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In the *Bacillus stearothermophilus* DnaB-DnaG Complex, the Activities of the Two Proteins Are Modulated by Distinct but Overlapping Networks of Residues

Jenny Thirlway and Panos Soultanas*

Centre for Biomolecular Sciences (CBS), School of Chemistry, University of Nottingham, University Park, Nottingham NG7 2RD, United Kingdom

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We demonstrate the primase activity of *Bacillus stearothermophilus* DnaG and show that it initiates at 3′-ATC-5′ and 3′-ATT-5′ sites synthesizing primers that are 22 or 23 nucleotides long. In the presence of the helicase DnaB the size distribution of primers is different, and a range of additional smaller primers are also synthesized. Nine residues from the N- and C-terminal domains of DnaB, as well as its linker region, have been reported previously to affect this interaction. In *Bacillus stearothermophilus* only three residues from the linker region (I119 and I125) and the N-terminal domain (Y88) of DnaB have been shown previously to have direct structural importance, and I119 and I125 mediate DnaG-induced effects on DnaB activity. The functions of the other residues (L138, T191, E192, R195, and M196) are still a mystery. Here we show that the E15A, Y88A, and E15A Y88A mutants bind DnaG but are not able to modulate primer size, whereas the R195A M196A mutant inhibited the primase activity. Therefore, four of these residues, E15 and Y88 (N-terminal domain) and R195 and M196 (C-terminal domain), mediate DnaB-induced effects on DnaG activity. Overall, the data suggest that the effects of DnaB on DnaG activity and vice versa are mediated by distinct but overlapping networks of residues.

The bacterial helicase (DnaB)-primase (DnaG) interaction signifies completion of the priming event at the replication origin (*oriC*) and plays an instrumental role in lagging strand synthesis regulating the repeated priming events necessary for Okazaki fragment formation (7, 15). A distinct C-terminal helicase-interacting domain of DnaG (known as P16) mediates this interaction structurally and functionally (2, 21). The recently determined structures of P16 from *Bacillus stearothermophilus* and *Escherichia coli* DnaG revealed that it consists of two subdomains, a six-helix bundle (subdomain C1) that is essential for stimulation of DnaB activity and a helical hairpin (subdomain C2) that mediates binding to DnaB (13, 19). DnaG interacts with a linker region that joins the N- and C-terminal domains of DnaB and induces the formation of threefold symmetric rings with mainly three DnaG molecules interacting with one DnaB hexamer (20). The surprising structural homology between the C1 subdomain of P16 and the N-terminal domain of *E. coli* DnaB suggested that the former may structurally and functionally replace the latter in the threefold symmetric DnaB when it is complexed with DnaG (6, 16, 19). The N-terminal domains of alternate DnaB monomers are believed to interact with the C-terminal domains of neighboring monomers around the threefold symmetric ring (23). In the DnaB-DnaG complex, the role of the N-terminal DnaB domains may be "replaced" by the structurally homologous P16 domains of DnaG (16, 19). Alternating C1 subdomains of three DnaG molecules interact with neighboring C-terminal domains of DnaB around the ring. These interactions are functionally significant because they are essential for stimulation of DnaB. In fact, a fragment that contains the last helix of the C1 subdomain and the C2 subdomain is able to bind to DnaB but cannot elicit a stimulatory effect in the same manner that full-length P16 does. The proposed structural model implies that residues on the surfaces of both the N- and C-terminal domains, as well as the linker region of DnaB, may be structurally and/or functionally significant for the activity of the DnaB-DnaG complex (16). In vitro experiments with the *E. coli* DnaB and DnaG proteins showed that DnaB modulates the activity of DnaG in several ways. One role of DnaB is to transport the bound DnaG to its recognition sites (5, 7, 12). In addition, DnaB also modulates primer synthesis in a manner independent of its translocation ability. It relaxes the specificity of the primase, stimulates its activity, and reduces the length of primers to between 10 and 14 nucleotides (1, 7, 8, 10, 24). Given the striking differences in the stability of the *B. stearothermophilus* DnaB-DnaG complex, it is not clear whether these observations apply only to *E. coli*.

Mutagenesis studies with conserved residues in *E. coli* and *Salmonella enterica* serovar Typhimurium DnaB proteins have highlighted the importance of nine residues for the activity of the DnaB-DnaG complex (3, 11, 18). In the absence of a stable DnaB-DnaG interaction in these systems, it was not possible to determine directly which of these residues mediate structural and/or functional aspects of this interaction. However, in *B. stearothermophilus* this complex is stable, and a study of the equivalent mutations in this system revealed some structural and/or functional roles (20). Five of these residues (E15, Y88, I119, I125, and L138) are conserved in *B. stearothermophilus*

* Corresponding author. Mailing address: Centre for Biomolecular Sciences (CBS), School of Chemistry, University of Nottingham, University Park, Nottingham NG7 2RD, United Kingdom. Phone: 44-(0)-115-9513525. Fax: 44-(0)-115-8468002. E-mail: panos.soultanas@nottingham.ac.uk.

DnaB, while the other four residues are T191, E192, R195, and M196 instead of the D212, D213, K216, and K217 residues in *E. coli*, as assessed by sequence comparisons. Only three of these residues (Y88, I119, and I125) were found to have a direct salt-dependent effect on DnaB-DnaG binding and on the ability of DnaG to stimulate the function of DnaB (20). The question of the roles (if any) of the other six residues remains unanswered.

In this study we investigated this question by examining the effects of all nine DnaB mutants on the activity of DnaG using an in vitro general priming assay. We demonstrated for the first time the general priming activity of *B. stearothermophilus* DnaG and found that it is ATP dependent. Its interaction with DnaB reduces the specificity and length of primers. We found that the E15A, Y88A, and E15A Y88A DnaB mutants are unable to reduce the specificity and length of the synthesized primers despite the fact that they interact with DnaG, while the R195A M196A DnaB mutant inhibits the general priming activity of DnaG.

These data in combination with our previous work (17, 20) suggest that four of the residues (E15, Y88, R195, and M196) are part of a network of residues on the surfaces of the N- and C-terminal domains of DnaB that are responsible for the allosteric effects of DnaB on the activity of DnaG. On the other hand, Y88, I119, and I125 are part of a network of residues on the surfaces of the N-terminal domain and the linker region of DnaB that mediate the allosteric effects of DnaG on the activity of DnaB. Therefore, the functional effects of DnaB on DnaG and vice versa are mediated by two distinct but overlapping networks of residues.

MATERIALS AND METHODS

Mutagenesis and protein purification. The mutagenesis procedures and DnaB protein purification procedures used have been described elsewhere (17, 20). Briefly, wild-type and mutant DnaB proteins were overexpressed in *E. coli* BL21(DE3) and purified with a combination of Blue Sepharose, Mono-Q, Hi-Trap heparin, and Superdex S-200 chromatography columns. DnaG was purified with a combination of Hi-Trap heparin, Source-Q, and Superdex S-75 chromatography columns, as described elsewhere (2). Protein concentrations were determined spectrophotometrically using values for specific absorbance at 280 nm of 0.418 and 0.66 for the DnaB and DnaG proteins, respectively. The only exceptions were the Y88A and E15A Y88A mutants, whose specific absorbance was 0.934. All specific absorbance values were calculated using the following formula: A_{280} (1 mg/ml) = $(5690W + 1280Y + 120C)/M$, where W, Y, and C are the numbers of Trp, Tyr, and Cys residues in a protein having a mass of M, respectively, and 5690, 1280, and 120 are the extinction coefficients for these residues, respectively. All mutant proteins were chromatographically stable and exhibited normal oligomerization states (hexamers) under either low-ionic-strength (100 to 250 mM NaCl) or high-ionic-strength (500 mM NaCl) conditions in a manner similar to wild-type DnaB.

General priming assays. General priming assays were carried out in the presence or absence of DnaB (0.2 to 60 nM, referring to the hexamer) with 360 nM DnaG and 5 μM 66-mer oligonucleotides S1 to S5 in a mixture containing 25 mM Tris (pH 7.5), 1 mM dithiothreitol, 10 mM $MgCl_2$, 13.3 μM nucleoside triphosphates (ATP, GTP, and CTP), and 0.5 μM [α-^{32}P]UTP (400 Ci/mmol) at 50°C. Samples were removed from the reaction mixture at different times, and the reaction was terminated by addition of stop buffer (0.4% [wt/vol] sodium dodecyl sulfate, 40 mM EDTA, 8% [vol/vol] glycerol, 0.1% [wt/vol] bromophenol blue). Excess unincorporated [α-^{32}P]UTP was removed with MicroSpinTM S200 HR spin columns (Amersham Biosciences) used according to the manufacturer's instructions. Primers were resolved in a 12% sequencing gel. Gels were dried under a vacuum, and analysis was carried out with a phosphorimager. Reactions with [γ-^{32}P]ATP were carried out in a similar manner.

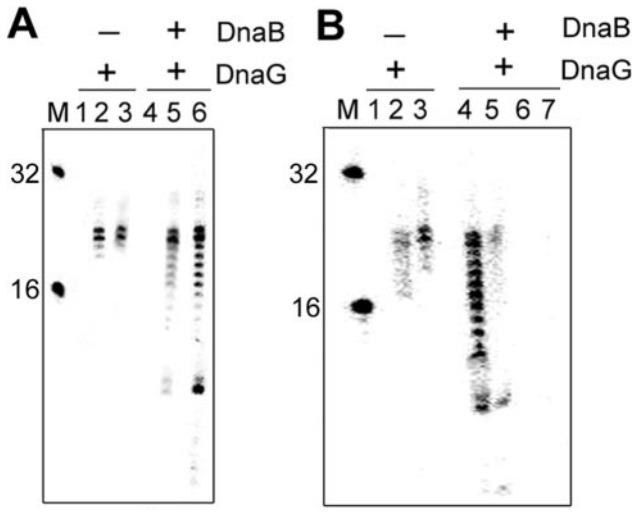


FIG. 1. General priming activity of DnaG. (A) General priming assays with substrate S1 and DnaG or DnaG plus DnaB, as indicated at the top. Reactions were carried out for 10, 30, and 40 min (for DnaG, lanes 1 to 3, respectively; for DnaG plus DnaB, lanes 4 to 6, respectively) at 50°C. DnaB modulates the activity of DnaG. In the presence of DnaB the distribution of primers synthesized by DnaG is different. In lanes 1 to 3, primers that are 22 or 23 nucleotides long are visible, whereas in lanes 4 to 6 a range of additional smaller primers are visible. (B) The relative molar concentrations of the two enzymes are critical for maximal modulation of the priming activity. Lanes 1 to 3 (10, 20, and 40 min, respectively) show a time course of the priming reaction in the absence of DnaB. Lanes 4 to 7 show the 40-min results for reactions performed in the presence of various concentrations of DnaB (lane 4, 2 nM; lane 5, 0.2 nM; lane 6, 20 nM; lane 7, 60 nM). All reactions were carried out with oligonucleotide substrate S1 at 50°C and 360 nM DnaG. Lane M contained oligonucleotide size markers.

RESULTS

DnaB modulates the activity of DnaG. The activity of *B. stearothermophilus* DnaG has not been demonstrated previously. In order to demonstrate this activity, we utilized various random oligonucleotides available in our lab (data not shown) and finally identified an active substrate (oligonucleotide S1) that allowed us to establish assay conditions for this activity and show that it is ATP dependent. DnaB modulates the size of primers synthesized by DnaG (Fig. 1A and B). In priming reactions that last for 40 min DnaG forms mainly primers that are 22 or 23 nucleotides long. In the presence of DnaB the size distribution of primers is different. The primers vary from 12 or 13 to 22 or 23 nucleotides long, and 5- or 6-nucleotide primers are also visible. Therefore, *B. stearothermophilus* DnaB, like *E. coli* DnaB, appears to regulate the size distribution of the primers synthesized by DnaG. As observed previously in the *E. coli* system, in *B. stearothermophilus* the ratio of DnaB to DnaG is critical for this regulatory effect. Varying the concentration of DnaB (0.2 to 60 nM, referring to hexamers) with a fixed concentration of DnaG (360 nM) revealed that the optimal effect of DnaB on the activity of DnaG was observed with 2 nM DnaB hexamer (Fig. 1B). Increasing the concentration of the DnaB hexamer to 60 nM (equivalent to one DnaB hexamer per three DnaG monomers) resulted in apparent inhibition of the DnaG activity, in a manner similar to that observed previously for the *E. coli* proteins. This is an important observation

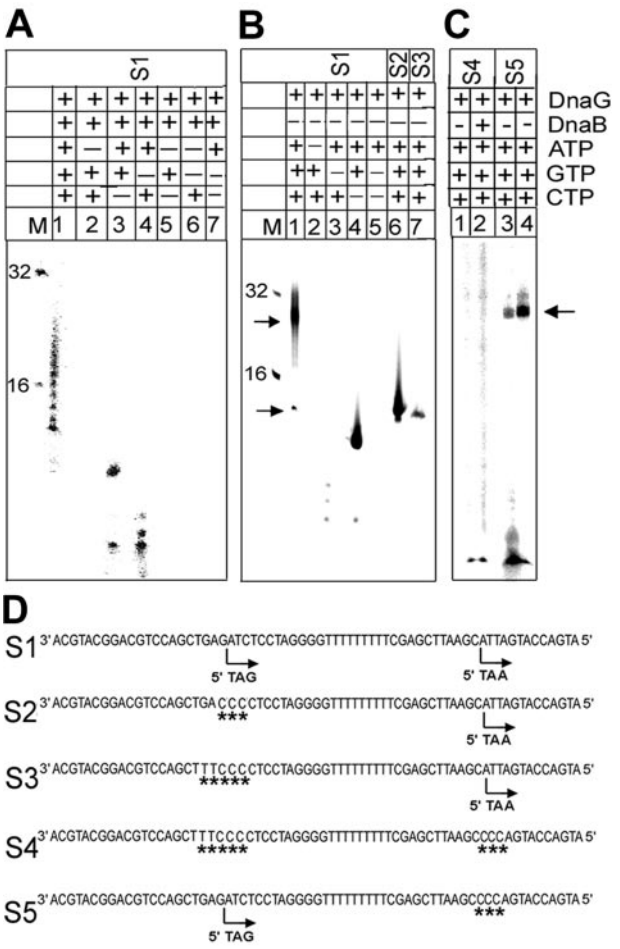


FIG. 2. General priming assays with substrate S1 and depleted combinations of nucleotides, as indicated at the top. Reactions were carried out as described in Materials and Methods in the presence of 360 nM DnaG for 60 min at 50°C, either in the presence (A) or in the absence (B) of 2 nM DnaB. Lanes 6 and 7 in panel B show reactions with oligonucleotide substrates S2 and S3, respectively. The arrows in lane 1 in panel B indicate primers initiating from the 5′-ATC-3′ (top arrow) and 5′-ATT-3′ (bottom arrow) sites. (C) Priming reactions with substrates S4 and S5. Most reactions were carried out for 60 min at 50°C with 360 nM DnaG in the absence of DnaB. Lane 2 shows the results of a reaction performed in the presence of 2 nM DnaB. The reaction with substrate S4 gave no primers (lanes 1 and 2). Lane M contained oligonucleotide size markers. (D) All the oligonucleotide substrates used in this study. Initiation sites are indicated by arrows, and sequence differences compared to substrate S1 are indicated by asterisks.

with direct relevance for the in vivo regulation of priming activity, as explained in more detail in the Discussion. Since the relative concentrations of the DnaB hexamer (2 nM) and the DnaG monomer (360 nM) were optimal under our conditions, we performed all our experiments under these conditions, unless indicated otherwise.

Initiation sites of *B. stearothermophilus* DnaG. In the absence of ATP no primers were synthesized either in the absence or in the presence of DnaB (Fig. 2). In the absence of either CTP or GTP very small primers were initiated both in the presence and in the absence of DnaB; they could not be extended beyond eight nucleotides when CTP was absent and beyond four nucleotides when GTP was absent (Fig. 2A and B). In the absence of any combination of two nucleoside triphosphates no primers were initiated (Fig. 2A and B). The 8-mer primers synthesized in the absence of only CTP indicated that there is a gap between the initiation site and the first guanine that the primase encounters during primer synthesis. In the S1 oligonucleotide there are only two guanines that are eight nucleotides apart, and these residues are in the sequence 3′-GATCTCCTAG-5′ (Fig. 2D). If priming initiates at 3′-ATC-5′, then in the absence of GTP only a dinucleotide is synthesized. However, we detected di-, tri-, and tetranucleotides (Fig. 2A, lane 4, and Fig. 2B, lane 3). An explanation for this is given below. To test the importance of 3′-ATC-5′, we mutated the 3′-GATC-5′ sequence to 3′-CCCC-5′ in substrate S2 and also the 3′-GAGATC-5′ sequence to 3′-TTCCCC-5′ in substrate S3 (Fig. 2D). Using these substrates, we discovered that the size of the primers synthesized was reduced even after 60-min reactions, and only 13-mer primers were synthesized (Fig. 2B, lanes 6 and 7). Therefore, mutating this part of the sequence in S1 did not inhibit primer synthesis but resulted in synthesis of smaller primers. One possibility is that there may be two initiation sites in substrate S1 (3′-ATC-5′ being one) and that the mutations of 3′-ATC-5′ in substrates S2 and S3 have resulted in switching priming to the second site. When we examined the sequence of S1 for another site similar to 3′-ATC-5′, we identified the 3′-ATT-5′ sequence, which, if utilized, would result in synthesis of 13-mer primers.

To examine this possibility directly, we mutated the sequences of both putative initiation sites in substrate S4 and also tested an additional substrate (S5) that carried only the 3′-ATC-5′ initiation site (Fig. 2C). Using S4 as the substrate produced no primers after 1 h in the presence or absence of 2 nM DnaB (Fig. 2C, lanes 1 and 2), compared to the 22- and 23-mers synthesized after 1-h reactions with S5 in the absence of DnaB (Fig. 2C, lanes 3 and 4). These data confirmed directly that DnaG initiates primer synthesis from two sites in S1, 3′-ATC-5′ and 3′-ATT-5′. The former site appears to be the primary initiation site, while the latter appears to be a secondary site. In fact, the 13-mer primer synthesized from this secondary initiation site on substrate S1 was only barely visible after 1 h compared to the much larger amounts of 22- and 23-mer primers synthesized from the primary initiation site (Fig. 2B, lane 1). Therefore, the fact that di-, tri-, and tetranucleotides were detected in the absence of CTP with substrate S1 (Fig. 2A and B) can be explained by initiation at the 3′-ATT-5′ secondary site, which could be extended further only by one more nucleotide before a guanine was encountered in the template (Fig. 2D).

Priming from both the 3′-ATC-5′ and 3′-ATT-5′ sites required an adenine at the second position and was also consistent with the absence of any detectable primers when ATP was omitted from the reaction mixture. We also carried out priming reactions using [γ-^{32}P]ATP, and we observed no primer synthesis (data not shown), indicating that ATP is not the initiating nucleotide but is instead required at the second position of the primers. If ATP were the initiating nucleotide, then all primers would have been labeled with a single radioactive [γ-^{32}P]ATP at the 5′ end.

Overall, these data show directly that DnaB regulates the length of primers synthesized by DnaG and that *B. stearother-*

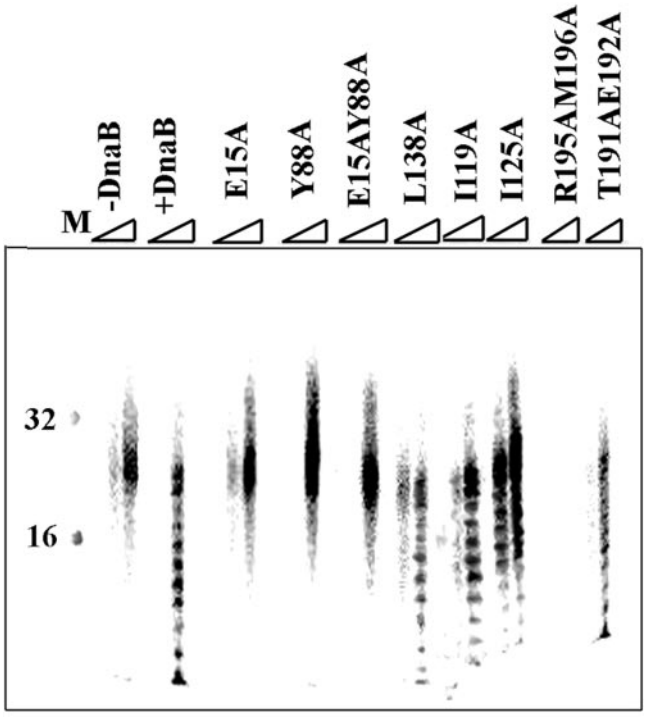
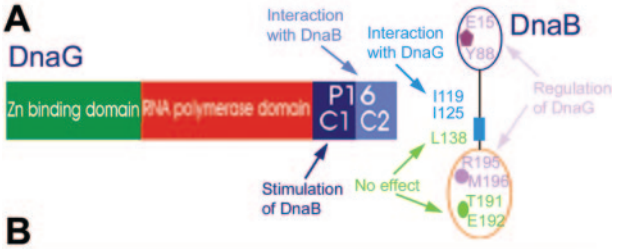


FIG. 3. Effects of DnaB mutants on the activity of DnaG. Time course priming reactions (10, 20, and 40 min) were performed with substrate S1 in the presence of 2 nM DnaB mutants, as indicated at the top. Control reactions in the presence and absence of wild-type DnaB are indicated by +DnaB and −DnaB, respectively. Lane M contained size markers.



B

Protein	Interaction with DnaG		DnaB activity		DnaG activity
	Low salt	High salt	ATPase	Helicase	
wtDnaB	+	+	+	+	+
E15A	+	+	+	+	-
Y88A	+	-	+	+	-
E15AY88A	+	-	+	+	-
I119A	*	-	-	-	+
I125A	*	-	-	-	+
L138A	+	+	+	+	+
T191AE192A	+	+	+	+	+
R195AM196A	+	+	+	+	*

FIG. 4. (A) Schematic representation of the domain organization of DnaB and DnaG and summary of the interaction interface between the two proteins. DnaG consists of three domains, a Zn-binding N-terminal domain (14), a central polymerization domain (9), and the C-terminal P16 domain that interacts with DnaB (13, 16, 19). The P16 domain consists of two subdomains, Subdomains C1 and C2. C2 structurally mediates the interaction with DnaB, while C1 mediates the functional stimulation of DnaB (16, 19). DnaB consists of N- and C-terminal domains (2, 9, 22). Residues from the flexible hinge region that connects the two domains are involved directly in the interaction (20), whereas the roles of all nine residues used in this study are indicated. (B) Table summarizing data from this work and from our previous work (20) on the biochemical properties of all nine DnaB mutants. Three main properties were examined. The second and third columns indicate the effects of the mutations on the stability of the DnaB-DnaG complex in low- and high-salt conditions (20). A strong complex is indicated by a plus sign, and a weak complex is indicated by an asterisk, whereas formation of no complex is indicated by a minus sign. The fourth and fifth columns summarize the effects on the "DnaG-to-DnaB" functional stimulation (i.e., ATPase and helicase activities) (20). A plus sign indicates that a DnaB mutant can be stimulated, whereas a minus sign indicates that a mutant does not have this ability. The last column indicates the effects on the "DnaB-to-DnaG" functional modulation (i.e., primase activity) (this study). A plus sign indicates that a DnaB mutant is able to modulate primer length, and a minus sign indicates that a mutant does not have this ability. The asterisk indicates that the DnaB mutant totally inhibits primer synthesis by DnaG.

mophilus DnaG can initiate primer synthesis from 3′-ATC-5′ and 3′-ATT-5′ sites. The 3′-ATC-5′ site is the primary site, while the 3′-ATT-5′ site is a secondary site utilized mainly in the absence of the 3′-ATC-5′ site.

DnaB mutants affect the activity of DnaG. Previous mutagenesis studies with the *E. coli* and *S. enterica* serovar Typhimurium systems have implicated nine DnaB residues in the interaction of this protein with DnaG (3, 11, 18). In *B. stearothermophilus* DnaB the equivalent mutations (E15A, Y88A, I119A, I125A, E15A Y88A, L138A, T191A E192A, and R195A M196A) were examined, and only Y88, I119, and I125 were shown to be involved directly in this interaction; the last two residues were also important for the functional stimulation of DnaB activity by DnaG (20). The roles of the other residues are not known since they did not affect the interaction with DnaG and also the DnaG-mediated stimulation of DnaB activity (20). Here we investigated the possibility that these residues may be important in mediating the DnaB-induced effects on the activity of DnaG.

The effects of these DnaB mutants on the activity of DnaG were examined using the general priming assay and substrate S1 (Fig. 3). We discovered that mutants with residues in the N-terminal domain of DnaB (E15A, Y88A, and E15A Y88A) were unable to regulate the size of the primers synthesized by DnaG, while mutants with residues in the linker region of DnaB (I119A, I125A, and L138A) were still able to regulate primer size just like wild-type DnaB. One double mutant (R195A M196A) with mutations in the C-terminal domain of DnaB totally inhibited the activity of DnaG. The T191A E192A double mutant was the same as wild-type DnaB. Although the experiments shown in Fig. 3 were carried out under the optimal conditions (2 nM DnaB hexamer and 360 nM DnaG monomer), it should be emphasized that in the case of the defective mutants we carried out similar experiments and varied the concentrations of the DnaB mutant proteins but we did not detect any effect on the DnaG activity over a broad range of concentrations (0.2 to 20 nM) (data not shown). All data from this study are summarized and compared to previous data (20) in Fig. 4.

DISCUSSION

DnaB modulates the activity of DnaG. We confirmed that *B. stearothermophilus* DnaB modulates DnaG in a manner similar to that observed for the *E. coli* proteins by reducing the length

of the primers synthesized (Fig. 1). The ratio of DnaB to DnaG is important for maximal modulation, since too little DnaB results in suboptimal modulation, whereas too much DnaB totally inhibits the primase activity (Fig. 1B). The same effect was observed with the *E. coli* proteins (8). The precise explanation for this observation is unclear, but it may have great functional importance for the bacterial helicase-primase complex. In view of the previously reported variability of the stoichiometry of the DnaB-DnaG complex (20), one explanation could be that the large excess of DnaG over DnaB hexamer (180:1 molar excess) is necessary to force all three DnaG molecules on the DnaB hexamer to produce the $DnaB_6$-$DnaG_3$ complex (active complex), whereas increasing the concentration of the DnaB hexamer relative to the DnaG concentration could result in more $DnaB_6$-$DnaG_2$ and $DnaB_6$-$DnaG_1$ complexes (inactive complexes) with no priming activity, despite the relative excess of DnaG over DnaB. Recently, workers have suggested a model in which primase molecules bound to the helicase regulate each other's priming activity by "*trans* interactions" between the zinc-binding domain of one primase molecule and the RNA polymerase domain of a neighboring molecule (4). Therefore, DnaG has two priming modes, a "*cis* mode" in the absence of the helicase and a "*trans* mode" in the presence of the helicase (4). Longer primers are synthesized in the "*cis* mode," whereas smaller primers are synthesized in the "*trans* mode." The more DnaG molecules associated with the helicase, the more the "*trans* mode" of priming becomes dominant. This model could explain the inhibitory effect at high DnaB concentrations as more DnaB hexamers sequester DnaG molecules in $DnaB_6$-$DnaG_1$ and $DnaB_6$-$DnaG_2$ complexes and therefore inhibit the formation of the $DnaB_6$-$DnaG_3$ active complex. The relative concentrations of DnaB and DnaG may also be an important regulatory factor that could affect the activity of the complex during DNA replication in vivo.

Interestingly, ATP has been reported to be the initiating nucleotide in *E. coli* (8), and our initial data suggested that it may also be the initiating nucleotide in *B. stearothermophilus*. However, we discovered that *B. stearothermophilus* DnaG initiates primer synthesis from 3′-ATC-5′ and 3′-ATT-5′ sites, and although ATP is essential, it is therefore incorporated at the second position of all the primers. Our attempts to radioactively label the primers with [γ-^{32}P]ATP failed to produce labeled primers, which is compatible with the requirement for an ATP nucleotide at the second position rather than the first position.

DnaB-DnaG interaction network. The DnaB-DnaG interaction in *B. stearothermophilus* is stable and provides an opportunity to dissect the precise roles of residues that participate in this interaction (2, 20). Such residues may contribute directly to the binding energy or may be functionally important, transmitting allosteric effects from one protein to the other and vice versa. A common network or entirely different networks of residues may be responsible for mediating these modulatory effects. In previous papers nine mutants of DnaB have been implicated in this interaction in *E. coli* and *S. enterica* serovar Typhimurium (3, 11, 18). The equivalent mutations were studied in *B. stearothermophilus*, and only mutations in Y88, I119, and I125 were found to affect directly the stability of the DnaB-DnaG complex in a salt-dependent manner (20). I119 and I125 are also involved in mediating the stimulatory effects of DnaG on the activity of DnaB. The rest of the DnaB mutants can still bind to DnaG apparently with the same affinity as the wild-type DnaB and are all stimulated by DnaG. Do these residues play a role in the function of the complex? For example, one possibility is that these residues constitute a separate network that mediates only the effects of DnaB on the activity of DnaG and not vice versa. Alternatively, is there an overlap of residues in the networks that mediate these effects? In order to investigate this possibility, we employed a general priming assay to examine the effects of our DnaB mutants on the activity of DnaG (Fig. 3).

Residues from the N- and C-terminal domains of DnaB modulate DnaG activity. All of the DnaB mutants in this study were able to bind to and translocate along DNA, hydrolyzing ATP in the process (20), and thus their effects on DnaG are unlikely to be translocation dependent. We discovered that only residues from the N-terminal (E15 and Y88) and C-terminal (R195 and M196) domains of DnaB affect its ability to modulate the DnaG activity. I125 residues from the hinge region (I119 and L138) of DnaB did not affect the modulation of DnaG activity. The absence of a functional effect of the "hinge mutants" on the activity of DnaG supports the notion that the hinge region plays a structural role rather than a functional role in the helicase-primase complex (20). A summary combining data from this work and from our previous work (20) is shown in Fig. 4. The data indicate that in the *B. stearothermophilus* DnaB-DnaG complex the activities of the two proteins are modulated by distinct but overlapping networks of residues.

The interaction interface appears to be extensive, with residues on the surfaces of both the N- and C-terminal domains of DnaB playing functional roles (Fig. 4). This is consistent with the recent model for the architecture of the complex based upon the structural homology of the N-terminal and DnaB-interacting domains of DnaB and DnaG, respectively (16, 19). A network of spatially conserved residues on the surfaces of the structurally homologous N-terminal domain of DnaB and the P16 domain of DnaG may form the helicase-primase interaction interface (16). Three of the residues that we studied are conserved in the *B. stearothermophilus* DnaB protein (E15, Y88, and I119) and in the *E. coli* DnaB protein (E33, Y104, and I135). They are situated on the same surface in the nuclear magnetic resonance structure of the DnaB N-terminal domain (22). In fact, E15 is also conserved in the P16 domain of the primase. It is tempting to speculate that this side faces the bound P16 primase domain in the complex, and if this is the case, then the equivalent surface of the P16 primase domain is the surface interacting with the helicase, thus mediating the functional effects between the two proteins in the complex. This region is in the basic surface close to the C-terminal hairpin (subdomain C2) of P16 (19). The surface distant from the C2 subdomain is strongly acidic, giving the distinct bipolar nature of P16. The surface of the N-terminal domain of DnaB is not bipolar but is predominantly acidic. The bipolar nature of P16 makes it an ideal spacer molecule "sandwiched" between the two domains of adjacent helicase molecules in the ring. Therefore, the position of the putative interacting network is consistent with the previous suggestion that P16 acts as a spacer between the N- and C-terminal domains of adjacent

DnaB molecules in the threefold symmetric form of the DnaB hexamer (19).

We demonstrated the primase activity of *B. stearothermophilus* DnaG and identified the initiating priming sites. The length of primers is modulated by the DnaB helicase, and the functional effects of DnaB on DnaG are mediated by a network of residues that is distinct from but somewhat overlapping with the network mediating the DnaG-to-DnaB effects. Therefore, the helicase is not merely a landing pad for the primase and can actively modulate priming activity.

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