

Published in final edited form as:

Structure. 2005 June ; 13(6): 839–844. doi:10.1016/j.str.2005.04.006.

## The Bacterial Helicase-Primase Interaction: A Common Structural/Functional Module

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### Abstract

The lack of a high-resolution structure for the bacterial helicase-primase complex and the fragmented structural information for the individual proteins have been hindering our detailed understanding of this crucial binary protein interaction. Two new structures for the helicase-interacting domain of the bacterial primases from *Escherichia coli* and *Bacillus stearothermophilus* have recently been solved and both revealed a unique and surprising structural similarity to the amino-terminal domain of the helicase itself. In this minireview, the current data are discussed and important new structural and functional aspects of the helicase-primase interaction are highlighted. An attractive structural model with direct biological significance for the function of this complex and also for the development of new antibacterial compounds is examined.

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The interaction between the bacterial replicative ring helicase (DnaB) and the primase (DnaG) is instrumental during the priming and elongation stages of DNA replication. Loading of DnaB at the bacterial replication origin (*oriC*) and subsequent recruitment of DnaG via a transient interaction with DnaB signify the end of the initiation (priming) and onset of the elongation stages of DNA replication. This interaction is needed repeatedly to regulate the cyclic synthesis of Okazaki fragments during lagging strand synthesis (Tougu and Marians, 1996a). In *Escherichia coli*, DnaG acts distributively by dissociating and reassociating to synthesize each primer for Okazaki fragment synthesis (Tougu et al., 1994). Alternatively, DnaG may remain bound to the newly synthesized primer and the single-strand binding protein (SSB) and subsequently is competed off and released from the DNA by the  $\chi$  subunit of the DNA polymerase holoenzyme III (pol. III; Yuzhakov et al., 1999). In contrast to the weak and transient interaction in *E. coli*, the *Bacillus stearothermophilus* DnaB-DnaG complex is stable, implying that the two proteins remain permanently associated and that DnaG does not leave the replisome during lagging strand synthesis (Bird et al., 2000).

Both proteins in the complex modulate each other's activities. DnaB affects the initiation specificity, stimulates primer synthesis, and reduces the size of the primers synthesized by DnaG (Lu et al., 1996; Johnson et al., 2000; Bhattacharyya and Griep, 2000; Mitkova et al., 2003), while DnaG stimulates the ATPase and helicase activities of DnaB (Bird et al., 2000). The structural details of this interaction have been somewhat limited. A 16 kDa carboxy-terminal domain (P16) of DnaG mediates structurally and functionally the interaction with DnaB (Tougu et al., 1994; Bird et al., 2000). DnaG interaction sites have been reported to reside on the surfaces of the amino-terminal (Chang and Marians, 2000) and carboxy-terminal (Lu et al., 1996) domains of *E. coli* DnaB, as well as the linker region that connects the two domains in the *Salmonella typhimurium* and *B. stearothermophilus*

DnaB proteins (Maurer and Wong, 1988; Stordal and Maurer, 1996; Thirlway et al., 2004). The lack of structural information for the DnaB-DnaG complex and the incomplete structural information for the individual proteins are hindering our efforts to understand the molecular details that underpin this essential interaction. Although there are no high-resolution structures available for both intact proteins, crystal structures have been reported for the amino-terminal domains of *E. coli* DnaB (Fass et al., 1999; Figure 1A) and *B. stearothermophilus* DnaG (Pan and Wigley, 2000; Figure 1B), as well as the central polymerization domain of *E. coli* DnaG (Keck et al., 2000; Podobnik et al., 2000; Figure 1B). The solution structure of the amino-terminal domain of *E. coli* DnaB is also available (Weigelt et al., 1999; Figure 1A). Crucially, the structure of the carboxy-terminal DnaB-interacting domain (P16) of DnaG has been a mystery until now that crystal and NMR structures have been reported for the *E. coli* and *B. stearothermophilus* P16 domains, respectively (Oakley et al., 2005; Syson et al., 2005).

### **P16 Is a Structural Homolog of the N-Terminal Domain of DnaB**

Both P16 structures revealed two subdomains, a smaller carboxy-terminal helix hairpin and a larger amino-terminal helical bundle that is structurally homologous to the unique amino-terminal domain of DnaB (Figure 2A). *E. coli* P16 crystallized as a dimer with two different conformers differing in the state of a long helix ( $\alpha 5$ ) that connects the two subdomains. NMR spectroscopy studies revealed that the kink near M542 in conformer II is a crystallization artifact and in solution P16 is mainly a monomer with a regular  $\alpha 5$  helix except for residues 522–527, as observed for conformer I (Oakley et al., 2005). Therefore, the dimerization of *E. coli* P16 observed in the crystal structure is likely to have no biological significance. The monomeric solution structure of *B. stearothermophilus* P16 also supports this notion. However, whether other bacterial primases are monomeric or dimeric and whether dimerization is functionally relevant still remain to be established.

*Thermus aquaticus* DnaG is unusual in that it is a stable dimer in solution. Biophysical analysis shows that the helicase-interacting domain (HID) mediates this dimerization. The crystal structure of the HID has revealed the dimerization interface is distinctly different from that seen in the *E. coli* structure. It will be of interest to see if comparison of this *Thermus aquaticus* HID structure with the structures discussed in this review give any further insight into the helicase-primase interaction (personal communication Scott Bailey and Thomas A. Steitz, Yale University).

### **The Two Subdomains of P16 Have Distinct Functions**

P16 is sufficient to elicit the full stimulatory effects on the activity of DnaB (Tougu et al., 1994; Bird et al., 2000) and mutagenesis studies have localized the DnaB interaction epitope of DnaG at the extreme carboxyl terminus (Tougu and Mariani, 1996a, 1996b) in what we now know is a helix hairpin. This module interacts with DnaB when detached from the amino-terminal subdomain but this interaction is nonfunctional, as it does not elicit the characteristic stimulatory effect on the ATPase activity of DnaB (Syson et al., 2005). The larger amino-terminal helical bundle, which is structurally homologous to the amino-terminal domain of DnaB, appears to be essential for a functional interaction and the stimulation of DnaB activity. Therefore, the two subdomains of P16 have distinct functions. The helix hairpin at the carboxyl terminus mediates the interaction of DnaG with DnaB structurally, while the rest of P16 mediates the functional effects on the activity of DnaB.

### **A Model for the DnaB-DnaG Complex and the Activation of DnaB**

Based upon the structural homology between the amino-terminal subdomain of P16 and the amino-terminal domain of DnaB, an attractive model has been proposed to explain how

DnaG interacts and activates DnaB (Syson et al., 2005; Figure 2B). Albeit speculative, the model is compatible with current structural and biochemical data and as we shall see below, it provides a testable framework for further biochemical studies to verify (or not) its validity. The ring DnaB helicase adopts 6-fold and 3-fold symmetric conformations referred to as  $C_6$  and  $C_3$  (Yu et al., 1996; Patel and Picha, 2000). The biological significance of these conformations remains unclear but electron microscopy studies revealed that  $C_3$  (considered to be a trimer of dimers) is defined by a characteristic interaction of the amino-terminal domain of one monomer with the carboxy-terminal domain of the neighboring monomer within a dimeric unit. This interaction is repeated three times (once for each dimeric unit) around the  $C_3$  ring (Yang et al., 2002; Figure 2C). The importance of the amino-terminal domain in modulating the  $C_6$  to  $C_3$  ring transition has also been highlighted by its solution and crystal structures (Weigelt et al., 1999; Fass et al., 1999) and by biochemical evidence (Biswas et al., 1994). The structural similarity between the amino-terminal subdomain of P16 and the amino-terminal domain of DnaB suggests that the former may be the functional equivalent of the latter in the DnaB-DnaG complex. The carboxy-terminal two-helix hairpin of DnaG could interact with the linker that joins the two domains of DnaB, and the amino-terminal subdomain of P16 could displace the structurally homologous amino-terminal domain of DnaB while at the same time maintaining the interactions that preserve the  $C_3$  ring conformation of the DnaB ring. The structural flexibility between the two subdomains of P16 is compatible with this model. In the *B. stearothermophilus* P16 the two subdomains have limited interaction, suggesting that their connection is highly mobile, allowing independent motions between the two subdomains (Syson et al., 2005). Similarly in the *E. coli* P16, the long  $\alpha 5$  helix that connects the carboxy-terminal hairpin to the rest of P16 in conformer I is flexible and could again allow independent mobility of the two subdomains (Oakley et al., 2005). Although conformer II is not the major conformer in solution and has been attributed to crystal packing forces (Oakley et al., 2005), a tantalizing question is whether it could have a biological relevance. For example, could conformer II correspond to the P16 conformation once bound to DnaB? Even more intriguingly, could the differences in the connectivity of the carboxy-terminal hairpin to the rest of P16 in the *E. coli* and *B. stearothermophilus* proteins explain the fact that the former forms a weak and transient complex whereas the latter forms a stable complex with DnaB? These are speculative suggestions that could be answered by hybrid P16 proteins where the amino-terminal helical bundle of the *E. coli* P16 could be fused to the hairpin of the *B. stearothermophilus* P16 and vice versa. Obviously, the high-resolution crystal structure of the DnaB-DnaG (or P16) complex will also provide key information to interrogate this model.

The proposed model predicts the presence of a spatially conserved and functionally equivalent network of surface residues on the structurally homologous amino-terminal domain of DnaB and amino-terminal subdomain of P16. Such a network of residues on the surface of DnaB will be crucial for the integrity of the  $C_3$  ring conformation, while on the surface of DnaG it will also be involved in mediating both the integrity of the  $C_3$  ring conformation and the stimulatory effects on the ATPase activity of DnaB in the complex. This model could potentially explain the observation that in *B. stearothermophilus*, binding of DnaG to DnaB induces exclusively the  $C_3$  ring conformation and is also consistent with the DnaB<sub>6</sub>-DnaG<sub>3</sub> complex observed in *E. coli* and *B. stearothermophilus* (Mitkova et al., 2003; Bird et al., 2000), as well as the DnaB<sub>6</sub>-DnaG<sub>2</sub> and DnaB<sub>6</sub>-DnaG<sub>1</sub> complexes observed in *B. stearothermophilus* (Thirlway et al., 2004). The “freezing” of DnaB in the  $C_3$  ring conformation is not a unique feature of DnaG binding, as the same has been observed when the helicase loader DnaC binds to DnaB (Barcena et al., 2001).

## A Network of Spatially Conserved Surface Residues Could Be the Key to the Molecular Details of the DnaB-DnaG Interaction

The poor primary sequence conservation between P16 and the amino-terminal domain of DnaB makes it almost impossible to identify a network of spatially conserved surface residues simply by an amino acid sequence comparison. However, with the *E. coli* and *B. stearothermophilus* P16 structures now available, structural superpositions of the atomic coordinates between the two P16 structures and the amino-terminal domain of *E. coli* DnaB can be carried out. These comparisons identified a strikingly conserved network of surface residues that could potentially decipher the molecular details that underpin the DnaB-DnaG interaction. Structural comparisons between *E. coli* and *B. stearothermophilus* P16 proteins with the amino-terminal domain of DnaB, followed by an amino acid sequence comparison between *E. coli* and *B. stearothermophilus* P16 proteins, are shown in Figure 3A. Spatially conserved residues were identified by these comparisons and these are shown in Figure 3B. These residues form a strikingly conserved network on the surfaces of the P16 proteins and the amino-terminal domain of *E. coli* DnaB and could form the interaction hot spot of the DnaB-DnaG association. Remarkably, they are reasonably well conserved in DnaB proteins from 14 bacterial species (Figure 3C). Two of these amino acids (E33 and D82 in *E. coli*) are part of a tetrad of residues that were found to be completely conserved in the DnaB amino-terminal domains from different organisms (Weigelt et al., 1999). Interestingly, two other residues (E31 and Y104) that have been reported to mediate subtle effects upon the *E. coli* DnaB-DnaG interaction (Chang and Marians, 2000) reside very close to residues (A32, E33 and E107, L108) involved in the putative interaction network (Figures 3B and 3C). The equivalent tyrosine (Y88) in *B. stearothermophilus* DnaB was also reported to directly affect the DnaB-DnaG interaction when mutated to an alanine (Thirlway et al., 2004). All the residues of the key network of contacts proposed here should be prime targets for mutagenesis studies to examine their contributions to the C<sub>6</sub> to C<sub>3</sub> (and vice versa) ring transitions, the DnaG to DnaB and DnaB to DnaG modulatory effects. These observations raise a number of important questions: (1) Are the residues shown in Figure 3 essential for the C<sub>3</sub> ring conformation and do they affect the ATPase and helicase activities of DnaB? (2) Which of these residues participate directly in the functional modulation of the DnaB activity by DnaG? (3) Which of these residues participate in the functional modulation of the DnaG activity by DnaB? (4) Are the P16 domain of DnaG and amino-terminal domain of DnaB functionally interchangeable? (5) Can we develop small molecules that interfere with this network to abolish the interaction?

## Evolution of the Bacterial Primase-Helicase Systems

Although the bacterial primase and helicase activities reside on separate polypeptides, some bacteriophages like T7, T3, and P4 possess a single polypeptide with both primase and helicase activities in separate amino- and carboxy-terminal domains, respectively (Frick and Richardson, 2001; Patel and Picha, 2000). Phylogenetic analysis of the primases and associated helicases has indicated a common origin for all one-component primase-helicase systems (Ilyina et al., 1992). Such systems may have arisen either by fusion of separate ancestral primase and helicase genes or by initial duplication of an ancestral gene encoding a bifunctional primase-helicase gene followed by divergence with one gene retaining the primase and the other the helicase activity. The striking structural homology between the carboxy-terminal domain of DnaG and the amino-terminal domain of DnaB supports a slightly different scenario whereby the gene duplication applies only to the linker region connecting the primase and helicase activities in the ancestral bifunctional gene, followed by gene separation leaving one copy of the linker region at the carboxyl terminus of the primase and another at the amino terminus of the helicase. Subsequent divergence of the linker region resulted in two structurally/functionally homologous domains on separate DnaG and

DnaB polypeptides (Figure 4). Early on in evolution, some bacteriophages like T7 and T3 would have assimilated the ancestral bifunctional bacterial gene in their genomes, whereas later on in evolution other bacteriophages like T4 would have assimilated the separate activities.

The separation of the two activities has also resulted in architectural differences between the binary DnaB-DnaG and the bifunctional one-component complexes. The latter has an obvious 1:1 stoichiometry for the two linked activities whereas the former has mainly a DnaB<sub>6</sub>-DnaG<sub>3</sub> stoichiometry (Bird et al., 2000; Mitkova et al., 2003), with a minority of stoichiometrically different complexes (DnaB<sub>6</sub>-DnaG<sub>2</sub> and DnaB<sub>6</sub>-DnaG<sub>1</sub>) also present in the *B. stearothermophilus* system (Thirlway et al., 2004). Separation of the helicase domain of the bifunctional T7 gp4 protein resulted in a helicase that crystallized as a ring hexamer (Sawaya et al., 1999; Singleton et al., 2000), whereas the full-length bifunctional T7 gp4 protein crystallized as a heptamer despite the presence of a mixed population of hexamers and heptamers in solution (Toth et al., 2003). The biological significance of these mixed oligomers in both the one-component bacteriophage and two-component bacterial primase-helicase systems is not clear at present. They may (or not) all be functionally competent but utilized for different functions during DNA replication. More juxtaposed primases relative to the associated helicase may simply increase, while fewer primases may decrease, the rate of primer synthesis if required. Indeed, the full-length T7 gp4 protein exhibits better primer synthesis activity than the isolated primase fragment (Frick and Richardson, 1999). A slower rate of primer synthesis may be required during primosomal assembly and initiation either at *oriC* or in the restart replisome, relative to normal elongation.

## The Helicase-Primase Interaction: A Target for Antibiotic Development

DNA replication is the most basic of functions in all biology and should be a prime target for antibiotic development. It is the target of the bactericidal fluoroquinolone class of antibiotics that interferes with the DNA gyrase and topoisomerase IV activities, but there are no other marketed drugs targeting other components of the replication machinery. Novel inhibitors have been reported for the PolC of Gram-positive bacteria (Daly et al., 2000) and for the binary interaction between a prototypic pair ORF104 and DnaI (the putative helicase loader in Gram-positive bacteria) in *Staphylococcus aureus* (Liu et al., 2004).

Both the DnaB and DnaG proteins, as well as their binary interaction, are essential for bacterial survival. Inhibition of either activity (or the formation of the DnaB-DnaG complex) will be detrimental to bacterial survival and thus these proteins should be legitimate targets for antibiotic development. Specific nucleotide analogs and also small molecules that target the primase activity or primase-helicase interaction have been reported (Moore et al., 2002; Hegde et al., 2004; Zhang et al., 2002). Our increasingly better understanding of protein-protein interfaces and the existence of interaction “hot spots” (Halperin et al., 2004) render protein complexes feasible targets for the development of novel antagonistic peptidomimetics and small-molecule inhibitors (Cochran, 2000, 2001; Zhao and Chmielewski, 2005). The new P16 structures and also the potential identification of a spatially conserved interaction network of residues on the surfaces of P16 and the amino-terminal domain of DnaB provide us with new leads for the development of antagonist small molecules that could interfere with this network, thus abolishing the essential primase-helicase interaction. Experimental screening and structure-based virtual screening approaches will benefit from the recent determination of the new P16 structures.

## Epilogue

The new P16 structures and their structural homology to the amino terminus of DnaB suggest a common structural/functional module. In the absence of the primase the amino-

terminal domain of the helicase is the active module, whereas in the presence of the primase this role is taken up by the carboxy-terminal domain of the primase. New lines of investigation are now obvious to test this notion and could also refocus rational drug development approaches that target this ubiquitous and essential bacterial interaction.

## Acknowledgments

I thank Max Paoli for his valuable comments on the manuscript. Ed Egelman has kindly provided the C3 DnaB model shown in Figure 2. This work was supported by the BBSRC (BB/C500579/1). I thank Drs. Scott Bailey and Thomas A. Steitz for communicating their findings on the *Thermus aquaticus* primase prior to publication.

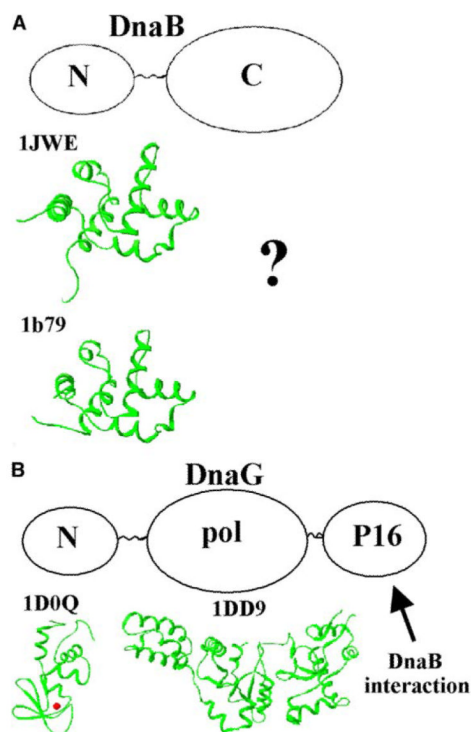
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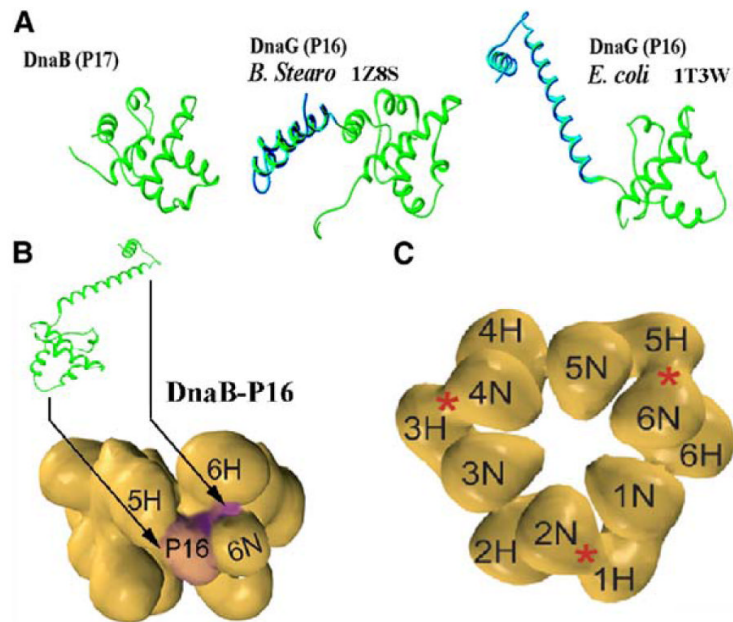


**Figure 1.**

Structural Information for the Bacterial Helicase and Primase Proteins

(A) A schematic representation of the two-domain structure of the DnaB helicase and the solution (Protein Data Bank code 1JWE) and crystal (PDB code 1b79) structures of the amino-terminal domain of *E. coli* DnaB.

(B) A schematic representation of the domain structure of the DnaG primase and the crystal structures of the amino-terminal (PDB code 1D0Q) and central polymerization (PDB code 1DD9) domains of the *B. stearothermophilus* and *E. coli* DnaG proteins, respectively. The Zn atom in the amino-terminal domain is indicated by a red dot. Structures for the DnaB-interacting carboxy-terminal P16 domain from the *E. coli* and *B. stearothermophilus* DnaG proteins have been solved recently (see Figure 2) and are the subject of this minireview.



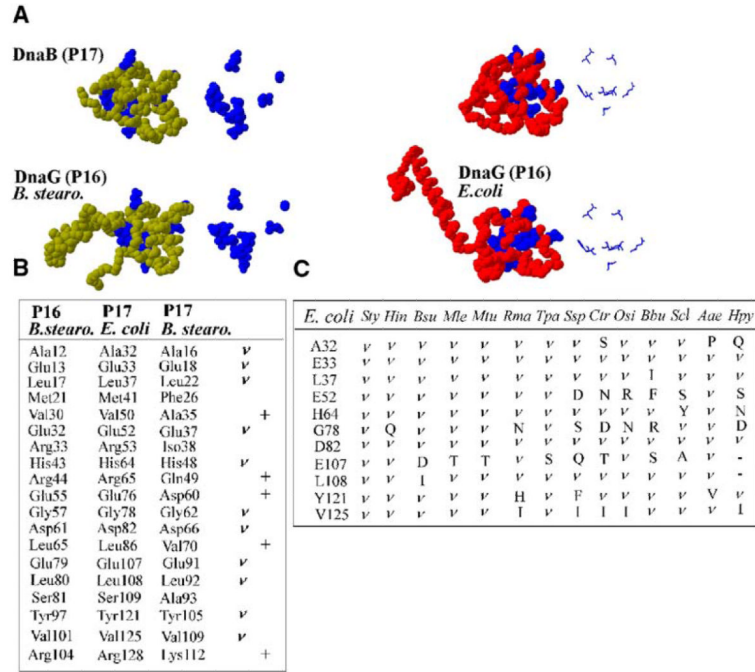
**Figure 2.**

A Model for the Architecture of the Bacterial Helicase-Primase Complex

(A) The P16 structures of the *B. stearo* (*B. Stearo* PDB code 1Z8S) and *E. coli* (PDB code 1T3W) DnaG proteins consist of two subdomains: a carboxy-terminal hairpin (cyan) and an amino-terminal helical bundle (green). The latter is structurally similar to the amino-terminal domain (P17) of the *E. coli* DnaB.

(B) A speculative model for the interaction of P16 with the C<sub>3</sub> ring of the DnaB helicase. P16 binds to the linker region that joins the two domains of DnaB via its carboxy-terminal hairpin and the amino-terminal subdomain of P16 displaces the structurally/functionally similar P17 domain of DnaB, thus maintaining the C<sub>3</sub> ring conformation.

(C) A view of the C<sub>3</sub> DnaB ring along the 3-fold symmetry axis. The ring adopts a trimer of dimers conformation with three amino-terminal domains (2N, 4N, and 6N) making contacts with neighboring carboxy-terminal domains (1H, 3H, and 5H) as indicated by red asterisks (Yang et al., 2002).



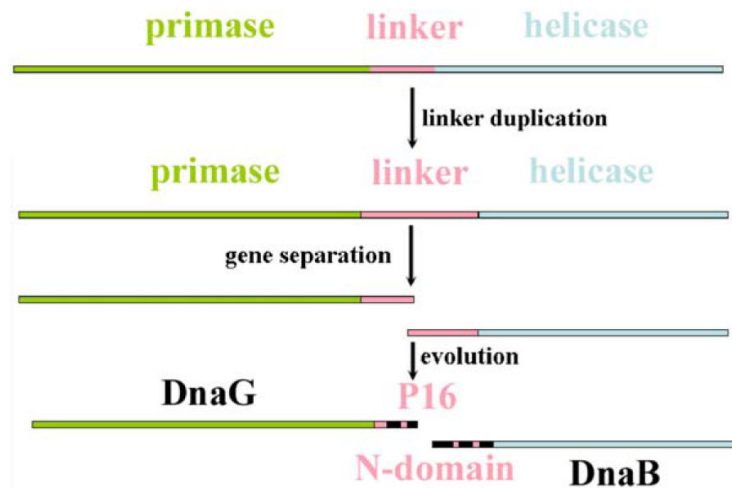
**Figure 3.**

The Putative Helicase-Primase Interaction Network

(A) The locations of spatially conserved residues (shown in blue) on the surfaces of the *E. coli* P17 domain (top left and right), the amino-terminal subdomain of the *B. stearothermophilus* P16 (green), and the equivalent subdomain of the *E. coli* P16 (red).

(B) The network of surface residues that could potentially participate in the DnaB-DnaG interaction. Identical residues in all three proteins (shown in blue in [A]) are indicated by ✓ and similar residues by +.

(C) The identical residues (✓) from (B) are reasonably well conserved in the amino-terminal domains of many bacterial DnaB helicases (Sty, *S. typhimurium*; Hin, *H. influenzae*; Bsu, *B. subtilis*; Mle, *M. leprae*; Mtu, *M. tuberculosis*; Rma, *R. marinus*; Tpa, *T. pallidum*; Ssp, *Synechocystis*; Ctr, *C. trachomatis*; Osi, *O. sinensis*; Bdu, *B. burgdorferi*; Scl, *S. clavuligerus*; Aae, *A. aeolicus*; Hpy, *H. pylori*). Conservation is indicated by ✓, whereas nonconserved residues are reported.



**Figure 4.**

**Evolution of Separate Helicase and Primase Genes**

Schematic diagram showing the evolution of two separate primase and helicase activities from a single ancestral gene. The ancestral gene encoded a bifunctional protein with the primase at the amino terminus (green) and the helicase at the carboxyl terminus (blue) linked together by a linker region (pink). Duplication of the linker region followed by gene separation and divergence resulted in two separate DnaG and DnaB proteins.