Replication forks are arrested at sequence-specific replication termini primarily, perhaps exclusively, by polar arrest of helicase-catalyzed DNA unwinding by the terminator protein. The mechanism of this arrest is of considerable interest. This paper presents experimental evidence in support of four major points pertaining to termination of DNA replication. First, the replication terminator proteins of both Escherichia coli and Bacillus subtilis are helicase-specific contrahelicases, i.e. the proteins specifically impede the activities of helicases that are involved in symmetric DNA replication but not those involved in conjugative DNA transfer and rolling circle replication. Second, the terminator protein (Ter) of E. coli blocks not only helicase translocation but also authentic DNA unwinding. Third, the replication terminator protein of Gram-positive B. subtilis is a polar contrahelicase of the primosomal helicase PriA of Gram-negative E. coli. Finally, the blockage of PriA-catalyzed DNA unwinding was abrogated by the passage of an RNA transcript through the replication terminator protein-terminus complex. These results are significant because of their relevance to the mechanistic aspects of replication termination.

Specific termination sites of DNA replication exist in the plasmid R6K (1–3) and the bacteria Escherichia coli (4, 5) and Bacillus subtilis (6, 7). Sites that arrest replication forks have also been reported in eukaryotes such as Epstein-Barr virus (8), and in ribosomal DNAs of yeast (9, 10), plant (11), and humans (12). The yeast centromeres are also known to arrest replication fork movement (13).

In prokaryotes, the replication fork arrest is polar and is mediated by the interaction of replication terminator proteins with the terminus sequence (6, 7, 14–16).

The replication terminator protein of E. coli (called Ter or Tus) and that of B. subtilis (called RTP)1 are polar contrahelicases, i.e. these proteins inhibit the activities of DnaB helicase of E. coli in only one orientation of the terminus sequence with respect to the origin (17–20). Although several groups have reported that Ter protein of E. coli (17, 21) impedes the activity of DnaB but not that of helicase II, others believe that the terminator proteins are general road blocks on DNA that block the passage of many if not all helicases (22–24). The resolution of the question of helicase specificity of replication terminator proteins has an obvious bearing on the mechanism of the contrahelicase activity. Blocking of many helicases that are involved in other functions such as DNA repair, transcription, conjugative transfer of DNA etc., might imply that the interactions of Ter and RTP with their respective binding sites called + and IR sequences (or BS3) are all that might be necessary to elicit polar impedence of helicase activity. In contrast, helicase-specific block might imply, in addition to DNA-terminator protein interaction, also helicase-terminator protein interaction. The crystal structure of RTP of B. subtilis has been solved at 2.6-Å resolution (25). The protein has a postulated tripartite DNA binding domain that envisages contact with the minor groove of DNA by the N-terminal arm and the α2-β3 pleated sheets and major groove contact by the α3 region of the protein. The crystal structure suggests an exposed hydrophobic patch near the α2-β3 region as a possible surface for interaction with helicases.

The Ter protein also blocks replication forks of SV40 and antagonizes the helicase activity of the T-antigen (26, 27). We have recently discovered that Ter and RTP interact with DnaB helicase and SV40 T-antigen.2 Hiasa and Marians (21) have reported that purified Ter, in vitro, can block DnaB translocation but not authentic unwinding of DNA duplex. The implication is that blocking of DNA unwinding requires the participation, along with Ter, of other proteins. Recently, we have discovered that both Ter and RTP are also polar anti-transcriptases and block RNA chain elongation by E. coli, T7, and SP6 RNA polymerases.3 The present investigation was mainly driven by the need to answer two questions: (i) are the contrahelicase activities of Ter and RTP helicase-specific? and (ii) do the terminator proteins by themselves, without the assistance of other proteins, block authentic helicase-catalyzed DNA unwinding? In addition to answering the two questions posed above, the results presented in this paper also demonstrate that RTP of Gram-positive B. subtilis, impeded the activity of PriA helicase of Gram-negative E. coli. Furthermore, the PriA-blocking activity of RTP was abrogated by the passage of an RNA transcript through the terminus (BS3)-RTP complex.

MATERIALS AND METHODS

Purification of Proteins and Enzymes—Ter, DnaB (17), and RTP (28) were purified as published. Rep helicase (29) was a generous gift from Tim Lehman (Washington University, St. Louis, MO). Single strand DNA binding protein (SSB) was from a commercial source (U.S. Bio.

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1 The abbreviations used are: RTP, replication terminator protein; SSB, single strand DNA binding protein; DTT, dithiothreitol; bp, base pair; PAS, primosome assembly site.

2 B. K. Mohanty and D. Bastia, manuscript in preparation.

3 B. K. Mohanty, T. Sahoo, and D. Bastia, manuscript in preparation.
Helicase I was purified according to a modification of a published procedure (30). The cells were harvested and lysed with lysozyme (1 mg/ml) in 50 mM Tris-HCl, pH 8.0, 10% sucrose, 1 mM DTT, 1 mM EDTA. The cleared lysate was precipitated with (NH₄)₂SO₄ (0.24 g/ml). The precipitate was spun down, dissolved in buffer A (25 mM imidazole-Cl, pH 6.8, 1 mM DTT, 1 mM EDTA, 15% glycerol, 50 mM NaCl) and dialyzed against the same buffer. The dialysate was loaded onto a Bio-Rex 70 column equilibrated with buffer A. After washing, the protein was eluted with 10 column volumes of a gradient of 0.05–1 M NaCl in the same buffer. The protein was eluted with a 0–0.75 M NaCl gradient in buffer B. The protein was eluted with a 0–0.75 M NaCl gradient, and peak fractions were taken through the (NH₄)₂SO₄ precipitation and the dialysate was loaded onto a Bio-Rex 70 column equilibrated with buffer C. The column was washed and eluted with a 20–400 mM linear (NH₄)₂SO₄ gradient in buffer C.

For assaying PriA helicase, the recombinant M13 single-stranded DNA was annealed to the specific single-stranded DNA and after purifying through Sepharose CL-4B columns were used for helicase assay. The enzyme was then purified by successive fast flow Q-Sepharose chromatography close to homogeneity (see Fig. 1). All gradients for the above mentioned steps were prepared in buffer D (10 mM NaCl, 0.1 mM EDTA, 15% glycerol), loaded onto a P11-phosphocellulose column, and eluted with a 0–1 M NaCl gradient in buffer D. The further purification involved S-Sepharose, hydroxylapatite, and a Mono-S step in sequence. PriA was purified from a plasmid (pMP8) were induced, and the purification was carried out through a DEAE-Sephadex step according to the published procedure (30).

The enzyme was then purified by successive fast flow Q-Sepharose, heparin-agarose and Mono-Q fast protein liquid chromatography columns. The purity of the preparations was monitored by SDS-PAGE on 10% gels and stained with Coomassie blue. The radioactive bands were detected on autoradiography. The percentage of oligonucleotide released was quantitated by PhosphorImager analysis or by Cerenkov counting of radioactivity present in each lane. The concentrations of helicase I and Rep were kept constant at 0.8, 1.2, and 1.6 pmol of RTP, respectively; lanes M–N, mp18B3 with DnaB and 0 and 0.4 pmol of RTP, respectively. Note that there is no detectable blockage of Rep helicase activity by RTP-B3 complex in either orientation, whereas DnaB activity is impeded. Top right panel, the radioactivity present in each band shown in the top left, was quantitated with a PhosphorImager scanner. The percentages confirm the conclusion stated above. Bottom left panel: lane A, mp19 substrate without Ter and without helicase I; lanes G–I, mp19 with 100 fmol of helicase I and 0, 0.5, 1.0, 1.5, 2.0, 2.5, and 3.0 pmol of Ter, respectively; lane I, mp18 with 400 fmol of Rep and 0, 0.4, 0.8, 1.2, and 1.6 pmol of RTP, respectively; lanes M–N, mp18 with 100 fmol of helicase I and 0, 0.5, 1.0, 1.5, 2.0, 2.5, and 3.0 pmol of Ter, respectively. Note there is no detectable blockage of helicase I activity by Ter in either orientation of +. Bottom right panel, the bands shown in the bottom left panel were quantitated with a PhosphorImager and plotted. The data support the conclusion made above.

RNA chain synthesis proceeded toward the BS3 site. The orientation of the BS3 site in this substrate was such that R TP-B3 complex would block PriA activity but not T7 RNA polymerase movement and RNA chain elongation (Fig. 8, top panel). An oligonucleotide complementary to the BS3 site was annealed to the single-stranded DNA and extended with Sequenase (modified T7 DNA polymerase, U.S. Biochemical Corp.) in the presence of dNTPs, [α-32P]dATP, ddCTP, and ddGTP to give partial duplex substrate of various lengths.

Helicase assay—Standard reaction mixture (in a total volume of 20 μl) for DnaB, Helicase I, and Rep helicase contained 10 fmol of the DNA substrate in 50 mM Tris-HCl pH 7.5, 4 mM MgCl₂, 2 mM ATP, 50 mM potassium glutamate, 50 μg/ml BSA, and 5 mM DTT. Indicated amounts of Ter or RTP were added to the substrate and placed on ice (in the case of Ter) or at room temperature (in the case of RTP) for 15–20 min. After adding the helicase (DnaB, Rep, Helicase I, or PriA) the mixture was incubated at 37 °C for 10 min (in case of Helicase I, Rep, and PriA) or 15 min (for DnaB). Reactions were terminated by the addition of dye mixture containing SDS-EDTA-sucrose and bromophenol blue. Analysis was done by electrophoresis through 6–8% polyacrylamide gel in Tris borate-EDTA buffer and autoradiography with Kodak XAR film. The percentage of oligonucleotide released was quantitated by PhosphorImager analysis or by Cerenkov counting of radioactivity present in the bands excised from the gel after autoradiography. For PriA helicase assay the reaction mixture contained, in addition to the above components, 0.5 μg of SSB/reaction. Mapping of the minimal effective terminator sequence required for Ter protein to bind and block DnaB-mediated oligo release was done by eluting the released bands in the gel with the radioactive bands (bracket and arrow in Fig. 6) and resolving it in a sequencing gel alongside a sequence ladder generated by the same oligo single-stranded DNA template. The coupled helicase transcription assay (PriA-T7 RNA polymerase) was carried out in a reaction mixture...
Ter (Tus) protein of both Ter and RTP? Previous work had established the fact that specific, implying specific recognition of replicative helicases by helicase that translocates on DNA, or is the block helicase—conducted to answer the following question. Do the terminator trahelicases—in a polar fashion (Fig. 2, DNA unwinding by Rep helicase. In contrast, DnaB was blocked in both orientations with respect to the direction of the helicase substrate contained the terminus of E. coli DnaB helicase was used as a positive control. The helicase purities of the various enzymes and proteins used in the experiments described in this paper are shown in Fig. 1. In the first series of experiments we kept the molar concentration of Rep helicase at 400 fmol (Fig. 1. In the first series of experiments we kept the molar concentration of helicase I was not impeded by Ter (kept constant at a concentration of protein:substrate up to 60:1) in either orientation of the terminus.

Results

Replication Terminator Proteins Are Helicase-specific Contrahelicases—The experiments described in this section were conducted to answer the following question. Do the terminator proteins act as polar clamps on DNA that block almost any helicase that translocates on DNA, or is the block helicase-specific, implying specific recognition of replicative helicases by both Ter and RTP? Previous work had established the fact that Ter protein of E. coli (17, 18) and RTP of B. subtilis (19, 20) impeded the activity of the E. coli replicative helicase, DnaB. We wished to investigate, using a wide range of concentrations of other helicases and the two terminator proteins, whether non-replicative helicases, i.e., helicase I and Rep helicase, are impeded by RTP and Ter. Non-replicative helicases are helicases that are not involved in Cairns-type or symmetric DNA replication. The purities of the various enzymes and proteins used in the experiments described in this paper are shown in Fig. 1. In the first series of experiments we kept the molar concentration of Rep helicase at 400 fmol (~58 ng) and varied the molar ratios of RTP over the DNA substrate from 40 to 160. DnaB helicase was used as a positive control. The helicase substrate contained the terminus of B. subtilis called BS3 (IRI) in both orientations with respect to the direction of the helicase translocation. The results shown in the autoradiogram and its quantitative analysis (Fig. 2, top) revealed that under the wide range of experimental conditions used, RTP failed to block DNA unwinding by Rep helicase. In contrast, DnaB was blocked in a polar fashion (Fig. 2, top left, lanes M and N). Similar experiments performed using Ter protein gave identical results (data not shown). We also performed helicase assays using a fixed amount (100 fmol = 18 ng) of helicase I and a wide range of ratios of Ter to DNA substrate (50–300). The results again showed that helicase I was not impeded by Ter (Fig. 2, lower panel). Similar experiments were also performed with RTP and yielded similar results (data not shown). Thus neither Ter nor RTP impeded the catalytic activity of Helicase I when bound to the respective terminator sequences present in either orientation.

In view of the reports in the literature that terminator proteins block many helicases (18, 24), we wished to confirm our observations by keeping the concentrations of RTP and Ter constant and changing the ratios of helicase I to the substrate over a wide range. The helicase assays again showed that neither the activity of Rep helicase (molar ratios of 5–40; RTP 40-fold molar excess over DNA substrate) nor of helicase I (molar ratio of enzyme over substrate 5–60; RTP present in 40-fold molar excess over substrate) was impeded by RTP (Fig. 3).

Fig. 3. The activities of both helicase I and Rep helicase are not impeded by RTP. The RTP concentration was kept fixed (RTP: substrate = 40:1), but the Rep and helicase I concentrations were varied over a wide range. Top left and right panels, the activity of Rep helicase was not impeded over a wide range of concentrations of the helicase (molar excess of enzyme to substrate up to 40-fold) in both orientations of the terminus of B. subtilis (BS3). Bottom left and right panels, the activity of helicase I was not impeded by RTP (kept constant at a concentration of protein:substrate up to 40:1) over a wide range of concentrations of helicase I (helicase:substrate up to 60:1) in either orientation of the terminus.

Fig. 4. Autoradiogram of an 8% polyacrylamide gel showing response of helicase I-mediated DNA unwinding to increasing concentrations of Ter protein. Substrate for helicase assay with extended heteroduplex region across the r site in M13 mp18r and M13 mp19r was prepared as depicted in the figure (top panel). Lane M, double-stranded DNA marker; lane A, mp18r without helicase I and without Ter; lanes B–E, mp18r with helicase I and increasing concentrations of Ter (0, 0.2, 0.4, and 0.8 pmol); lane F, mp19r without helicase I and without Ter; lanes G–J, mp19r with helicase I and increasing concentrations of Ter (0, 0.2, 0.4, and 0.8 pmol); lane K, mp19r with DnaB only; lane L, mp18r with DnaB and 0.4 pmol of Ter; lane M, mp19r with DnaB only; lane N, mp19r with DnaB and 0.4 pmol of Ter. Note there was no blockage of helicase I activity in either orientation by Ter in contrast to polar blockage of DnaB activity (positive controls).
experimental conditions.

We endeavored to test whether Ter (or RTP) might block the activity of Helicase I on a DNA substrate that had a heteroduplex region of only a certain length. Using a oligonucleotide primer and the circular single-stranded DNA of M13 mp18/19, we generated a population of DNA substrates that contained various lengths of heteroduplex regions from < 100 bp to > 2000 bp (Fig. 4, top). We performed helicase assays in the absence and presence of various concentrations of Ter. DnaB helicase was used as a positive control. The results showed that whereas DnaB was impeded in a polar fashion by Ter (Fig. 4, bottom), lanes k and l, the activity of Helicase I was not impeded, regardless of the length of the heteroduplex region present in the substrate (Fig. 4, lanes B–E, and G–J), and in both orientations of the \( \tau \) sequence.

RTP Impedes the Activity of PriA Helicase in a Polar Mode—To extend our previous observation that RTP of Gram-positive B. subtilis blocks the replicative helicase DnaB of Gram-negative E. coli (19, 20), we wished to test whether RTP also impedes the activity of PriA helicase of E. coli. PriA translocates in a 3′ → 5′ direction on DNA, unlike DnaB that moves in a 5′ → 3′ direction (21, 32). We constructed a helicase substrate that had a PAS (Fig. 5A) and the BS3 site in both orientations (BS3 and BS3rev). The helicase assays showed that RTP of B. subtilis failed to block the activity of PriA on the BS3 substrate; DnaB activity in contrast was impeded (Fig. 5B, top). However, in the BS3rev substrate, RTP impeded the helicase activity of PriA but not of DnaB (Fig. 5B, bottom). The quantitation of the autoradiogram shown (in Fig. 5B) is shown in Fig. 5C. Thus a 20-fold molar excess of RTP over DNA substrate was able to block, in a polar fashion, the activity of 40 fmol of PriA.

The Ter Protein, Unaided by Other Replication Proteins, Can Block Not Only Helicase Translocation but Also Authentic DNA Unwinding—Previous work by Hiasa and Marians (21) had suggested that the Ter (Tus) protein, unaided by other replisomal proteins, was able to block DnaB translocation but not authentic unwinding of long stretches of double-stranded DNA (>250 bp). The conclusions of Hiasa and Marians (21) were based on the observation that Ter by itself could impede the DnaB-catalyzed unwinding of short DNA duplexes of up to 100 bp but failed to block the unwinding of duplexes over 250 bp and, secondly, that the half-maximal concentration of ATP required for unwinding of short duplexes was the same as that needed for DnaB translocation. In contrast the half-maximal concentration of ATP required for DnaB-catalyzed unwinding was 10-fold more than that needed for enzyme translocation. The implication was that Ter by itself can not block helicase-catalyzed unwinding ahead of a replication fork but needed the assistance of other proteins for this activity. We wished to reexamine this point by constructing helicase substrates that had a wide range of lengths of double-stranded DNA that include the terminus \( \tau \) in both orientations. We used M13 mp18/19, single-stranded DNA circles with a primer that was extended in the presence of a dideoxynucleotide triphosphate (ddNTP) and \((\alpha^{32}P)\)dATP and T7 DNA polymerase. A set of extension products, ranging in length from ~50 to ~1500 nucleotides was thus generated (Fig. 6, top). The two sets of substrates M13 mp18/19; thus generated were incubated with DnaB and ATP in the absence and presence of a range of concentrations of Ter protein. The results showed that, in the M13 mp18/19 substrates, all the extension products that had stopped short of the \( \tau \) sequence were released by DnaB, whereas all DNA chains that included \( \tau \), ranging in length from ~150 to greater than 1500 bp, were blocked from release by DnaB, thus generating a “footprint” of release (Fig. 6, bottom, lanes C–G). In contrast, in the M13 mp19/19 substrate, Ter protein was unable, as expected, to block DnaB activity, and a ladder of products from ~50 to ~1500 nucleotides were released (Fig. 6, bottom, lanes I–N). These experiments showed that there was no length dependence of Ter in its ability to impede DnaB activity in a polar fashion. Thus, we concluded...
that Ter protein by itself was capable of impeding both DnaB translocation and duplex unwinding, unaided by any other replisomal proteins.

The Minimal Effective Sequence of \( t \) That Promotes Contrahelicase Activity—The “helicase activity footprint” described above provided us with an approach to determine the minimum critical nucleotide sequence of \( t \) that, when present in the double-stranded form, could elicit contrahelicase activity of Ter. The experiment described in Fig. 6 was performed separately, using all four ddNTPs, and the regions marked by the bracket (Fig. 6, bottom) were eluted and resolved in a sequencing gel with appropriate sequencing ladder as markers. This experiment allowed us to determine the precise right boundary of a “minimal effective length” of the \( t \) sequence (Fig. 7, top). Thus the extended primer had to copy the critical T residue on the template, thus generating the complementary sequence 5′-ACTTTAGTTACACATA-3′ to be able to elicit contrahelicase activity. All extension products short of the last A residue failed to elicit contrahelicase activity of the Ter protein (Fig. 7, bottom).

The Contrahelicase Activity of RTP against PriA Helicase Is Abrogated by an Invading RNA Transcrip—We have previously shown that Ter and RTP are also DNA sequence-specific anti-transcriptases, i.e., the proteins are able to block, in a polar fashion, RNA chain elongation by several prokaryotic RNA polymerases. We have also shown that the contrahelicase activity of Ter and RTP directed against DnaB, that translocates in a 5′→3′ direction on DNA, is abrogated by an invading RNA transcript. We wished to examine if RNA transcription would have the same effect on the ability of RTP to impede a helicase that translocates in the 3′→5′ direction, i.e., PriA. We constructed a set of substrates by primer extension on the M13 mp18BS3rev template that included a PAS as shown in Fig. 8 (top). The substrate included a T7 promoter capable of directing a RNA transcript clockwise into the BS3rev terminus. The family of partial heteroduplexed substrates was coated with SSB, and helicase assays were performed with purified PriA. The results showed that PriA, in the presence of ATP was able to unwind the DNA, thus releasing a ladder of extension products (Fig. 8, bottom, lanes A and B). RTP blocked the release of the ladder (Fig. 8, bottom, lanes C and D). Turning on the T7 primer extension

\[5′ \rightarrow -C \rightarrow 3′\]

\[5′ \rightarrow -A \rightarrow 3′\]

\[5′\text{ACTTTAGTTACACATA}-3′\] blocked

\[3′\text{TGAAATCAATGTGATGAAATAAAAA}-5′\] unwind

FIG. 6. Autoradiogram of a nondenaturing 6% polyacrylamide gel showing polar contrahelicase activity of Ter protein independent of the length of the double-stranded region in a heteroduplex containing interstitially located Ter-binding site (†). Top, the helicase substrates with M13 mp18- (Ter-active) and M13 mp19- (Ter-inactive) templates were constructed as described in the figure. Bottom: lane M, double-stranded DNA marker; lane A, boiled M13 mp18- substrate; lane B, M13 mp18- without Ter and without DnaB proteins; lanes C-G, M13 mp18- substrate with 400 ng of DnaB and increasing amounts of Ter protein (0, 0.4, 0.8, 1.2, and 1.6 pmol, respectively); lane H, boiled M13 mp19- substrate; lanes I-N, M13 mp19- substrate with 400 ng of DnaB and increasing amounts of Ter protein (0, 0.4, 0.8, 1.2, and 1.6 pmol). Note that Ter- complex blocks DnaB-catalyzed unwinding of heteroduplex DNA containing the Ter binding site in a polar fashion and is independent of the size of the oligonucleotide.

FIG. 7. Autoradiogram showing precise right boundary of the minimum effective terminator sequence required for Ter protein to bind and mediate contrahelicase activity with respect to DnaB. Lanes a, c, g, and t represent extended primer across the † site effectively released by DnaB in the presence of Ter protein (shown by bracket and arrow in Fig. 6). Lanes A, C, G, and T are sequence ladder across † site generated using the same primer used for extension and helicase assay. Note when chain extension (i.e. heteroduplex region) is beyond the “T” at the 3′ end (bracketed) of the sequence shown in the bottom panel, Ter protein is able to effectively block DnaB activity in a polar fashion (lanes D-G in Fig. 6).
We have critically examined this issue using the replicative primosomal helicase PriA of E. coli and the Rep helicase and Helicase I of E. coli as representatives of non-replicative helicases. Non-replicative helicases implies those that do not participate in symmetric or “Cairns type” DNA replication. It is worth remembering that Helicase I is involved in conjugal DNA transfer, whereas Rep helicase is involved in rolling circle DNA replication of the φX174 family of phages (33, 34).

We have found that RTP of B. subtilis acted as a polar contrahelicase of PriA, thus complementing our earlier results with DnaB of E. coli (19, 20). In contrast, we detected no blockage of either Rep or Helicase I by Ter or RTP over a wide range of terminator-to-substrate and helicase-to-substrate ratios. Thus we could not confirm an earlier report claiming polar blockage of Helicase I by Ter protein (23). On the basis of our results reported in this paper, we believe that Ter and RTP are specific contrahelicases of the replicative helicases DnaB and PriA, and in the case of Ter, of T antigen of SV40 (23, 26, 27). Implicit in these results is the idea that the arrest of replication forks at the terminus involves specific interaction between replicative helicase and terminator protein(s). Consistent with this notion is our recent discovery that a point mutation at a residue believed to be in the helicase blocking domain of RTP, as suggested from its crystal structure (25), does not interfere with DNA binding but abolishes the ability of the resultant RTP to block DnaB helicase.4

A second issue addressed in this paper is whether Ter, unaided by any other protein, can block authentic, helicase-catalyzed DNA unwinding. Our results conclusively show that over a wide range of ~50 to greater than 1500 bp of duplex DNA, DNA unwinding by DnaB is abolished by Ter, thus complementing our earlier results with RTP (20). Thus we were unable to confirm the conclusions of Hiasa and Marians (21) that Ter by itself can block helicase translocation but not authentic DNA unwinding.

We have reported that invasion of a terminus, in vitro, by an RNA transcript, released arrested PriA helicase. This observation provides further support of our proposition that transcriptional invasion of a replication terminus is detrimental to the termination process. The RNA transcripts are halted before entering the terminus by the RNA chain anti-elongation activity of RTP and Ter in a polar fashion.

The Ter protein of E. coli and RTP of B. subtilis bear little or no homology in the primary amino acid sequence, yet both proteins block the same replicative helicases of E. coli, namely DnaB and PriA (17, 19, 20). On the basis of crystallography of RTP and mutational analysis, there are already preliminary indications of the surface of RTP that is involved in helicase blocking.5 Although Ter protein has been refractory to crystallization, mutational analysis of its coding region should enable one to localize the helicase-blocking region of the protein. Future work will be directed at localizing the RNA polymerase-blocking domain on RTP and Ter as well as the interaction surface on DnaB that recognizes Ter and RTP. Such studies should illuminate further the mechanism of replication termination.

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