The Interaction between Helicase and Primase Sets the Replication Fork Clock*

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The synthesis of an Okazaki fragment occurs once every 1-2 s at the Escherichia coli replication fork and requires precise coordination of the enzymatic activities required. We have shown previously that the primase is recruited anew from solution for each cycle of Okazaki fragment synthesis and that association of primase with the replication fork is via a protein-protein interaction with the helicase, DnaB. We describe here mutant primases that have an altered interaction with DnaB and that direct the synthesis of Okazaki fragments of altered length compared to the wild-type. The mutant primases were deficient only in their ability to participate in replication reactions where their entry to the DNA was provided by the initial protein-protein interaction with DnaB. The primer synthesis capacity of these proteins remained unaffected, as was their ability to interact with the DNA polymerase III holoenzyme. Neither replication fork rate nor the efficiency of primer utilization was affected at replication forks programmed by the mutant enzymes. Thus, the interaction between DnaG and DnaB at the replication fork is the primary regulator of the cycle of Okazaki fragment synthesis.

At the replication fork, lagging-strand synthesis is cyclical in nature. Okazaki fragments (1–2 kb) are synthesized by the DNA polymerase III holoenzyme (pol III HE; Ref. 1) are primed by 10–12-nt-long oligoribonucleotides (2) synthesized by the primase (DnaG; Ref. 3). The leading- and lagging-strand polymerase assemblies at the fork (composed of the catalytic core (aeu) (4)) linked to the dimeric processivity factor β (5)) are coupled in space (6) by physical association with a dimer of the α subunit (7, 8, 33). Upon encountering the 5’-end of the previous primer on the penultimate Okazaki fragment, the polymerase must dissociate from the lagging-strand template and cycle to a newly synthesized primer to begin synthesis of the next Okazaki fragment.

The question of what sets the cycle of Okazaki fragment synthesis was first addressed when Alberts et al. (9) advanced the “trombone model” of a replication fork. In this model, Okazaki fragment size was determined by the position of the first primer on the lagging-strand template relative to the advancing fork, the rate of polymerization of the coupled leading- and lagging-strand polymerases was equal, and the signal to prime was termination of synthesis of the penultimate Okazaki fragment. Because templating of Okazaki fragment size to that of the first one produced was inherent to this model, it could not account for the subsequent observation (10) that the size of the lagging-strand product could be varied at an active fork.

Selick et al. (10) therefore modified their model, suggesting that if the signal to prime remained the release of the lagging-strand polymerase from the just completed Okazaki fragment, size variation could be accommodated if the lagging-strand polymerase synthesized DNA at a faster rate than the leading-strand polymerase and paused between termination of synthesis and release from the template. This would allow for variation in the distance on the lagging-strand template between two primers. Footprinting experiments of the bacteriophage T4 polymerase and its accessory proteins on a hairpin primer-template supported this model (11). However, recently, Hacker and Alberts (12) demonstrated that the release of the polymerase from the nascent strand followed a first order kinetic decay with no lag when the polymerase encountered the 5’-end of what would be the previous Okazaki fragment at a replication fork.

Stukenberg et al. (13) showed that the pol III HE behaved in a fashion similar to the T4 polymerase in that the core-β assembly bound to the nascent strand dissociated when it encountered the 5’-end of a DNA strand annealed to the same template, but not when it was stalled by deprivation of deoxyribonucleoside triphosphates. At the replication fork, this would make the lagging-strand core available for recycling to the next primer. Whereas this spontaneous action of the polymerase may be the trigger for dissociation of core from β, it is not clear how this relates to the regulation of Okazaki fragment length. We have shown (14) that, just as for the T4 forks, Escherichia coli forks reconstituted in vitro can also be made to vary the size of the Okazaki fragments synthesized. Thus, what determines cycling of the lagging-strand polymerase and setting of Okazaki fragment length are likely to be two related but separate questions, answers to which might lie in the properties of two or more distinct protein complexes in the replisome.

We found that Okazaki fragment size at replication forks reconstituted in vitro was governed by the frequency of primer synthesis and the efficiency with which the primers were used to initiate nascent lagging-strand synthesis (15). We postulated that priming frequency at the replication forks was not determined by the properties of the lagging-strand polymerase, but by the distributive interaction of the primase with the replication fork with respect to the Okazaki fragment cycle. This interaction was suggested to be between primase and the replication fork helicase, DnaB (16).

That an interaction between DnaG and DnaB might exist had been suggested previously (17), yet physical proof of such an interaction had remained elusive, presumably because of its
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weak and transient nature. We used functional domain analysis of primase by limited proteolysis coupled with assay in reconstituted replication reactions in vitro to identify the C-terminal 16 kDa of primase as the DnaB interaction domain (18). As described in an accompanying article (19), analysis of the 16-kDa domain showed that deletion of the C-terminal 16 amino acids resulted in an enzyme that could act as a bona fide primase during DnaB-independent replication, yet was completely unable to support DnaB-dependent DNA replication presumably because of the loss of its DnaB interaction site. Additional deletion analysis showed that removal of the extreme C-terminal 8 amino acids from p16 abrogated its interaction with DnaB.

We describe here the identification of single amino acid residues at the extreme C terminus of primase that, when mutated to alanine, result in proteins with altered interactions with DnaB. These point mutations do not affect the ability of DnaG to behave as a bona fide primase in DnaB-independent replication reactions, whereas they display altered properties in DnaB-dependent replication reactions. For this reason, the results correspond to alterations in the size of the lagging-strand product, suggesting that Okazaki fragment length is indeed determined primarily by the interaction between DnaG and DnaB at the replication fork. We present a model that integrates the relationship between the polymerase and priming cycles on the lagging-strand side of the replication fork.

MATERIALS AND METHODS

Construction and Purification of Mutant p16 Proteins and DnaGs—Alanine substitution mutants of p16 were constructed by polymerase chain reaction (PCR) using the following primers and pET3d-p16 (18) as the template. The C-terminal primer for p16-T573A, p16-L574A, p16-N575A, and p16-Q576A was 5'-TCCCTCTATGAATGGCAGGATCCTTATCCGACCATTA-3'. The N-terminal primers were: p16-T573A, 5'-CGCTGTCGACCTGGTCAACCAAGCAGG-3'; p16-L574A, 5'-GCCCTTCGACCTGGTCAACCAAGCAGG-3'; p16-N575A, 5'-GCCCTTCGACCTGGTCAACCAAGCAGG-3'; and p16-Q576A, 5'-GCCCTTCGACCTGGTCAACCAAGCAGG-3'. The C-terminal PCR primer for p16-E577A, p16-L578A, and p16-K581A was 5'-AAGCTTATGCGCAACATGCCAGAGGCCGTTT-3'. The N-terminal PCR primers were: p16-E577A, 5'-GATGAGCTCACCCTCTCTTTCTCTGCGCCCATCTC-3'; p16-L578A, 5'-GATGAGCTCACCCTCTTTCTCTGCGCCCATCTC-3'; and p16-K581A, 5'-GATGAGCTCACCCTCTTTCTCTGCGCCCATCTC-3'. All the PCR products were digested with Sacy and BamHI, and the resulting DNA fragments were recovered from a polyacrylamide gel and ligated to Sad- and BamHI-digested, alkaline phosphatase-treated pET11d-p16. Candidate clones were recovered after transformation into DH5α, and the presence of the desired mutation was confirmed by dideoxy sequencing.

DnaG T573A, DnaG Q576A, and DnaG K580A were constructed by digesting the respective p16 mutant constructs with Sad and Sphi. The DNA fragment containing the mutated region of the DnaG open reading frame was then purified by sedimentation through a 10–40% neutral sucrose gradient in 1 M NaCl. The fragment was then ligated to Sad- and Sphi-digested, alkaline phosphatase-treated pET3d-dnaG (20). Candidate clones were recovered after transformation into DH5α, and the presence of the desired mutation was confirmed by dideoxy sequencing.

Wild-type and mutant p16 constructs were transformed into B834(ΔDE3) (Novagen) and overexpressed by induction for 2.5 h at 37 °C with 0.4 mM isopropyl-1-thio-β-galactopyranoside in medium containing 500 μM ampicillin. Purification of p16 proteins was as described previously (18).

Wild-type and mutant DnaG constructs were transformed into BL21(ΔDE3) (Novagen) and overexpressed by induction with 0.4 mM isopropyl-1-thio-β-galactopyranoside for 2.5 h at 37 °C in 1.8 M sodium chloride containing 500 μM ampicillin. Purification of DnaG proteins was as described previously (18).

Replication Proteins and Assays—Primosomal proteins, the E. coli single-stranded DNA-binding protein (SSB), and the pol III HE were described in an accompanying article (19), as were the G4 single-stranded (circular) (ss(c)) → replicative form (RF), general priming, and αX174 ss(c) → RF replication reactions. Tailed form II DNA template was prepared according to Mok and Marians (21). Rolling circle replication reaction mixtures (12 μl) containing 50 mM Hepes-KOH (pH 7.9), 12 mM MgOAc, 10 mM dithiothreitol, 5 μM ATP, 80 mM KCl, 100 μg/ml bovine serum albumin, 0.42 μM tailed form II DNA (as molecules), 1.1 μM SSB, 3.2 mM DnaB, 56 mM DnaC, 28 mM DnaT, 2.5 mM PrIa, 2.5 mM PrIb, 2.5 mM PrIc, 28 mM pol III HE, and wild-type and mutant primases as indicated in the figure legends were incubated at 30 °C for 2 min. DNA, poly(dT), and the dNTPs to 40 μM, and the incubation continued for 15 min. [α-32P]dATP was then added to give a specific activity of 2000–4000 cpm/μmol and the incubation continued for 10 min. The reactions were terminated by the addition of EDTA to 40 mM. An aliquot of the reaction mixture was acid-precipitated for the determination of total DNA synthesis and the DNA products analyzed by alkaline agarose gel electrophoresis as described by Wu et al. (14).

Determination of Replication Fork Rates—Standard reaction mixtures were incubated for 6 min after the addition of label to establish active replication forks. A 10-fold excess of 5-methyl-dCTP was then added to the reaction mixtures. The reaction was terminated after an additional 25-s incubation by the addition of a 25-fold excess of ddGTP over dCTP. Nascent DNA was then digested with Alul, HpaII, and Hhal, which cannot cleave hemimethylated DNA but do cleave nonmethylated DNA. DNA products were analyzed by electrophoresis through horizontal 0.5% agarose gels at 1.6 V/cm for 24 h. The length of the largest DNA fragment was determined by comparison with the DNA size standards, and the replication fork rate was equal to this length (in nucleotides) divided by 25 s. The control reactions were identical except that the DNA was not digested with the restriction enzymes.

Determination of the Efficiency of Primer Utilization—Standard rolling circle DNA replication reactions containing the concentrations of wild-type and mutant primases as indicated and [α-32P]UTP and [α-32P]dCTP each at 10,000 cpm/μmol were incubated for 10 min at 30°C. RNA and DNA products were recovered by phenol-chloroform extraction and ethanol precipitation after treatment of the replication mixture with alkaline phosphatase (2 units) and analyzed by electrophoresis through a 15% (19), acrylamide/bisacrylamide) polyacrylamide gel containing 50% urea using 100 mM Tris borate (pH 8.3) as the electrophoresis buffer. The label now appears in two populations, as free 10–12-nt primers and as primer that has been used to initiate Okazaki fragment synthesis. The latter population barely enters this high percentage gel. The efficiency of primer utilization is calculated from the ratio of the label appearing as free primer to the total radioactivity on the gel as determined by phosphorimagery analysis.

RESULTS

Point Mutations in the Extreme C Terminus of Primase Affect Its Ability to Interact with DnaB—In an accompanying article, we report that the extreme C-terminal 8 amino acids of primase are required for interaction with DnaB (19). To localize the amino acids involved in the DnaG-DnaB interaction, 8 of the 9 C-terminal amino acids of primase were replaced with Ala. Our strategy was to first construct the eight alanine substitution mutants within the context of p16 (the C-terminal domain of primase that can be overexpressed and purified from E. coli and which inhibits the primase-DnaB interaction; Ref. 18) in order to determine whether any of them showed an altered DnaG-DnaB interaction in vitro. Mutations that endowed p16 with such a biochemical phenotype would then be introduced to full-length primase in order to study their effect on Okazaki fragment synthesis at the replication fork.

The C-terminal 9 amino acids of primase are: T37LNLQEL-AKK283. With the exception of Ala279, these were all substituted with Ala in our p16 overexpression vector and the resulting mutant proteins, as well as wild-type p16, were purified (Fig. 1A). L578A was completely insoluble, so we constructed L578F, which had a somewhat better solubility. Nevertheless, only partially purified preparations of L578F and K581A were used in the assays described below because of their limited overexpression.

To analyze the ability of mutant p16 or mutant primases (see
below) to interact with DnaB, two different replication assays were used that require DnaB to attract DnaG to the ss(c) DNA template: general priming (22) and \( \phi X 174 \) ss(c) RF DNA replication (23, 24). In the former reaction, DnaB bound to naked DNA serves as a target for DnaG. Once attracted to the DNA in this manner, DnaG can synthesize a primer that can be utilized by the pol III HE to prime complementary strand synthesis. This reaction is inhibited if the DNA is coated with SSB. Under these circumstances, an enzymatically more complex reaction is required to develop a target for DnaG binding. In the \( \phi X \) ss(c) → RF DNA replication reaction, PriA, PriB, PriC, DnaT, DnaC, and DnaB are required to first form the preprimosome on the DNA (25). Primase can then bind to DnaB present in the preprimosome and become activated to synthesize a primer on the DNA. Primase alone is essentially inactive for primer synthesis in either of these assays.

Wild-type p16 is a potent inhibitor of these reactions because it competes with primase for binding to DnaB (Fig. 1B). p16 by itself is devoid of any primase or DNA binding activity (18). The eight mutant p16 proteins could be divided into four classes based on their ability to inhibit \( \phi X \) ss(c) → RF DNA replication (Fig. 1B). T573A, N575A, and E577A were moderately better inhibitors of replication than wild-type p16, indicating that these mutant proteins might interact somewhat more strongly with DnaB than the wild-type protein. L574A, L578F, and K581A inhibited replication to a comparable extent as wild-type p16, suggesting that changing these amino acids to Ala (or Phe in the case of L578F) did not affect the primase-DnaB interaction significantly. K580A showed a moderately reduced inhibition of replication when compared to wild-type p16, indicating that the ability of K580A to interact with DnaB was weaker than that of wild-type p16. Finally, Q576A showed a severely reduced inhibition of replication compared to wild-type p16, indicating a drastic loss in the ability of Q576A to interact with DnaB.

One mutation from each class was chosen for characterization in the context of full-length primase. Thus, we generated the T573A, K580A, and Q576A mutations in the full-length primase open reading frame, overexpressed, and purified these mutant proteins concurrently with wild-type primase (Fig. 2A). The activity of these mutant proteins was compared to that of the wild-type protein in the general priming reaction (Fig. 2B). As was the case when the mutation was in the form of p16, the T573A primase displayed a moderately better interaction with DnaB, as inferred from its ability to support general priming somewhat better (30–40% increase in specific activity) than wild-type primase. K580A primase showed a moderately reduced interaction with DnaB, as inferred from its somewhat reduced ability (70–140% decrease in specific activity) to support general priming compared to the wild-type protein, consistent with the behavior of the K580A p16. Finally, just as Q576A p16 showed a severely reduced ability to interact with DnaB, the ability of the Q576A primase mutant to support general priming compared to the wild-type p16 was extremely compromised (4–12-fold reduction in specific activity).

The T573A, Q576A, and K580A Mutations Do Not Affect...
The effects observed with the mutant primases could be a result of an alteration in the primer synthetic activity of the enzyme. To assess this, the activity of each mutant protein was determined in the bacteriophage G4 ss(c) → RF priming and replication reaction (Fig. 3). G4 ss(c) → RF DNA replication is DnaB-independent (28). Rather than requiring the assistance of DnaB to gain entry to the ss(c) DNA for subsequent priming, primase is capable of recognizing a hairpin structure at the G4 origin of complementary strand synthesis by itself, provided that the DNA is coated with SSB. The enzyme then synthesizes a specific 29-nt primer that is elongated by the pol III HE (27).

All three mutant primases were fully active in supporting G4 ss(c) → RF replication (Fig. 3A). In fact, the T573A primase had a 2-fold higher specific activity than wild-type primase. In addition, the primers synthesized by the mutant primases were indistinguishable from the one synthesized by the wild-type primase (Fig. 3B). Therefore, these point mutations at the C terminus of primase affect neither its primer synthetic capability nor its ability to support replication, suggesting that potential interactions with DNA, SSB, and the pol III HE have not been affected adversely.

Thus, we have engineered point mutations in primase that affect selectively its ability to interact with DnaB, the replication fork helicase, without affecting the ability of these mutant proteins to act as bona fide primases. This gave us the means to determine the effect of the interaction between the replication fork helicase and primase on Okazaki fragment length.

The DnaG-DnaB Interaction Sets the Period of the Okazaki Fragment Cycle—We examine replication fork action in a rolling circle DNA replication system supported by a specialized tailoned form II DNA template in the presence of the pol III HE, SSB, and the primosomal proteins (14). Replication forks that form on the template support sustained DNA synthesis, generating long (>0.5 Mbp) multigenome length duplex tails consisting of a continuous leading strand annealed to discontinuous Okazaki fragments. This system accurately mimics the underlying assumptions of this model are: (i) that the strength of the DnaG-DnaB interaction correlates directly with priming frequency, (ii) that the rate of replication fork progression, and thus the rate at which template is generated, is constant, and (iii) that the DnaG-DnaB interaction is the major determinant of Okazaki fragment length during a cycle of Okazaki fragment synthesis.

This issue was addressed by examining the variation in Okazaki fragment size as a function of the primase concentration in the rolling circle replication system (Fig. 4). The behavior of the mutant proteins was consistent with both the observations described in the previous sections and with the model.

If Okazaki fragment length were set by the interaction between DnaG and DnaB, the replication fork helicase, the ratio of Okazaki fragment size as a function of the primase concentration would approach 15 kb in size, it became difficult to distinguish them from leading-strand product. At a concentration equal to those for the wild type, the Q576A primase directed the synthesis of fragments that were nearly 14-fold longer. At 320 nM primase, a concentration of the wild type that still generates fragments of minimum length, the Q576A protein directed the synthesis of fragments that were nearly 14-fold longer.

Whereas the data with the mutant primases are consistent with our model, there are alternative explanations for the observed changes in Okazaki fragment size with respect to the wild type. Because fragment size is dictated by the distance between two successful initiations on the lagging-strand template, if the frequency of primer synthesis and the efficiency of primer utilization remained constant, a mutant primase-induced variation in the rate at which template was generated (i.e., the rate of replication fork movement) could also lead to alteration in Okazaki fragment size.

To assess this, we determined the rate of replication fork movement at active forks reconstituted with the wild-type and mutant primases (Fig. 5). We used a methodology that we had developed previously (14). Active replication forks are pulse-labeled with S-methyl-dCTP and the reaction is terminated shortly after the addition of the pulse-label. The S-methyl-dCMP incorporated renders the region of the DNA tail synthe-
sized during the pulse resistant to the action of restriction endonucleases such as HaeIII that have dC residues in their recognition sequence. The DNA is digested with a mixture of these enzymes. This effectively removes all non-methylated DNA synthesized before the pulse. The 32P-labeled material is now electrophoresed through denaturing gels, and the length of the sharpest trailing edge represents the longest distance traveled by a replication fork during the pulse period.

Fork speed was assessed at concentrations of the mutant primases where they generated Okazaki fragments of distinctly different sizes. In all cases, the size of the leading strands synthesized during the pulse period were the same (Fig. 5). The slight variation observed could not account for the almost 15-fold difference expected when comparing the Q576A primase to the wild type if alterations in replication fork rate were the basis for observed changes in the size of the Okazaki fragments.

It is possible, for example in the case of the Q576A primase, that the enzyme actually associates with the fork with the same affinity as the wild type and synthesizes primers at the same frequency, but the mutant primase interferes with the ability of the lagging-strand polymerase to cycle to the new primer terminus, leading to a decrease in the efficiency of primer utilization. We have shown previously that this will lead to the generation of larger Okazaki fragments.

To assess this possibility, we turned again to another technique we had developed previously (15). Rolling circle replication reactions are performed in the presence of [α-32P]CTP and UTP, which results in the labeling of the primers. The DNA products are analyzed by electrophoresis through high percentage denaturing polyacrylamide gels. The label appears as two populations. One migrates as free primers 10–12 nt in length, whereas the other population does not enter the gel. The latter population represents primers that have been elongated into Okazaki fragments. Thus, the efficiency of primer utilization can be calculated from these data.

This analysis was performed for the wild type and the three mutant primases. The concentration of each was adjusted so that the replication forks in each reaction were generating Okazaki fragments of different size (Fig. 6). In each case, the efficiency of primer utilization was identical (80–85%). We therefore conclude that the observed differences in the size of the Okazaki fragments formed by replication forks directed by the mutant primases is the result of alterations in the interaction between the primase and DnaB that lead to a change in the overall period of the Okazaki fragment cycle.

**DISCUSSION**

The replication fork is a complex structure at which the action of upward of 20 different proteins must be coordinated precisely to ensure the accurate and ordered duplication of the chromosome. The regulatory mechanisms that govern replication fork action are likely to be drawn from two major sources: (i) the programmed behavior of individual proteins or protein complexes and (ii) protein-protein interactions that act either as a trigger for induction of a particular activity or as a recruit-
The synthesis of Okazaki fragments that were of the typical minimum size if it was present at a much higher concentration. Thus, alterations in Okazaki fragment size can be correlated directly with the affinity of the primase for the replication fork, suggesting that the cycle of primase binding DnaB, synthesizing a primer, and leaving the replication fork, is the primary regulator of Okazaki fragment size. (This will be referred to below as the primase cycle.) The primase mutations exhibit no demonstrable effect in any system on the pol III HE. Thus it is unlikely that the observed changes in Okazaki fragment size can be attributed to an alteration in an interaction between the polymerase and the primase or to an altered ability of the primase to receive a signal from the polymerase. In fact, the one known interaction between the polymerase and the primase at the replication fork appears to be operating normally. This is the limitation of primer length to 10–12 nt that arises because of an interaction between the lagging-strand polymerase and the primase as it is synthesizing the primer (28). This can be seen in Fig. 6, where the unused primers are of the appropriate length.

Therefore, Okazaki fragment size is determined by a protein-protein interaction between DnaG and DnaB and not by the properties of the lagging-strand polymerase. To endow the replication fork with maximum efficiency, it is likely, as we have suggested previously (16), that the primase cycle and the lagging-strand polymerase cycle (i.e. nascent strand synthesis, termination of synthesis, dissociation of core from β, release from the template, and transit of the core to the new primer terminus) overlap (Fig. 7). Offsetting the synthesis of the primer and release of the polymerase from the penultimate Okazaki fragment ensures that a new primer will be available immediately for resumption of nascent strand synthesis. The construction of a processive core-β assembly requires the action of the γ-complex of the HE to place β on the primer terminus (29–31). Our recent studies show that the γ-complex remains associated with the pol III HE at active replication forks (33); thus, because β is used stoichiometrically for lagging-strand synthesis (14), offsetting the cycles also allows the placement of β onto the primer prior to the lagging-strand core becoming available for cycling.

It has been demonstrated that the core and β will dissociate when the polymerase runs into the 5′-end of a DNA strand on the template (13). Because this is identical to the situation on the lagging-strand template when the polymerase encounters the previous Okazaki fragment, it has been suggested that this is the trigger that keys recycling. Whether the lagging-strand polymerase at a bona fide replication fork operates in this manner remains to be determined. However, it is unlikely that this event can be the trigger for synthesis of a new primer. It would be difficult to fit the data described here to such a model. In addition, our previous observation (15) that the efficiency of primer utilization decreases dramatically when the concentra-
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DnaG: \text{WDD}LAKNI\text{AEQTFTEDSLNHMFDSLLELRO}^{551}

DnaB: \text{WDDVAERVADDYTTRPHRFHTEMARLOE}^{50}

Given the multiple protein-protein interactions that must occur at the replication fork, perhaps this indicates a common interacting partner for these proteins.

One of the more fascinating aspects of replication forks is how the molecular architecture might contribute to function. Essential protein-protein interactions that have been identified thus far include: (i) coupling of the leading- and lagging-strand polymerases by a dimer of the \( \gamma \) subunit of the holoenzyme (33), (ii) coupling of the replication fork helicase with the polymerase via an interaction between DnaB and \( \gamma \) (32), (iii) limitation of primer length as a result of an interaction between primase and the lagging-strand polymerase (28), and (iv) the interaction between DnaB and DnaG that attracts primase to the replication fork described here. Presumably other such interactions will be uncovered as investigative efforts continue.

In light of this complexity and the speed at which the enzymatic tasks must be accomplished, it seems likely that the proteins at the fork are highly spatially coordinated, forming a molecular clockworks that dictates the temporal sequence of events. The fact that the DnaB interaction site on primase lies within the last 9 or so C-terminal amino acids suggests that the disposition of this site relative to the rest of the protein can be modulated rather freely. It is tempting to speculate that the C terminus of primase might be inserted into the primase interaction site on DnaB, thus securing contact between these two proteins. Perhaps all interacting components at an active replication fork are always held in place by at least one interaction with another fork component. This would ensure the proximity of all necessary DNA sites and enzymatic activities. For example, DnaG could maintain contact with DnaB as it initiates synthesis of the new primer. The action of the \( \gamma \)-complex to load \( \beta \) on to the primer would serve to catch the site of the primer on the lagging-strand template from the primase-DnaB complex. This could induce primase to leave while also freeing DnaB for interaction with another primer. Thus, the next primer site would remain with the advancing replication fork at all times, even if the DNA loop separating the new primer from the previous one is 2 kb or more (Fig. 8).

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The sequence T\text{NQELAK}^{581} that forms the C terminus and DnaB interaction site of primase has a limited homology to a region in DnaB, T\text{GYDDLNNK}^{530}. It is not known whether this region is functionally significant in DnaB, or where the primase interaction site is. There is also significant homology in sequence and predicted secondary structure between DnaB and another region of primase in the 16-kDa C-terminal domain.
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