The Extreme C Terminus of Primase Is Required for Interaction with DnaB at the Replication Fork*

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We have shown previously that a protein-protein interaction between DnaG and DnaB is required to attract the primase to the replication fork. This interaction was mediated by the C-terminal 16-kDa domain (p16) of the primase. A screen was developed that allowed the detection of mutant p16 proteins that did not interact with DnaB. Various mutagenesis protocols were used to localize this interaction domain to the extreme C terminus of the primase. A mutant primase missing only the C-terminal 16 amino acids was isolated and its activities examined. This mutant enzyme was fully active as a primase, but was incapable of interacting with DnaB. Thus, the mutant primase could not support DNA synthesis in either the general priming reaction or during primer synthesis. Therefore, the mutant primase could not support DNA synthesis in replication reactions that included DnaG: general priming, phiX174 single-stranded circular (ss(c)) → replicative form (RF) DNA replication, or at the replication fork. The C-terminal 16-kDa domain (p16, amino acids 434–581) did not exhibit any independent enzymatic activities and could not bind DNA. However, p16 inhibited replication reactions that were dependent on DnaB. In the presence of primase at the replication fork, p16 interfered with Okazaki fragment synthesis. As the concentration of p16 was increased in the reaction, the Okazaki fragments increased in size. Because Okazaki fragment size is directly proportional to the frequency of primer synthesis (12), this indicated that p16 was competing with DnaG for a site at the replication fork that was required to attract primase. Because p16 only interfered in replication reactions that included DnaB and did not bind DNA on its own, we concluded that DnaG associated with the replication fork via a protein-protein interaction with DnaB.

Our previous studies of primase structure resulted in the definition of two stable domains by partial trypsinolysis that could be isolated and assayed independently (11). The N-terminal 49-kDa domain (p49, amino acids 1–433) retained primer synthetic capability but could not function as a primase in any assay that included DnaB: general priming, phiX174 single-stranded circular (ss(c)) → replicative form (RF) DNA replication, or at the replication fork. The C-terminal 16-kDa domain (p16, amino acids 434–581) did not exhibit any independent enzymatic activities and could not bind DNA. However, p16 inhibited replication reactions that were dependent on DnaB. In the presence of primase at the replication fork, p16 interfered with Okazaki fragment synthesis. As the concentration of p16 was increased in the reaction, the Okazaki fragments increased in size. Because Okazaki fragment size is directly proportional to the frequency of primer synthesis (12), this indicated that p16 was competing with DnaG for a site at the replication fork that was required to attract primase. Because p16 only interfered in replication reactions that included DnaB and did not bind DNA on its own, we concluded that DnaG associated with the replication fork via a protein-protein interaction with DnaB.

We have proposed that it is the period of the primase cycle, set by the affinity of DnaG for DnaB, that is the primary regulator of Okazaki fragment size at the replication fork (2, 3). By having the primase cycle offset from the polymerase cycle, a completed primer carrying a new β subunit will be available immediately upon release of the lagging-strand core from the just-completed Okazaki fragment. Thus, maximum efficiency will be maintained. Alternative models, where the dissociation of the lagging-strand polymerase from the completed Okazaki fragment is the trigger for primer synthesis (13), leave the polymerase waiting unnecessarily for the resumption of nascent strand synthesis. In order to address these issues, we have sought to generate mutant primases that have an altered affinity for DnaB. We report here the localization of the DnaB interaction domain on primase to its extreme C terminus. A mutant DnaG lacking only its extreme C-terminal 16 amino acids is fully functional as a primase, but is completely incapable of supporting DNA synthesis in replication reactions that require DnaB. Alanine cluster mutagenesis and deletion analysis in p16 allowed the further localization of the interaction domain to the extreme C-terminal 8 amino acids in primase.

Many different tasks must be coordinated by the enzymes that comprise the replisome in Escherichia coli in order to ensure the rapid, accurate, and ordered duplication of the chromosome (1). Lagging-strand synthesis is a particularly complicated process that requires the cyclical repetition of many different reactions in a defined temporal sequence. The lagging-strand cycle itself consists of two related but independent cycles that are offset with respect to one another (2, 3). The polymerase cycle describes: (i) nascent strand synthesis, (ii) termination of Okazaki fragment synthesis, requiring dissociation of the DNA polymerase III core (aeu) (4), containing the polymerase (5) and 3′ → 5′ exonuclease (6) activities from the δ processivity factor (7), (iv) transit of the polymerase from the 3′-terminus of the just-completed Okazaki fragment to the new primer terminus, and (v) resumption of nascent strand synthesis. The primase cycle describes: (i) binding of primase to the replication fork, (ii) initiation of primer synthesis, (iii) limitation of primer length via an interaction between the DNA polymerase III holoenzyme (pol III HE)† and the primase (8), (iv) assembly of the initiation complex on the primer terminus (the placement of β on the primer by the action of the γ-complex (9, 10)), and (v) the dissociation of primase from the fork.

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† The abbreviations used are: pol III HE, the E. coli DNA polymerase III holoenzyme; ss(c), single-stranded (circular); RF, replicative form; SSB, the E. coli single-stranded DNA-binding protein; SOE, splicing by overlap extension; PCR, polymerase chain reaction; nt, nucleotide(s); ORF, open reading frame; IPTG, isopropyl-β-D-galactopyranoside.

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and Herrick (14). Maxiprep and Midiprep DNA purification kits were from Qiagen.

FF, Superose 12, Superose 6, and Mono Q were from Pharmacia Biotech Inc. Seekein ME, NuSieve, and GCG LMP agarose were from FMC. Single-stranded DNA-cellulose was prepared by the method of Alberts and Herrick (14). Maxiprep and Midiprep DNA purification kits were from Qiagen.

Plasmids pET3c, pET3d, pET11d, and pET15d were from Novagen, as was bacteriophage M13. Site-directed mutagenesis was performed using either conventional PCR methodology, or splicing by overlap extension (SOE; Ref. 25) PCR methodology.

The detailed form I item template DNA was prepared as described by Mok and Marians (11). The pol III HE was reconstituted from pol III* and mutant p16 proteins and primases were purified as described by Tougu and Marians (17). Oligonucleotides were synthesized by the Sloan-Kettering Microchemistry facility.

The E. coli single-stranded DNA-binding protein (SSB) was prepared according to Minden and Marians (18). PriA, PriB, PriC, DnaT, DnaB, and DnaC were prepared according to Marians (19). Wild-type and mutant p16 proteins and primases were purified as described by Tougu and Marians (11). The pol III HE was reconstituted from pol III* and as described (20).

E. coli Strains, Bacteriophage, and Recombinant DNA Techniques

E. coli DH5α and DH5αF- (Life Technologies, Inc.) were used for all subcloning procedures. E. coli BL21 (DE3), BL21 (DE3)pLysS, and BL21 (DE3) pLysS, which were used for overexpression of proteins, were from Novagen, as was bacteriophage λCE6. E. coli C600 (DE3) was constructed as described by Novagen using an E. coli C600 laboratory stock. Bacteria were routinely grown in LB medium (21) supplemented with 0.4% glucose. Ampicillin was added to 0.5 mg/ml in liquid medium and 0.1 mg/ml in plates as indicated. Chloramphenicol was added to 25 μg/ml. Minimal M9 media and plates were as described (21). Competent cells were prepared by CaCl2 treatment, and transformations were performed as described (22). Alternatively, competent cells were prepared as described and used for electroporation (23).

Restriction endonuclease digestions, ligation reactions, alkaline phosphatase treatment of DNA, and 5'-end-labeling of DNA with [γ-32P]-ATP and T4 polynucleotide kinase were as described by Maniatis et al. (24). PCR was performed using either the Flu, Taq, or Vent™ DNA polymerases according to each manufacturer’s instructions. Between 1 and 100 ng of DNA were used as template. Primer concentrations were 1 μM unless specified otherwise. Typical reactions were incubated for 25–35 cycles in either a Perkin Elmer-Cetus model PE9600 or a Hybaid Omnigene (Denville Scientific) thermal cycler.

PCR incubation conditions were (i) 45 s at 95 °C for denaturation, (ii) annealing for 30 s at a temperature 5 °C below the lower of the two Tm values of the primers in use, and (iii) extension at 72 °C for 1 min.

DNA sequencing was by the dye-deoxy method as recommended by Amersham. Sequence ladders were separated by electrophoresis at 60 watts constant power through 6% polyacrylamide gels (19:1 acrylamide:bisacrylamide) containing 50% (w/v) urea using 100 mM Tris borate (pH 8.0), 1 mM EDTA as the electrophoresis buffer. Gels were dried before autoradiography.

Site-directed mutagenesis was performed using either conventional PCR or splicing by overlap extension (SOE; Ref. 25) PCR methodology.

Construction of Mutant p16 Proteins and Primases

For generation of mutants by conventional PCR, a mutagenic primer was used in combination with a wild-type primer to generate mutant DNA products that were purified from the wild-type starting material by recovering the fragment after electrophoresis through 8% polyacrylamide (19:1 acrylamide:bisacrylamide) gels using Tris borate electrophoresis buffer. These DNA fragments were digested with the indicated restriction endonucleases and then ligated with appropriately digested expression vectors. Recombinant plasmids were recovered after transformation as described above.

In the generation of mutants using the SOE-PCR methodology, oligonucleotides containing the desired mutations were used in a first PCR to generate mutant 5' and 3' DNA arms that were then purified from the wild-type starting material by electrophoresis as described above. The 5’ and 3’ DNA arms containing the desired mutations and the extreme flanking primers were then combined for a second PCR resulting in the full-length final DNA product containing the desired mutation. These DNA fragments were then joined with expression vectors as described above.

The specific primers used in the construction of the genes encoding the various mutant p16 proteins are described in Table I. Primer combinations used in conventional and SOE-PCR are presented in Table II.

All p16 mutants that were generated by PCR utilized pET3d-p16 as a template. All mutagenized p16 PCR products were digested with the Ncol and BamHI restriction enzymes, followed by ligation with Ncol- and BamHI-digested, alkaline phosphatase-treated pET3d.

p16A1 was constructed by digesting pET3d-dnaG with Ndel, followed by self-ligation of the 4.8-kilobase pair DNA fragment containing vector sequences as well as 121 nt encoding the C-terminal 41 amino acids of p16. The ligated DNA was transformed into E. coli DH5α, and candidate clones were screened by restriction endonuclease digestion of miniprep DNA.

Alanine cluster mutagenesis of the C-terminal 16 amino acids of p16 was performed using conventional PCR to construct open reading frames (ORFs) encoding p16A1-136 (E566-L579), p16A1-137 (L574-E577), and p16A1-138 (L578-K581). SOE-PCR was used to construct p16A1-1 (E570-T573).

All p16 mutant constructs were transferred into E. coli DH5α. Correct deletion mutants of p16 were identified by restriction analysis of miniprep DNA. All other p16 mutant DNAAs were first identified by expected size in restriction endonuclease analysis and were then sequenced utilizing primer 18 (Table I).

The C-terminal 16 amino acids of p16 were also subjected to random mutagenesis using a library of partially randomized oligonucleotides covering this region of the ORF. Two populations were used. The first (3% doping) was synthesized using a nucleoside mix containing 91% of the specified nucleotide residue in the primase sequence and a 3% (3% doping) was synthesized using a nucleoside mix containing 91% of the specified nucleotide residue in the primase sequence and a 3% substitution with each of the other 3 nucleotides. For the second oligonucleotide population (6% doping), the respective values were 82% specified and 6% substitution. The gel-purified oligonucleotides were used for PCR to generate a population of DNA fragments carrying mutagenized p16 ORFs. These DNAAs were then digested with Ncol and BamHI and ligated with similarly digested pET3d DNA that had been

Table I

| Primer | Nucleotide sequence |
|--------|--------------------|
| (5’→3’) |                        |
| 1      | AAGCATTAGCAGCAATGCGAGAGAGGCGTTT |
| 2      | TCCCTCCTAGTGGATGGCGATCTGACCTTAA |
| 3      | CCAAGGCTCGAGGGGATCTAGTGGTTAACC |
| 4      | CAGTTCCAGGCAGCGGATCAACATTTGGTGA |
| 5      | GTAATGATGACGTCATTTATTTTGGACAGCAGG |
| 6      | CGTTAATGCGCAATTCGGTTTAAATGCCCAGG |
| 7      | TGGCCAGCTCTTCAGGACTGCTGTTAACCAGG |
| 8      | TCCTGGTGTTAAACAGACGATCGCTTGCCGTT |
| 9      | GAAGACGCTCGAGTGGTCCAATTTACAGGAG |
| 10     | AAAAGGATCCCTGAGTGCTCTTCTGCGAGACAGG |
| 11     | TTTGGATCCCTGAGGTCAAGCGCGCGAGCCTGCGTTTAA |
| 12     | TACCTTTCTGGCACATTTTGCAGAACAGTCAAG |
| 13     | GAAGAAGCTCGAGCTGAGTGGACATTACCAAAAC |
| 14     | GATTAATGCCAACCATGGCTGCCACCCTTGAA |
| 15     | AAACGGATCCCTAGTGGCAGAGAGCGGCGTTT |
| 16     | GTITAAATGCAGACTTGCGCTGACATTTAAAC |
| 17     | GAAGAAGCTCGAGCCTGACATTTACAGGAG |
| 18     | AAGCTCTCCGAGCTGCTTATTCAGA |
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| Mutant | PCR primers\(^a\) |
|--------|------------------|
| p16Δ1  | 1 + 3            |
| p16Δ2  | 1 + 4            |
| p16Δ3  | 1 + 5            |
| p16Δ4  | 1 + 6            |
| AΔ1    | 1 + 7            |
| AΔ2    | PCR 1: 1 + 8 and 2 + 9, PCR 2: PCR 1 products and 1 + 2 |
| AΔ3    | 1 + 10           |
| AΔ4    | 1 + 11           |
| MT1-10 | PCR 1: 1 + 12 and 2 + 13, PCR 2: PCR 1 products and 1 + 2 |
| MT1-10 | PCR 1: 1 + 14 and 2 + 15, PCR 2: products and 1 + 2, PCR 2: PCR 1 products and 1 + 2 |
| p16-parB | PCR 1: 1 + 16 and 2 + 17, PCR 2: PCR 1 products and 1 + 2 |

\(^a\) Primers are listed in Table I.

The standard reaction buffer was 50 mM HEPES-KOH (pH 8.3 at 30 °C), 10 mM MgOAc, 10 mM dithiothreitol, 0.01 mg/ml rifampicin, and 0.2 mg/ml bovine serum albumin. Incubations were at 30 °C (5 min for M13Gori ss(c) → RF assays, 15 min for general priming and dX174 ss(c) → RF assays). The coupled priming and replication reactions were stopped by the addition of 0.1 ml of 0.2 M NaPPi and 0.1 ml of 1 mg/ml heat-denatured salmon sperm DNA as carrier. Trichloroacetic acid-insoluble counts/min were then determined.

Replication Assays

The standard reaction buffer was 50 mM HEPES-KOH (pH 8.3 at 30 °C), 10 mM MgOAc, 10 mM dithiothreitol, 0.01 mg/ml rifampicin, and 0.2 mg/ml bovine serum albumin. Incubations were at 30 °C (5 min for M13Gori ss(c) → RF assays, 15 min for general priming and dX174 ss(c) → RF assays). The coupled priming and replication reactions were stopped by the addition of 0.1 ml of 0.2 M NaPPi and 0.1 ml of 1 mg/ml heat-denatured salmon sperm DNA as carrier.

Results

A Screen for Mutant p16 Proteins That Do Not Interact with DnaG—Our analyses of replication fork function had led us to propose that the interaction between DnaG and DnaB was the primary regulator of Okazaki fragment size at the replication fork (3). We were therefore interested in isolating mutants of primase that had an altered affinity for DnaB. To accomplish this, we attempted to localize the region of DnaG required for interaction with DnaB to a small subset of amino acid residues. Because the only assays for mutants of this type were the replication assays in vitro, we explored the possibility that the toxicity of expression plasmids carrying the p16 ORF could be used as a screen for mutant p16 proteins.

The expression system allows delivery of the T7 RNA polymerase required for overexpression through either infection with a bacteriophage λ carrying the T7 RNA polymerase gene, or by induction with IPTG of a T7 RNA polymerase gene controlled by the lac operator and carried on the host chromosome in a defective λ lysogen (26). λ infection is the most tightly controlled induction system, allowing the cloning of lethal genes in E. coli host strain BL21. There are varying degrees of leaky expression with the IPTG induction technique. If host strain BL21ΔADE3 is used, there can be a significant amount of expression of the target gene. This can be reduced if host strain BL21ΔADE3lysS is used. In this strain, the T7 lysozyme gene is carried on a plasmid that is compatible with the pET series. T7 lysozyme is a natural inhibitor of T7 RNA polymerase (26).

The pET3c-p16 plasmid is tolerated only in BL21, whereas plasmids expressing either p49 or full-length primase are tolerated in all of the expression strains (11). We reasoned that the toxicity of the p16 resulted from interference with Okazaki fragment synthesis as a consequence of the leaky expression of the ORF. Thus, we tested whether finding pET clones expressing p16 that were tolerated by BL21ΔADE3 would be a valid screen for p16 proteins that no longer interacted with DnaB.

A series of N- and C-terminal deletions were constructed in the p16 ORF (Fig. 1A). The relative toxicity of the expression clones was determined by comparing their transformation fre-
frequency in BL21 and BL21(ΔDE3). All of the deletion constructs were nontoxic (the ratio of transformation frequency in BL21(ΔDE3) to that in BL21 was nearly 1) (Table III). The relative expression levels and solubility of representative N- and C-terminal deletions of p16 were similar to that of the wild type (Fig. 1B); thus, the lack of toxicity was not a result of the mutant protein being sequestered in inclusion bodies in the cell. To ascertain that loss of toxicity did indeed correlate with the inability of the mutant p16 to interact with DnaB, p16Δ1, which had the smallest deletion that resulted in loss of toxicity, was overexpressed, purified, and analyzed for its ability to inhibit DnaB-dependent replication in vitro.

The mutant p16 fractionated during column chromatography in an identical fashion as the wild type and could be purified to the same extent (Fig. 2A), p16Δ1 had a markedly different effect than p16 in the DnaB-dependent general priming (Fig. 2C) and φX174 ss(c) → RF (Fig. 2D) replication reactions; whereas p16 inhibited both of these replication reactions, p16Δ1 showed no inhibition in either reaction, suggesting that deletion of the C-terminal 16 amino acids from p16 rendered it unable to interact with DnaB. In addition, the differences in the ability of p16Δ1 and p16 to inhibit DnaB-dependent replication correlated with the different toxicity of the expression constructs in BL21(ΔDE3), validating the use of the screen for detecting the inability of p16 to interact with DnaB in vitro.

Deletion of the C-terminal 16 Amino Acids of Primase Inactivates Its Ability to Interact with DnaB—To study the effect of deleting the C-terminal 16 amino acids within the context of full-length primase, an ORF expressing DnaGΔ1 was constructed as described under "Materials and Methods." DnaGΔ1 was purified to homogeneity (Fig. 3A). No differences were noted in the chromatographic behavior of DnaGΔ1 compared to the wild type. The activity of DnaGΔ1 was indistinguishable from that of the wild type in the DnaB-independent G4 ss(c) → RF DNA replication assay (Fig. 3B), indicating that the ability of DnaGΔ1 to act as a primase and to interact with the pol III HE had not been compromised by deletion of the C-terminal 16 amino acids. For comparison, the activity of p49 is also shown in the figure. Our previous studies had demonstrated that whereas this protein retained primer synthetic capability, removal of the p16 domain decreased the specific activity of the protein by about a factor of 7 in the G4 assay (11). Interestingly, DnaGΔ1 is as active as wild type in the G4 assay, suggesting that the region of the protein responsible for activating priming is located more N-terminal. In contrast to the results in the G4 assay, DnaGΔ1 was incapable of supporting either general priming (Fig. 3C) or φX174 ss(c) → RF DNA replication (Fig. 3D), indicating that loss of the 16 amino acids from the C terminus of primase inactivates its ability to interact with DnaB, thereby precluding primer synthesis in these systems.

Cluster to Alanine and Random Mutagenesis Further Defines the DnaB-Interaction Domain at the Extreme C Terminus of Primase—Two different mutagenesis techniques were used to derive more information on the organization of the amino acid sequences at the C terminus of primase that were required for interaction with DnaB: cluster to alanine mutagenesis and PCR mutagenesis using partially randomized primers. Alanine scanning has been used for mapping regions of interest in proteins to a small number of amino acids that could then be analyzed individually (27, 28). The most widespread variation of alanine scanning mutagenesis is based on mutagenizing a protein with functional significance is localized in this manner, single amino acid substitutions can be used for more precise analysis.

Four Ala cluster mutations were made in the C-terminal 16 amino acids of p16, generating p16A1-1–4 where the amino acid substitutions were: A4-1, E560ERL569 → A560AAA569; A4-2,
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**TABLE IV**

Relative transformation frequencies in *E. coli* BL21 and BL21(DE3) of various pET3d plasmids expressing mutated p16 proteins

| p16   | Mutation       | Transformation in BL21(DE3)* |
|-------|----------------|-----------------------------|
| A4-1  | E566GLRL569 → A566AAA569 | –                           |
| A4-2  | E570LWT573 → A570AAA573 | –                           |
| A4-3  | L574QOE577 → A574AAA577 | –                           |
| A4-4  | L578AKK581 → A578AAA581 | +                           |
| MT1   | L574 Stop      | +                           |
| MT2   | L574 Stop      | +                           |
| MT3   | L571 Stop      | +                           |
| MT4   | L571 Stop      | +                           |
| MT5   | E567 Stop      | +                           |
| MT6   | E567 Stop      | +                           |
| MT7   | R568C          | +                           |
| MT8   | L578F          | +                           |
| MT9   | Q576P, E577K, L578P | +                           |
| M10   | R568G, L569, L571V, N575H | +                           |
| Wild type | None           | –                           |
| p16Δ11| ΔE566-K581     | +                           |

*+, relative transformation efficiency near 1.0; –, relative transformation efficiency <0.005.

die (2), mutated 8 of the last 9 C-terminal amino acids of primase to Ala and used these proteins to prove our proposal that it was the interaction of DnaG with DnaB that is the primary regulator of Okazaki fragment size at the replication fork. It is useful to compare those results with the data reported here for the Ala cluster mutations in order to generate a functional map of the C-terminal region of primase.

We know that changing Asn575 and Glu577 to Ala strengthens the p16-DnaB interaction, that the Q576A substitution weakens it somewhat, and that the L574A substitution has no effect (2). Thus, the likely phenotype of the A4-3 cluster substitution would be the one observed, i.e. the substituted p16 would still be toxic. Whereas we have not analyzed the region defined by the A4-1 cluster substitution further, a similar situation could be true there, or, alternatively, this region is not a determinant of the primase-DnaB interaction.

A4-2 and A4-4 were not toxic. We know that the K580A substitution weakens the primase-DnaB interaction somewhat, whereas the K581A substitution has no effect (2). However, the L579A mutant p16 is completely insoluble at low salt (2), indicating that Leu579 is important for either the structure or correct folding of the region. We have also changed Leu579 to Phe. This mutant p16 behaves like wild type (2), indicating that the severe effect of mutating Leu579 to alanine likely results from the loss of the long and bulky side chain of leucine. The A4-4 p16 was overexpressed the least well of the four Ala cluster substitutions (Fig. 4A); thus, it seems likely that the effect of the L579A substitution was dominating the effect of the cluster substitution.

The A4-2 region, we would have expected this cluster substitution to remain at least as toxic as wild type in BL21(DE3). Therefore, the loss of toxicity observed with A4-2 must result from mutating either Gly570, Leu571, or Trp572 to Ala. We suspect that changing the bulky Trp572 to Ala perturbs the structure of the primase-DnaB interaction site, rendering the A4-2 cluster nontoxic because of its inability to interact with DnaB. This conclusion is supported by the ability of p16A4-2 to be overexpressed to high levels (Fig. 4A).

PCR mutagenesis with two different partially randomized oligonucleotide primer pools was also used to define the DnaB-interaction domain as described under "Materials and Methods." Mutant p16 proteins were identified using the toxicity screen (Table IV), and 10 clones that gave what appeared to be full-length proteins when expressed (Fig. 4B) were sequenced.
proteins, we have localized the region on DnaG between DnaB and the temporal sequence of events. These include: (i) an interaction between DnaB and DnaG that attracts the primase that serves to limit primer size to the typical 10–12 nt that is observed at the fork (8), and (iv) the interaction between DnaG and DnaB that attracts the primase to the replication fork (11).

The most informative mutations were nonsense ones that resulted in the truncation of the p16 ORF very close to the C terminus. Truncations of 8, 10, and 15 amino acids from the C terminus were nontoxic in the transformation assay (Table IV). Thus, because we have been able to establish that nontoxicity in the transformation assay correlates strongly with the inability of the nontoxic protein to associate with DnaB (30), (ii) coupling of the replication fork movement (30), (ii) coupling of the replication fork movement (30), and (iii) an interaction between the pol III HE and the primase that serves to limit primer size to the typical 10–12 nt that is observed at the fork (8), and (iv) the interaction between DnaB and DnaG that attracts the primase to the replication fork (11).

The DnaB-DnaG interaction is crucial for lagging-strand synthesis. The period of the primase cycle, which is governed by the association and dissociation of DnaG with the replication fork, is the key to regulation of Okazaki fragment size (2, 3). Through the use of various mutagenesis techniques, a screen in vivo for p16 proteins that could not interact with DnaB, and replication assays in vitro to assess the activity of mutant p16 proteins and primases, we have localized the region on DnaG required for interaction with DnaB to the extreme C terminus. Our results indicate that removal of only the last 8 amino acids from the C terminus of primase prevents it from interacting with DnaB.

This region of primase is likely to be relatively unconstrained in structure and probably moves freely in solution so that it can perhaps be captured by a suitable binding pocket on DnaB bound to DNA. Because the DnaB-DnaG interaction has not been demonstrated in solution, it seems likely that the reciprocal binding pocket on DnaB is not completely formed until it is bound to the DNA. This ensures, because the primase can only interact with the replication fork, that there will always be an adequate supply of free DnaG to keep the primase cycle running at maximum efficiency.

Interfering with the primase cycle in vivo presumably has significant consequences for the cell. A mutation in dnaG exists called parB (31, 32). Cells carrying this temperature-sensitive mutation exhibit a partition defect at the nonpermissive temperature. Our results indicate that removal of only the last 8 amino acids from the C terminus of primase prevents it from interacting with DnaB.

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