We have delineated the amino acid to nucleotide contacts made by two interacting dimers of the replication terminator protein (RTP) of Bacillus subtilis with a novel naturally occurring bipolar replication terminus by converting RTP to a site-directed chemical nuclease and mapping its cleavage sites on the terminus. The data show a relatively symmetrical arrangement of the amino acid to base contacts, and a comparison of the bipolar contacts with that of a normal unipolar terminus suggests that the DNA-protein contacts play an important determinative role in generating polarity from structurally symmetrical RTP dimers. The amino acid to nucleotide contacts provided distance constraints that enabled us to build a three-dimensional model of the protein-DNA complex. The model is consistent with features of the bipolar Ter-RTP complex derived from mutational and cross-linking data. The bipolar terminus arrested Escherichia coli DNA replication and DnaB helicase and T7 RNA polymerase in vitro in both orientations. RTP arrested the unwinding of duplex DNA on the bipolar Ter-DNA substrate regardless of the length of the duplex DNA. The latter result suggested further that the terminus arrested authentic DNA unwinding by the helicase rather than just translocation of helicase on DNA.

In many prokaryotic and some eukaryotic replicons, replication forks initiated at specific replication origins and moving bi-directionally are not terminated randomly but in regions delimited by polar replication termini (Ter).

**IMPLICATIONS FOR THE ORIGIN OF POLARITY OF FORK ARREST**

* This work was supported by a grant from NIGMS and a merit award from NIAID of the National Institutes of Health (to D. B.). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

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1 The abbreviations used are: Ter, polar replication termini; RTP, replication terminator protein; bp, base pairs, EPD, (S)-(2-pyridyl-thio)cysteaminy1-EDTA; IRI, inverted repeat 1.
**Bipolar Terminus**

**RECOMBINANT DNA CONSTRUCTS—**The pUC18-IRI (BS3) and pUC18-IRI (BS3) Rev. plasmids were constructed by transferring an XbaI-HindIII fragment that contained the IRI (unipolar Ter) from the pET22b-BS3 and pET22b-BS3 Rev. plasmids (11) into the pUC18 vector. The pUC18/19-Bipolar plasmids were constructed by cloning a 46-bp EcoRI-HindIII fragment containing the bipolar Ter site into the pUC18/19 vectors. The plasmids pET22b-IRI, pET22b-BS3 and pET22b-IRI Rev. (pET22b-BS3 Rev) contained a unipolar Ter site in opposite orientations. The pET22b-Bipolar and pET22b-Bipolar Rev. plasmids were constructed by cloning an NdeI-HindIII and an NdeI-EcoRI fragment from pUC18-Bipolar and pUC19-Bipolar plasmids, respectively, in the pET22b vector. M13mp19-Bipolar and M13mp18-Bipolar clones were made by cloning a 46-bp DNA fragment containing the bipolar terminus into the M13mp18 and M13mp19 vectors, respectively.

**RESULTS**

**METHYLPATATION PROTECTION ANALYSIS OF RTP-BIPOLAR TER COMPLEX—**Our first goal was to derive a picture of the arrangement of two interacting dimers of RTP at the bipolar Ter. A priori, a comparison of the bipolar Ter with a unipolar Ter (IRI) sequence showed significant homology at the core sequence and very little at the auxiliary sequence (Fig. 1A). A closer look suggested that the auxiliary site-equivalent of the bipolar Ter has significant sequence homology with core sequence, and thus the bipolar Ter may have two core sequences arranged in a head-to-head manner (Ref. 7, see Fig. 1A). To determine some of the key base to amino acid contacts between the two RTP dimers and the bipolar Ter DNA, the protein was complexed with 5' end-labeled DNA and treated with dimethyl sulfate as described (14), and the methylated bases were mapped after depurination and β-elimination in sequencing gels. The summary of the comparative methylation protection data is shown in Fig. 1B. A representative protection data of the top strand of the bipolar terminus is shown in Fig. 1C.

An examination of the guanine contacts of the bipolar Ter site showed a symmetrical pattern as contrasted with that of the normal unipolar Ter (IRI) site that showed more G contacts at its core sequence than the auxiliary site (Fig. 1B). This result prompted us to investigate in more detail the amino acid to nucleotide contacts between RTP and the bipolar Ter site as described below.

**COMPUTATIONAL MODELING OF THE RTP-BIPOLAR TER COMPLEX DNA COMPLEX—**Ideally, one needs to solve the x-ray crystal structure of the RTP-Ter complex to develop a detailed picture of how the protein interacts with DNA. However, the co-crystals of bipolar Ter-RTP complex that have been generated up to this time differ to ~6 Å. Although further improvement in resolution and the eventual determination of the structure looks promising, we decided to approach the problem in another way. We converted RTP to a site-directed chemical nuclease and used it to generate an amino acid to nucleotide cleavage.
map of the RTP-bipolar Ter complex. The structure of (S)-2-(pyridylthio)cysteaminyl-EDTA (EPD), the exchange reaction with cysteine-substituted RTP, and the expected pattern of cleavage when the iron atom contacts the major as contrasted with the minor groove of the DNA are shown in Fig. 2. The cleavage data were used along with computational methods described below, to construct a three-dimensional model of the RTP-bipolar Ter complex.

Such models have served useful purposes for studies of DNA-protein interaction in solution and provide a useful adjunct to the co-crystal structures of the complexes. Also, x-ray crystal structures provide a snapshot of the protein-DNA complex that is constrained by the crystal lattice, whereas affinity cleavage, while lacking the accuracy of a crystal structure, serves to illuminate the nature of the nucleotide to amino acids contacts both in real time and in solution (8). We generated affinity cleavage maps as described below. Distance constraints were obtained from the cleavage data and were used to generate a model of the RTP-bipolar Ter complex as described in detail in a later section.

**Affinity Cleavage Maps**—The residues 56, 59, and 63, belonging to the α3 DNA-recognition helix and the residues 16 and 74 from the α1 helix and the β2 strand, respectively were separately mutagenized to cysteines, and the resulting mutant forms were coupled to EPD. The protein–Fe–EDTA conjugates were used to cleave bipolar Ter DNA that was singly end-labeled at the 5’-ends after adding ascorbate to the reaction mixture. The cleavage pattern of some representative derivatives is shown in Fig. 3. Controls are described in the legend to Fig. 3. Nonderivatized RTP did not cleave DNA either in the presence or in the absence of ascorbate. Wild-type RTP treated with EPD did not cleave DNA with ascorbate. Derivatized RTP needed ascorbate to generate hydroxy radicals that in turn cleaved DNA.

It should be noted that the cleavages are expected to shift either to the 5’ or the 3’ side from the location of the iron depending on which groove of the DNA double helix contacted the iron atom (Ref. 16; see Fig. 2). Thus residue 74, upon derivatization, produced a cleavage pattern that was shifted to the 3’ side, consistent with the contact of the β2 strands at the minor groove of Ter DNA (Fig. 4). The residues 16 and 63 both contacted Ter DNA at the center (Figs. 3 and 4). This result is consistent with the fact that Arg-16 and Glu-63 are located only 9 Å apart in the crystal structure of RTP (5).

To make sure that the cleavage pattern was specific, we mutagenized the G residue of the bipolar Ter contacted by amino acid 56 to a T. This mutation on the bipolar Ter DNA caused the specific cleavage pattern to disappear and generated a more randomized and dispersed cleavage pattern in the mutant Ter (Fig. 3C), thus supporting the notion that the residue 56 to G contact was specific. It should be noted that the G contact described above is known to be necessary for stable binding of RTP to DNA (14). Residue 59, after derivatization, promoted several foci of cleavage on both strands of DNA (not shown) as contrasted with the simple cleavage patterns of derivatized 16 and 63 (Fig. 3, A and B).

**Description of the Molecular Model**—As in the previously published structure of the RTP-unipolar Ter (10), the residues 16 and 63 of each RTP monomer interacted with the center of symmetry within each Bipolar Ter subsite (Fig. 4). A, B and C, D refer to the monomeric subunits of the two dimers of RTP (Fig. 4). The computational modeling of RTP-bipolar Ter complex is shown in Fig. 5. The α3 helices of both dimers are inserted into the major groove of the DNA duplex; the anti-parallel β-ribbons straddle the phosphate backbone and insert into the minor groove. The N-terminal arms are also properly positioned for interactions with the phosphate backbone if they were dropped (the arms are raised by crystal packing forces in the crystal structure and therefore remain in that position in the model). The major groove is forced open by the insertion of the α3 helices, and this necessitated a global compensation in the DNA structure by a shrinkage of the minor groove. This distortion disrupts the normal base pairing. This distortion in the DNA predominated near the center of the RTP dimer and at the ends of the anti-parallel β-ribbon. Overall, there was only a slight net curvature of the DNA consistent with published results (17). The distortions to the DNA were more equally distributed throughout the entire terminus, rather than there being more severe distortion within the core subsite (Fig. 5). A feasible dimer-dimer interaction can be investigated by rotating one dimer by 8–10 Å around the DNA axis.

Whereas subsequent investigations are warranted, it is
tempting to postulate that the bipolar terminus exhibits the bipolar blockage of the replication fork, because it is composed of two strong-binding subsites. Examination of the sequence of this terminus reveals that these two subsites, called core A and core B, show a strong similarity to the core subsite within the IRI (unipolar Ter) sequence. The critical guanines found in the core B (IRIB) subsite are also found here in equivalent positions, suggesting that each subsite within the bipolar terminus is indeed a “core”-type of subsite. This strong/strong binding arrangement yields a plausible explanation for the behavior of this terminus. Also, the pattern of contacts within both the subsites of the bipolar terminus is symmetrical. Interestingly, there are no contacts between residue 74 and the DNA in the border region between the subsites in the bipolar terminus. This is in contrast to the IRI terminus where residue 74 of the D-monomer makes several contacts. Whereas the exact reasons for this difference in binding remains elusive, it does show that RTP binds to unipolar and bipolar termini differently.

Cooperative Binding of RTP to the Bipolar Terminus—A series of previous work has shown that two RTP dimers bind to the normal unipolar terminus in a cooperative manner (14, 18, 19). First, the higher affinity core site is filled by one dimer and then the overlapping, weaker auxiliary site is filled by the second dimer. We wished to compare the pattern of RTP binding to the bipolar terminus site with that of the unipolar site. The IRI fragment (Fig. 6, panel A, lane 1) showed the typical two-step mobility shift in the presence of RTP with the first shift corresponding to filling of the core site and with the second shift corresponding to filling of both the core and auxiliary site (Fig. 6, panel A, lanes 2–12). The fragment containing the bipolar terminus (Fig. 6, panel B, lane 1) when mixed with RTP also showed a two-step filling of its sites in a stepwise manner (Fig. 6, lanes 2–12). A very faint third shift was observed (7), which might be because of aggregation of more RTP dimers on the site.

Characteristics of in Vitro Replication Fork Arrest by IRI and Bipolar Termini—We wished to characterize the bipolar terminus with regard to its ability to arrest replication forks in vitro and also to map the points of arrest of the newly synthesized DNA. Previously we have used a surrogate in vitro replication system of E. coli (4, 14) to study the replication fork arrest by RTP because to date, active preparations of the DnaB homolog of B. subtilis have not been purified by any laboratory. Moreover, an efficient replication system that operates in crude extracts of B. subtilis has not been developed to date. We used four templates in this study. Fragments containing the IRI (TerI) site in either orientation were cloned in pUC18 plasmid and the bipolar terminus was cloned in pUC18 and pUC19 in either orientation with respect to origin of replication to generate four templates namely pUC18-IRI, pUC18-IRI Rev., pUC18-Bipolar, and pUC19-Bipolar for study (see “Experimental Procedures”).

The plasmid DNA templates were replicated in vitro using the E. coli cell extracts in the presence or absence of different concentrations of RTP and the products were analyzed in a denaturing urea-polyacrylamide sequencing gel. The pUC18-IRI Rev. template, containing the IRI site in nonfunctional orientation with respect to the origin, was replicated in the presence of various amounts of RTP (Fig. 7, panel A, lanes 1–4). As expected, RTP did not block the replication fork in this template (Fig. 7, panel A, lanes 2–4). On the other hand replication of the pUC18-IRI template containing the IRI site in functional orientation with respect to the replication origin, in the presence of various amounts of RTP showed a distinct termination product (Fig. 7, panel B, compare lane 1 with lanes 2–4). A major band of about 600 bases and a minor band

![Autoradiograms of the Fe–EDTA cleavage of labeled bipolar terminus DNA fragment](image-url)
between 500 and 600 bp were observed. We have titrated RTP concentrations and have noticed that at high concentrations of RTP, the intensities of the bands corresponding to the replication fork arrest in pUC18-IRI template were reduced, either because of multiple stops or because of overall inhibition of replication (data not shown).

The pUC18-Bipolar template replicated in the presence of 0.3, 0.6, and 2.4-fold RTP over template concentration showed a major blocked product smaller than 500 bases representing the leading strand block product and smaller blocked products of about 400 bases representing the lagging strand block products. At high RTP concentrations, the number of bands increased and total replication seemed to decrease. It may be noted that the RTP concentrations needed to show blockage in pUC18-Bipolar and pUC19-Bipolar templates was lower in comparison to that needed in pUC18-IRI and pUC18-IR Rev. templates.

We analyzed the labeled in vitro replication products further to determine the exact nucleotide sequence where replication was arrested in pUC18-IRI, pUC18-Bipolar, and pUC19-Bipolar templates. The pUC18-IRI template containing a unique XbaI site 140 bp upstream of the IRI site was digested with XbaI. The pUC19-Bipolar template was digested with PvuII enzyme. The digestion products were run in a denaturing urea-polyacrylamide sequencing gel along with pUC18-Bipolar sequencing ladder (data not shown). Fig. 11A shows the nucleotide sequence of products. At high RTP concentrations, the number of bands increased and total replication seemed to decrease. It may be noted that the RTP concentrations needed to show blockage in pUC18-Bipolar and pUC19-Bipolar templates was lower in comparison to that needed in pUC18-IRI and pUC18-IR Rev. templates.

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IRI, where the leading strand is arrested by the RTP-IRI complex. The stop site matches the border of the minimal effective sequence needed to arrest the helicase (14, 15). Fig. 11, panel B shows the nucleotide sequences on both sides of the bipolar terminus where replication fork is blocked by the RTP-Bipolar Ter complex.

Contrahelicase Activity at the Bipolar Terminus—We have shown previously that RTP arrests replication by inhibiting the activity of *E. coli* replicative helicase, DnaB (4, 14). We wished to investigate the arrest of helicase in both orientations of the bipolar terminus. M13mp18 and M13mp19 clones containing the IRI site have been described (4). The M13mp18-Bipolar and M13mp13-Bipolar templates were hybridized to 5′ end-labeled oligonucleotides to generate the forked helicase substrates and were assayed for DNA unwinding activity of DnaB in the absence and presence of RTP. The gels were quantitated with a phosphorimager, and data were plotted as a graph in Fig. 8. As expected, DnaB was arrested by RTP on the M13mp19-IRI template but not on the template M13mp18-IRI (4, 14). Unwinding was blocked on the M13mp19-Bipolar template (Fig. 8) as in M13mp19-IRI. However, in the case of the M13mp18-Bipolar template, RTP blocked oligonucleotide release by about 50% in contrast with only an ~10% release in the case of M13mp18-IRI. Thus, although the bipolar terminus blocked replication and helicase activity from both orientations, one orientation of the terminus blocked the helicase more efficiently than the other.

We further investigated helicase blockage by the bipolar terminus with heteroduplex substrates using a technique developed by us (14, 15). The M13 universal forward primer labeled at the 5′ position was annealed to the four single-stranded DNA templates and was extended by DNA polymerase (sequenase) in the presence of all four dNTPs and one ddNTP (Fig. 9A). Four sets of reactions were carried out, each containing a different ddNTP. Because equimolar amounts of DnaC stimulate DnaB helicase activity (20), we performed the helicase assays either with only DnaB or with DnaB in the presence of 0, 2.4, 4.8, and 9.6-fold RTP over DNA template, respectively.

Panel D shows reaction products obtained in the presence of 0, 2.4, 4.8, and 9.6-fold RTP over DNA template, respectively. Panel C, replication products of pUC18-Bipolar plasmid. Lanes 1–4 show reaction products obtained in the presence of 0, 2.4, 4.8, and 9.6-fold RTP over DNA template, respectively. Panel B, replication products of pUC18-IRI. Lanes 1–4 show reaction products obtained in the presence of 0, 2.4, 4.8, and 9.6-fold RTP over DNA template, respectively. Panel A, replication products of pUC18-IRI Rev. Lanes 1–4 show reaction products obtained in the presence of 0, 2.4, 4.8, and 9.6-fold RTP over DNA template, respectively.

FIG. 8. Quantitative analysis of the extent of DNA unwinding by DnaB helicase on M13mp19-IRI (unipolar), M13mp19-IRI Rev (unipolar-reversed orientation), M13mp19-Bipolar, and M13mp18-Bipolar Rev. partial duplex helicase substrates. Note that the M13mp18-Bipolar Rev. substrate was less efficient in arresting helicase activity than the M13mp19-Bipolar substrate. The unipolar (IRI) substrate shows the expected polarity of helicase arrest.

Fig. 7. Autoradiogram of a 6% polyacrylamide-8 M urea gel showing unidirectional fork blockage by IRI and bi-directional fork blockage by bipolar terminus in an *in vitro* replication assay using an *E. coli* *in vitro* replication system. Panel A, replication products of pUC18-IRI Rev. Lanes 1–4, reaction products obtained in the presence of 0, 2.4, 4.8, and 9.6-fold RTP over DNA template, respectively. Panel B, replication products of pUC18-IRI. Lanes 1–4 show reaction products obtained in the presence of 0, 2.4, 4.8, and 9.6-fold RTP over DNA template, respectively. Panel C, replication products of pUC18-Bipolar plasmid. Lanes 1–4, 0, 0.3, 0.6, and 2.4-fold RTP over DNA template, respectively. Panel D, replication products of pUC19-Bipolar template. Lanes 1–4, reaction products obtained in the presence of 0, 0.3, 0.6, and 2.4-fold RTP over DNA template, respectively. Panel E, molecular size markers (M). The arrows indicate bands generated by replication arrest by RTP.

Fig. 9. Ladder assay of arrest of helicase-catalyzed DNA unwinding at a bipolar terminus. A, diagram showing the substrate used in the experiment. The substrates were prepared by annealing the 5′-labeled M13mpUC forward primer to the M13 based single-stranded DNA templates containing the bipolar terminus in either orientation. In four separate reactions, a different ddNTP was included to randomly terminate the extension products. A nested set of extension products was thus generated. B, autoradiogram of an 8% polyacrylamide gel showing bi-directional blockage of DnaB by the bipolar terminus. Left panel (Top): lane A, M13mp19-Bipolar substrate plus DnaB and ATP; lanes B–F, same as in A except that 10, 20, 40, 80, and 160 fmols of RTP was included in the reaction mixture, respectively. Right panel (Bottom): lane G, M13mp18-bipolar substrate plus DnaB + ATP; lanes H–L contained substrate + DnaB + ATP and 10, 20, 40, 80, and 160 fmols of RTP, respectively. The band(s) corresponding to the highest molecular size DNA band released by the helicase were extracted from the gels from each of the four ddNTP reactions (arrow and bracket) and resolved in a DNA sequencing gel (see Fig. 10).
clones containing IRI or bipolar terminus were linearized with BsiPI and transcribed in the absence or presence of RTP.

RTP blocked T7 RNA polymerase-catalyzed transcription at IRI in a polar fashion (11). The pET22b-IRI (pET22b-BS3) template transcribed in the absence of RTP yielded a single major transcript, whereas in the presence of 0.35, 0.7, or 1.4-fold RTP, a truncated product was formed with increasing intensity (Fig. 12A, panel A, lanes 1–4). However, in the template pET22b-IRI Rev. (pET22b-BS3 Rev.) no major truncated transcript was formed in the absence or presence of RTP (Fig. 12B, lanes 1–4). When pET22b-Bipolar template was transcribed in the presence of 0.35, 0.7, or 1.4-fold RTP, a truncated transcript was generated (Fig. 12D, lanes 1–4) as in the case of pET22b-IRI. Similarly pET22b-Bipolar Rev. template showed generation of a truncated transcript in the presence of RTP (Fig. 12C, lanes 1–4) although with less efficiency than the pET22b-Bipolar template (compare the amount of full-length transcripts in lanes 2–4 of Fig. 12C with 12D). The transcription blocking efficiency of IRI and bipolar templates were quantitated with a phosphorimager and are shown in Fig. 12E, and the data support the conclusions stated above.

DISCUSSION

The first part of the work reported in this study was initiated with the primary objective of trying to understand the possible molecular basis of the polarity of arrest of the replication forks and the replicative helicase, by comparing the nucleotide to amino acid contacts of RTP bound to the bipolar terminus with that of the more ubiquitous unipolar terminus. We have also constructed a three-dimensional model of the RTP-bipolar Ter complex by combining the crystal structure of the apoprotein (5) and the affinity cleavage data. The model that resulted is consistent with mutagenesis and cross-linking data that suggested roles for the α3 helix, the β2 strand, and the N-terminal arm of RTP in DNA binding (10, 21). Thus the α3 helix appears to be the recognition helix that invades the major groove, and the β2 strand makes minor groove contacts. Unfortunately, attempts to derivatize the N-terminal arm and to generate affinity cleavage were unsuccessful.

Inspection of the bipolar Ter sequence of the plasmid pLS20 of B. subtilis did not provide definitive information on the molecular basis of polarity. The methylation protection data reported here did provide a preliminary picture of symmetrical contacts of RTP with the bipolar terminus but did not reveal precise amino acid to base contacts, which was obtained from the affinity cleavage data. The affinity cleavage method, used to construct models of DNA-protein complexes when the crystal structure of the apoprotein is already known, has proven to be a very useful adjunct to the DNA-protein co-crystal structures derived by x-ray crystallography (8, 9, 22). This study has revealed symmetrical base to amino acid contact in the bipolar Ter, as contrasted with the asymmetric and more frequent contacts with the core site and fewer contacts with the auxiliary site that were observed in the more ubiquitous unipolar Ter (10). This structural analysis strongly suggests that symmetrical RTP dimers appeared to generate polarity by asymmetric protein-DNA contacts in unipolar Ter and, in the bipolar Ter, the symmetric contacts abolished or reduced polarity. In contrast with RTP of B. subtilis, the Tus protein of E. coli caused polar arrest of helicases because of the inherent asymmetry of the monomeric protein bound to a single Ter site (23).

A careful comparison of the RTP-bipolar Ter complex with that of the RTP-unipolar Ter complex also revealed that the contacts seen in the two cases are different in some additional regards. For example, residue 74 that is located in the β2 strands of the two dimers contacts the central part of the unipolar Ter, but these contacts are not visible in the bipolar Ter.
FIG. 11. Sequence of IRI (unipolar) and bipolar termini showing the stop sites for replication fork and the boundary of minimum effective terminator sequence needed in the double-stranded form for contrahelicase activity. Bold horizontal arrows show the directions of replication fork progression. Vertical down-arrows show the stop sites of replication fork coming from the right of unipolar and the bipolar termini. Vertical up-arrows show the stop sites of replication fork coming from the left. The two stop sites in IRI are equivalent to the two right-most stop sites on the bipolar terminus. The filled triangles represent the minimum effective terminator sequences in double-stranded form needed for contrahelicase activity. The numbers refer to the G residues that were protected from methylation (see Fig. 1).

FIG. 12. Arrest of T7 RNA polymerase-catalyzed transcriptional elongation at IRI (unipolar) and the bipolar termini. A–D, autoradiograms of a 6% polyacrylamide–8 M urea gel showing arrest of T7 RNA polymerase transcription by RTP-IRI (unipolar) and RTP-Bipolar terminus complexes in either orientation. Panel A, transcription of pET22b-IRI template containing IRI (BS3) site in functional orientation. Panel B, pET22b-IRI Rev. containing IRI in nonfunctional orientation. Panel C, pET22b-Bipolar Rev. and panel D, pET22b-Bipolar. In all panels, lanes 1–4 contained 0.0, 0.35, 0.7, and 1.4-fold RTP over DNA template. Panel E, quantitations of the truncated (arrested) transcript formation at both the unipolar and bipolar termini. Note that the bipolar reverse orientation is somewhat less efficient in arresting T7 RNA polymerase than the other orientation.

Does DNA-protein contact directly and by itself promote polarity by strong DNA binding of RTP to the core and weaker binding to the auxiliary site, or does the mechanism also involve productive helicase-RTP protein-protein contact on one side and poorer or no contact at the other site? We believe that DNA-protein interaction is one factor, but not the only factor, in promoting polarity for the following reasons. First, there is a large amount of in vitro data that support RTP-DnaB interaction in vitro (24). Second, we have recently observed that a structurally asymmetric Ter site located near a replication checkpoint of B. subtilis arrests forks in a bipolar fashion, albeit inefficiently (25). The available data support the idea that appropriate protein-DNA interaction creates a conformation that allows helicase to contact the contrahelicase domain of RTP. Having a symmetrical Ter sequence would be one obvious way of achieving that end. It is however possible that different but quasi-equivalent RTP-DNA contacts at the core and auxiliary sites of a Ter site, such as was observed at or near the left checkpoint of B. subtilis chromosome, would generate a conformation necessary for RTP-helicase contact on both sides of the Ter site, thus promoting bipolarity of fork arrest.

The observation (Fig. 6) that the binding of the two dimers of RTP to the bipolar Ter seems to show cooperativity is consistent with our earlier reports that it is not enough to have two dimers of RTP merely binding to two tandem core sites to arrest a helicase but that the two dimers must be interactive (14). It should be noted that the helicase arrest experiment shows the minimal effective sequence needed to arrest the helicase from both directions. It does not show the precise location where the helicase actually stops. The site at which most of the newly synthesized DNA stop appears to be just inside the minimum effective sequence needed for helicase arrest. In this context, it might be useful to keep in mind that the behavior of the solo helicase at the terminus might not exactly duplicate that of the helicase associated with the replication complex in vitro.

Perhaps it is interesting that replication termini also arrest RNA polymerases in a polar fashion. However, the observation does not necessarily support the notion that any enzyme that slides on DNA or melts DNA would be arrested by RTP. Our observations reported previously (15) showed that several helicases that support rolling circle-type replication are not arrested by RTP.

The results of the helicase ladder experiment described here showed that RTP arrested DnaB helicase-catalyzed unwinding of DNA regardless of the size of the double-stranded region, from a few bases to more than one kilobase. If RTP merely blocked helicase translocation rather than authentic unwinding, then it would have been expected to inhibit only the release of smaller fragments of the ladder rather than the larger ones (14, 26). Inhibition of unwinding of partially double-stranded DNA regardless of the lengths of the fragments tends to be consistent with the idea that RTP arrested authentic DNA unwinding rather than just the translocation of the helicase.

Finally, the ability of RTP to arrest RNA polymerase and several helicases might suggest that RTP arrests these enzymes at the Ter site nonspecifically by binding tightly to DNA.
However, we have presented evidence elsewhere that is more consistent with the notion that RTP-mediated arrest not only involves RTP-Ter DNA interaction but also protein-protein interaction between the arresting and the arrested proteins (24, 25). Recent work from our laboratory with the Tus protein of *E. coli* has also demonstrated a critical role of the terminator protein-DnaB interaction in fork arrest. It appears likely that although Tus and RTP are structurally different, they arrest the replicative helicase by the same mechanism.

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doi: 10.1074/jbc.M010940200 originally published online January 16, 2001

Access the most updated version of this article at doi: 10.1074/jbc.M010940200

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