replication); lane 2, control ternary complex on the *Sma*I–*Hae*III fragment after replication with dUTP; lane 3, replication with dTTP and cutting with *Dra*I (the same bands were seen when non-replicated molecules were digested with all three restriction enzymes); lane 4, replication with dUTP and cutting with *Dra*I. *c*, As *b* but replication was without the DNA helicase. Lanes as in *b*.

METHODS. After replication on the CL-2B column-purified ternary complexes (~0.01 pmol) with either dUTP or dTTP for ~30 s, 10 units of *Hae*III were added and the incubation continued at 37 °C for 4 min (without helicase) or 1–2 min (with helicase). The DNA was cut with 10 units of *Sma*I at room temperature for 5 min. Where indicated, 10 units of *Dra*I were then added for another 5 min at 37 °C. The reaction was stopped by chilling on ice; heparin and Ficoll were added to 100 μg ml^{-1} and 3%, respectively. Samples were loaded on a 3% (Fig. 2*b*) or 4% (Fig. 2*c*) non-denaturing, neutral polyacrylamide gel (37.5: 1 acrylamide: bisacrylamide in 1 × TBE (89 mM Tris base, 89 mM boric acid, 2.5 mM EDTA)) for electrophoresis at room temperature for ~5 h at 11 V cm^{-1}. The gel was dried and autoradiographed. *In vitro* replication was done as described in Fig. 1*b* except that 0.2 mM non-radioactive dTTP or dUTP was used instead of [α-^{32}P]dTTP.

replicated. To determine this fraction, nascent RNA was labelled with [α-^{32}P]rUTP and ternary complexes were purified by gel filtration. Non-replicated circular DNA templates run as a defined band during electrophoresis on neutral agarose gel. Replication converts these molecules to circular molecules with long single-stranded tails, which migrate more slowly. Because only the RNA is labelled, the changing distribution of radioactive signals in the gel as a function of time reflects the efficiency of replication on templates bearing ternary complexes (Fig. 4*c*). Quantification of radioactivity at the position corresponding to the non-replicated template reveals that ~70% of the templates bearing a ternary complex have been replicated. Moreover, there appears to be no blockage of replication fork movement by the ternary complex, because no discrete bands corresponding to stalled structures appear on the gel, even in the absence of DNA helicase. We conclude that most of our DNA templates have undergone extensive DNA synthesis, and that the ternary transcription complexes bound to them remain functional for RNA chain elongation after the passage of replication forks.

Electron microscopic examination

As an independent test of our conclusions, we have used electron microscopy to examine the fate of the ternary complex after replication. The analysis should also reveal unanticipated products of replication, if any are formed. For each DNA molecule that undergoes rolling-circle replication, the extent of such replication is easily assessed by the length of its single-stranded DNA tail. In principle, the replication fork must have passed the ternary complex without displacing it from the template if a DNA molecule bearing such a complex has a single-stranded tail that is longer than the distance from the nick to the ternary complex (~170 nt).

The template for these replication reactions was the ternary complex purified on the CL-2B column. Before visualization, replication products were re-treated with 0.5 M NaCl and passed again through CL-2B to remove replication proteins. As a control, Fig. 5*a* shows a globular particle associated with the non-replicated circular template. Several lines of evidence suggest that this particle is the ternary complex: (1) it survives high-salt (0.5 M NaCl) treatment and CL-2B gel filtration; (2) it occupies the expected place on DNA cut at unique restriction enzyme sites (such as *Ssp*I in Fig. 5*b* and *Hin*dIII in Fig. 5*c*); (3) it disappears when rNTPs are added for several minutes (not shown); (4) nascent transcripts can be seen on brief incubation (30 s) with a low concentration of rNTPs (1 μM each) (Fig. 5*d*).

Replicated DNA molecules (Fig. 5*e–g*) bearing the ternary complex have tails of varying lengths that can exceed the size of the circular template. We randomly sampled 180 molecules to obtain the data in Fig. 5*i*, *j*. A similar fraction of the DNA molecules bear the ternary complex before and after replication (Fig. 5*i*), consistent with the finding that the by-passed ternary complex remains DNA-bound. Moreover, a significant fraction of templates bearing the ternary complex have tails longer than the size of the circular template (Fig. 5*j*), proving that at least

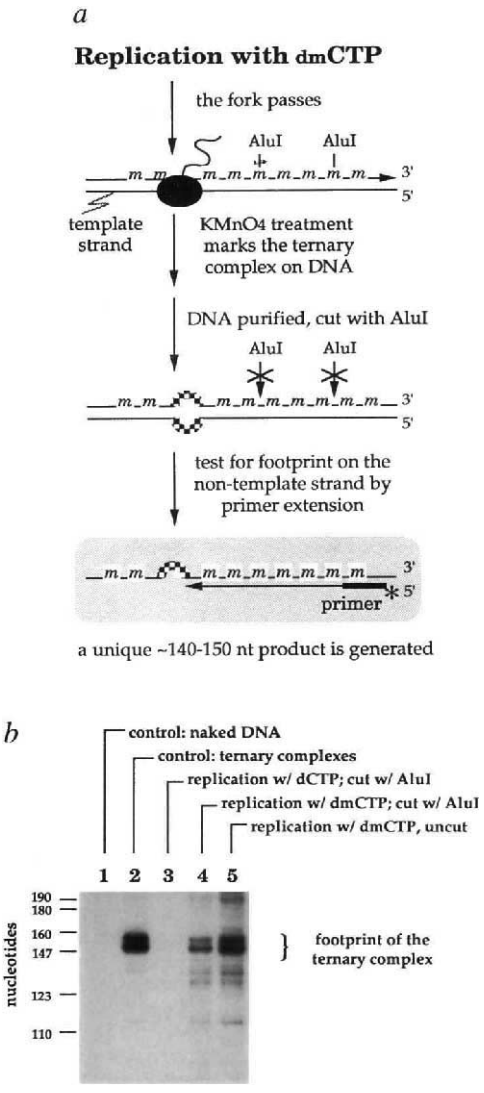


FIG. 3 Determination of the location of the ternary complex after replication. *a*, Outline of the experiment. $KMnO_4$ oxidizes regions of single-stranded DNA[41] in the ternary complex, and this footprint of the transcription bubble is observable by primer extension analysis only if DNA is resistant to cutting by *Alu*I at the sites shown. Resistance is conferred by incorporation of dmCMP ('m'). The asterisk represents the ^{32}P-label at the 5′ end of the primer. *b*, Primer extension analysis. Lane 1, naked DNA control; lane 2, ternary complex control, showing the position and signal intensity of the ternary complex in unreplicated DNA not cut with *Alu*I; lane 3, replication with dCTP and cut with *Alu*I; lane 4 replication with dmCTP and cut with *Alu*I; Lane 5 replicated with dmCTP, but not cut with *Alu*I.

METHODS. Nicked DNA (0.1 pmol) was incubated with 0.4 pmol RNA polymerase core supplemented with 2 pmol gene 55 protein and 0.8 pmol gene 33 protein; 40 μg ml^{-1} gene 44/62 protein, 20 μg ml^{-1} gene 45 protein, 1 mM dATP, 120 μM UpG, 5 μM rATP, rGTP, and rUTP, 220 μM 3′-*O*-methyl rCTP as chain terminator, 5% polyethylene glycol (3.3K), 33 mM Tris–acetate (pH 7.8), 250 mM potassium acetate, 10 mM magnesium acetate, 0.5 mM DTT and 100 μg ml^{-1} nuclease-free BSA. After incubation at 37 °C for 15 min, potassium acetate was diluted to 120 mM and replication was allowed to proceed at 37 °C for 4 min by adding 3 μg ml^{-1} gene 43 protein, 60 μg ml^{-1} gene 32 protein, 20 μg ml^{-1} gene 41 protein, 0.2 μg ml^{-1} gene 59 protein, 0.5 mM dGTP, 0.2 mM dCTP or dmCTP, 0.2 mM dTTP, with 50 μg ml^{-1} rifampicin present to prevent re-formation of ternary complexes by way of newly initiated transcription, and 10 units of *Hae*III added to limit the extent of DNA synthesis by linearizing the DNA template. $KMnO_4$ was then added to a final concentration of 5.1 mM. After 1 min at 37 °C, the $KMnO_4$ reaction was quenched with 5 μl 14 M β-mercaptoethanol. The sample was treated with 80 μg ml^{-1} proteinase K in the presence of 0.5% SDS for 30 min at 37 °C, followed by phenol–chloroform extraction and ethanol precipitation with 15 μg ml^{-1} glycogen as carrier. The pellet was dissolved in a buffer (10 mM Bis-Tris–propane–HCl, 10 mM $MgCl_2$, 1 mM DTT, pH 7.0) that allowed optimal digestion by *Alu*I (10 units) during 8 min at 37 °C. Primer extension was done with a 5′ end-labelled 19-nt single-stranded DNA complementary to the non-template strand; subsequent sample preparation and electrophoresis on a 10% polyacrylamide gel (37.5: 1 acrylamide: bisacrylamide) with 8 M urea in 1 × TBE were performed as described[42–44].

one round of replication has occurred. Thus, the replication fork is indeed able to pass the ternary complex without displacing it.

When we briefly added rNTPs to mixtures that had finished replication, nascent RNA was detected on many extensively replicated DNA templates (Fig. 5*h*), indicating that by-passed ternary complexes are functional. Finally, DNA structures other than those expected from rolling-circle replication were not observed, arguing against the possibility that any of the findings in this article are explained by some unanticipated replication mechanism.

Discussion

Our examination of the consequences of a collision between a replication fork and a codirectionally orientated, stalled RNA polymerase ternary transcription complex yields a surprising result: the replication fork passes the ternary complex after only a brief pause (estimated to last <1 s; Fig. 1*b*); the by-passed ternary complex not only remains bound to the DNA (Fig. 2) with a transcription bubble at its original DNA site (Fig. 3), but it is fully competent to resume RNA synthesis (Fig. 4). Electron microscope examination of the reaction products supports this conclusion at the single macromolecule level (Fig. 5).

Our results do not merely reflect a special property of T4 late gene transcription. We have repeated the experiment shown in Fig. 4 with a ternary transcription complex derived from initiation at a σ^{70} promoter by *E. coli* RNA polymerase (in the absence of any T4 protein) and have obtained the same result (data not shown).

A stalled ternary complex is an imperfect representation of true transcription intermediates, whose normal structures are likely to be kinetically determined[4]. But the recovery of full-length transcripts during a chase in which rolling circle replication is ongoing (Fig. 4*a*) suggests that many transcription intermediates (and not just our stalled ternary complex) can survive the replication fork; further evidence supporting this point will be presented elsewhere.

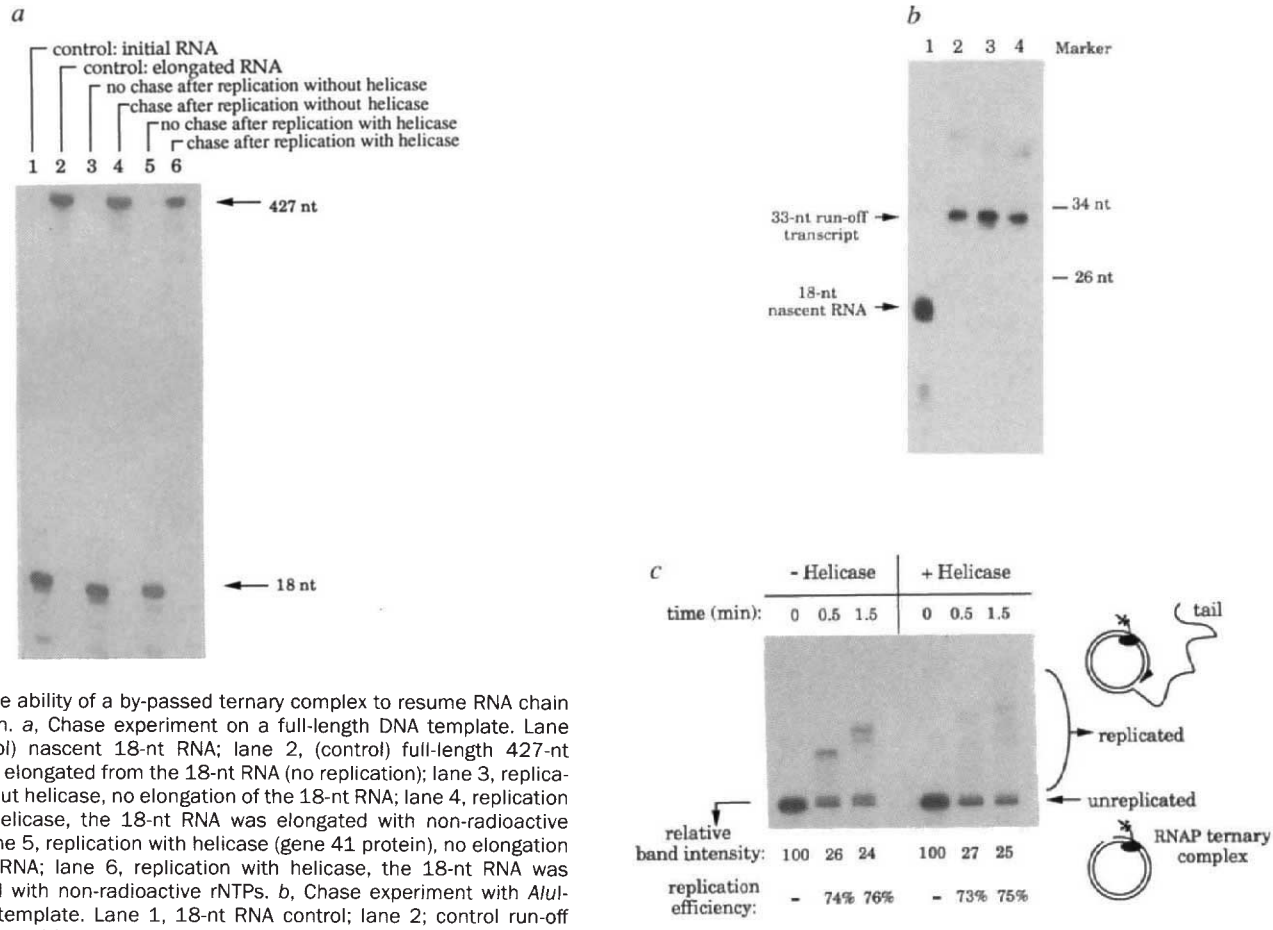


FIG. 4 The ability of a by-passed ternary complex to resume RNA chain elongation. *a*, Chase experiment on a full-length DNA template. Lane 1, (control) nascent 18-nt RNA; lane 2, (control) full-length 427-nt transcript elongated from the 18-nt RNA (no replication); lane 3, replication without helicase, no elongation of the 18-nt RNA; lane 4, replication without helicase, the 18-nt RNA was elongated with non-radioactive rNTPs; lane 5, replication with helicase (gene 41 protein), no elongation of 18-nt RNA; lane 6, replication with helicase, the 18-nt RNA was elongated with non-radioactive rNTPs. *b*, Chase experiment with *AluI*-cut DNA template. Lane 1, 18-nt RNA control; lane 2; control run-off transcript (~33-nt RNA); lane 3, run-off transcript after replication without helicase; lane 4, run-off transcript after replication with helicase. *c*, Determination of replication efficiency. Replication (with or without DNA helicase) proceeded at 37 °C for the time indicated. The replication efficiencies are calculated from the reduction of the radioactive signal (quantified using a PhosphorImager) at the position of the non-replicated molecules.

METHODS. *a*, *In vitro* replication was as described in Fig. 2 (except that the DNA was not linearized) for 5 min (without helicases) or for 2 min (with helicases), followed by the addition of cold rNTPs (0.5 mM rATP, 0.5 mM rGTP, 0.2 mM rCTP and 0.2 mM rUTP) to chase the nascent transcript at 37 °C for 8 min. Samples were then chilled on ice, treated with 2 units of DNase I (with $CaCl_2$ at a final concentration of 0.5 mM), phenol–chloroform extracted and electrophoresed on a 10% denaturing polyacrylamide gel. To rule out the possibility of RNA polymerase reassociation during the rNTP chase, this experiment has been repeated: (1) in the presence of rifampicin (30–50 μg ml^{-1}); (2) with synthetic oligonucleotides containing the promoter sequences in 10–20-fold molar excess over the template; (3) with yeast ribosomal RNA (40–100 mg ml^{-1}). These variations did not change the outcome of the experiment. *b*, As in *a*, except that RNA chains were elongated on templates that had been digested with 10 units of *AluI*. *c*, *In vitro* replication was done on the column-purified complex under the same conditions of the chase experiments described in *a* and *b*. The reaction was stopped by chilling the samples on ice at the indicated times. Heparin and Ficoll were added to concentrations of 100 μg ml^{-1} and 3%, respectively. Samples were then loaded on a 0.8% neutral agarose gel (non-denaturing) in 1 × TBE and electrophoresed at room temperature for 4–5 h at 7 V cm^{-1}. The gel was dried and autoradiographed or exposed to a PhosphorImager screen for quantitative analysis.

It is not obvious how two bulky enzyme complexes can pass one another in a non-destructive way. In particular, because the codirectionally moving DNA and RNA polymerases use the same DNA single strand as template, the replication apparatus almost certainly unwinds the end of the growing RNA transcript that is base-paired to DNA. How difficult is this likely to be? Recent structural studies on transcription complexes[17,18] suggest that growing RNA chains may be relatively loosely held at the 3′ end in a short DNA–RNA hybrid, and periodically transferred to a tighter binding site in *E. coli* RNA polymerase[17–25]. This would imply that: (1) the contribution of the RNA–DNA hybrid to the stability of the ternary complex need not be as great as previously thought[26,27], making its transient unpairing less daunting; (2) the RNA–DNA hybrid need not present an insurmountable barrier to the progression of a replication fork.

Although we cannot rule out the possibility that some property of the T4 DNA replication proteins is important for our observations (for example, tethering the RNA polymerase to the DNA molecule, thereby preventing its release as the DNA polymerase passes), it seems more likely that our results are intrinsic to the *E. coli* RNA polymerase, a multiple-subunit enzyme[28] that can undergo large conformational changes[29]. A schematic model of this type is shown in Fig. 6, where at least two DNA-interacting domains of the polymerase are present within the ternary complex, each individually detachable from the DNA without destroying the complex. When the replication fork invades the interior of the complex, it causes a momentary unpairing of the short RNA–DNA hybrid at the 3′ end of the nascent RNA in the ternary complex, but the other DNA-binding domain keeps the RNA polymerase attached to the daughter DNA helix. The nascent RNA remains bound to RNA polymerase, and it may ensure the maintenance of transcription fidelity by its specific hybridization back to the DNA template.

In contrast to the ternary transcription complex, a promoter-bound RNA polymerase that is not transcribing is displaced from the template after replication[30]. Compared to the very tight ternary complex that enables RNA polymerase to transcribe in a highly processive manner, promoter binding by RNA polymer-

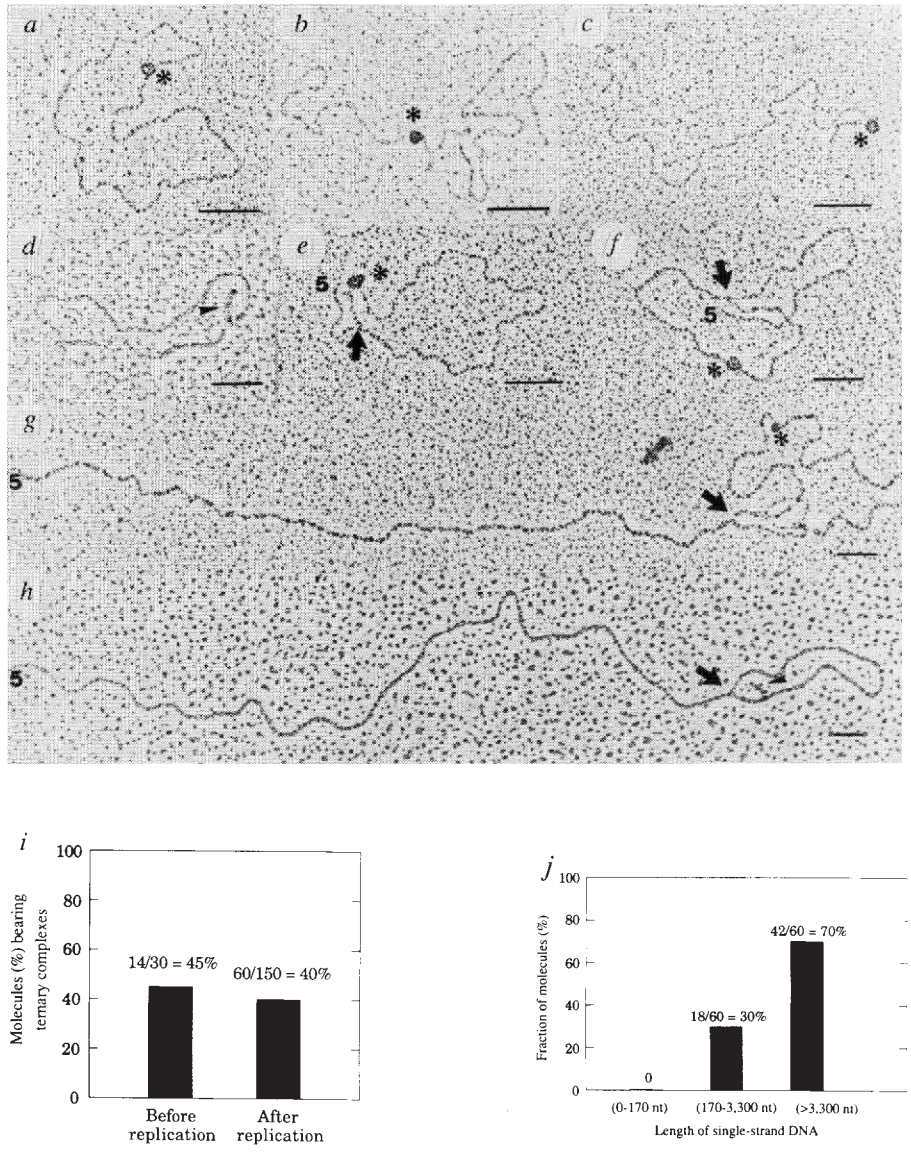


FIG. 5 Electron microscopic examination of replication products. *a*, A non-replicated molecule bearing a globular particle (the putative RNA polymerase ternary complex). *b* and *c*, Mapping the position of the globular particle by restriction enzyme digestion (*b*, *Ssp*I; *c*, *Hin*dIII). The relative distances from the particle to the two ends (long/short): for *Ssp*I, the measured ratio is 1.8 (±0.1) (expected 1.8); for *Hin*dIII, the measured ratio is 23 (±3) (expected 25). *d*, Production of nascent RNA. *e–g*, Various extents of replication take place on templates bearing a ternary complex. The ternary complex remains on the replicated DNA. *h*, Production of RNA on the extensively replicated DNA template. *i*, Comparison of the percentage of molecules bearing a ternary complex before and after replication. Randomly selected samples of 30 non-replicated and 150 replicated molecules were examined. *j*, Distribution of replicated molecules with tails of varying lengths. The distance from the gene 2 nick (replication origin) to the ternary complex is ~170 nt. If the replication fork stalls permanently before the ternary complex, no molecules should bear a tail exceeding this length. Scale bar, 0.1 μm. Arrows, replication forks. Asterisks, RNA polymerase (*a–c*, *e–g*). Arrowheads, nascent RNA (*d* and *h*). '5', the 5′ end of a displaced DNA tail.

METHODS. *In vitro* replication was done on CL-2B-column-purified ternary complex as described in the legend to Fig. 1*b*. The reaction was stopped by chilling the sample on ice, followed by the addition of NaCl to 0.5 M and passage through CL-2B to remove replication proteins. Electron microscopy studies were done on the radioactive fractions as follows: *a–c*, and *e–g*: 2–8 μl samples were applied to glow-discharged carbon grids for 2 min, dehydrated in 100% ethanol and uranyl acetate, and shadowed with platinum at an angle of 8 degrees[45]; *d* and *h*, rNTPs (1 μM each) were added to elongate the nascent 18-nt RNA at 37 °C for 30 s. The reaction was stopped with 20 mM Na_3-EDTA. Transcripts were crosslinked to DNA templates by ultraviolet light (254 nm) irradiation at 25 °C for 10 min at a distance of ~2 cm from a UVGL-25 lamp. Samples were spread with cytochrome *c* as described in ref. 46 before examining them with a Philips EM400 microscope.

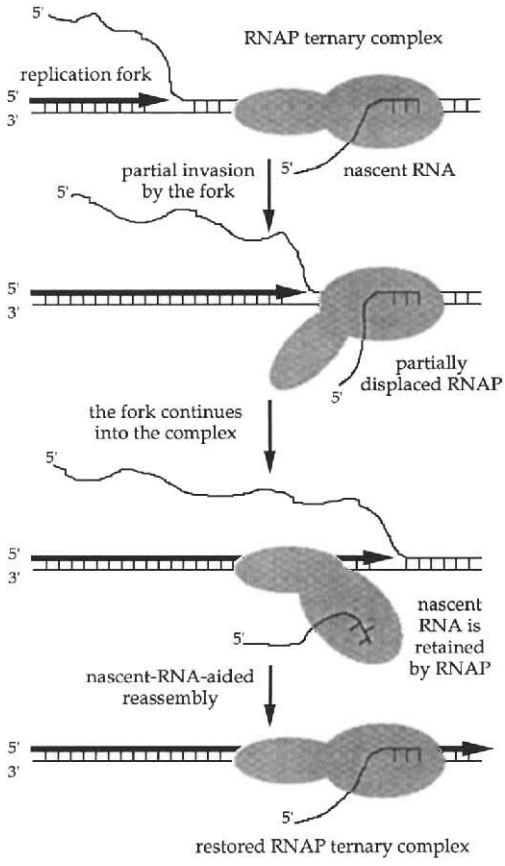


FIG. 6 A schematic model to account for some of the experimental observations. Well-separated DNA-binding domains might allow the *E. coli* RNA polymerase (RNAP) to retain its place as replication passes through. Replication proteins and the transcription bubble are not drawn. As described in the text, the retention of exact transcription register that we observed (Fig. 4*b*) is likely to involve base-pair reformation by the 3′ end of nascent RNA. In addition, the ternary complex contains about 17 base pairs of separated DNA strands[47] in the form of a transcription bubble. Because there is a substantial energetic cost to reforming this bubble[4], the passage of a replication fork through the ternary transcription complex might involve a reaction pathway that never entirely dissipates the DNA strand separation.

ase is a weaker interaction. Because it relies on hydrogen-bonding interactions with specific bases on both DNA strands[4], the separation of the two strands of the double helix during replication would be expected to destabilize the promoter complex. There is little energy investment during promoter binding by RNA polymerase, and its displacement is less costly to the cell.

Our results suggest the existence of a mechanism that allows the RNA polymerase and its attached transcript to survive the collision between the replication and transcription machineries. Because the *E. coli* and the eukaryotic nuclear RNA polymerases have evolved from a common ancestor and have homologous subunits that share amino-acid sequence homology[31,32], our results may also be relevant to the behaviour of these polymerases when a replication fork passes.

Our conclusions are entirely based on *in vitro* experiments done with highly purified proteins. Is there any *in vivo* evidence for or against a mechanism of this type? When the fate of the nascent transcript of a large *Drosophila* gene (Ubx) whose complete transcription takes longer than the time of a cell cycle was observed[33], DNA synthesis *in vivo* did not abort the nascent transcript (in this case, the orientation of the fork relative to the RNA polymerase movement is unknown). In contrast, electron microscopy has been used to show that the nascent transcripts of a rRNA gene of *E. coli* are displaced from the template when a replication fork invades the transcription unit from either direction[34]. But these rRNA transcripts are unusual in at least two aspects: they are attached to closely spaced RNA polymerase molecules, and they are modified by a set of specialized RNA-binding proteins[35]. We would predict that a different result would be obtained with other transcription units. Methods that permit a quantitative analysis, such as simultaneously probing the fork movement and nascent transcript production by nucleic acid hybridization, should be useful for examining this issue.

It is certainly possible that unidentified protein factors exist *in vivo* that modulate the basic mode of interaction between a replication fork and RNA polymerase observed in our experiments. Nevertheless, the fact that a ternary complex can survive a replication fork *in vitro* demonstrates a remarkable ability of RNA polymerase to cope with perturbing events during its elongation of RNA chains. □

Received 16 June; accepted 30 September 1993.

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ACKNOWLEDGEMENTS. We thank J. Barry and G. A. Kassavetis for advice and help, and K. Hacker, G. Sanders, J. Raff, C. Mirzayan, R. Schrock, R. V. Aroian and J. Cox for reading the manuscript. This work is supported by separate grants from the National Institute of General Medical Sciences to B.M.A. and E.P.G., and by the Howard Hughes Medical Institute (M.L.W.). B.M.A. is an American Cancer Society Research Professor.