The bacterial helicase (DnaB) – primase (DnaG) interaction: a common structural/functional module.

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Summary

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 *E. coli* and *B. 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 mini-review 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.

Introduction

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 signifies 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 *E. coli*, DnaG acts distributively by dissociating and re-associating 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 χ 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 *B.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 carboxyl-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 carboxyl-terminal (Lu et al., 1996) domains of *E. coli* DnaB, as well as the linker region that connects the two domains in the *S. 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; Fig. 1a) and *B. stearothermophilus* DnaG (Pan and Wigley, 2000; Fig. 1b), as well as the central polymerisation domain of *E. coli* DnaG (Keck et al., 2000; Podobnik et al., 2000; Fig. 1b). The solution structure of the amino-terminal domain of *E. coli* DnaB is also available (Weigelt et al., 1999; Fig. 1a). Crucially the structure of the carboxyl-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 homologue of the N-terminal domain of DnaB

Both P16 structures revealed two-subdomains, a smaller carboxyl-terminal helix hairpin and a larger amino-terminal helical bundle that is structurally homologous to the unique amino-terminal domain of DnaB (Fig. 2a). *E. coli* P16 crystallised as a dimer with two different conformers differing in the state of a long helix (α5) that connects the two

subdomains. NMR spectroscopy studies revealed that the kink near M542 in conformer II is a crystallisation artefact and in solution P16 is mainly a monomer with a regular α5 helix except for residues 522-527, as observed for conformer I (Oakley et al., 2005). Therefore, the dimerisation 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.

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 localised the DnaB-interaction eepitope of DnaG at the extreme carboxyl terminus (Tougu and Marians, 1996a; Tougu and Marians, 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 non-functional, 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, that 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 whilst 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; Fig. 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 six-fold and three-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 carboxyl-terminal domain of the neighbouring 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; Fig. 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 suggest that the former may be the functional equivalent of the latter in the DnaB-DnaG complex. The carboxyl-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 α5 helix that connects the carboxyl-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 tantalising 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 carboxyl-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 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 $DnaB_6$-$DnaG_3$ complex observed in *E. coli* and *B. stearothermophilus* (Mitkova et al., 2003; Bird et al., 2000), as well as the $DnaB_6$-$DnaG_2$ and $DnaB_6$-$DnaG_1$ 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 only 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 Fig. 3a. Spatially conserved residues were identified by these comparisons and these are shown in Fig. 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 fourteen bacterial species (Fig. 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 (Fig. 3b, c). The equivalent tyrosine (Y88) in *B. stearothermophilus* DnaB was also reported to affect directly 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_6 to C_3 (and vice versa) ring transitions, the DnaG to DnaB and DnaB to DnaG modulatory effects. These observations raise a number of important questions:

(i) Are the residues shown in Fig.3 essential for the C_3 ring conformation and do they affect the ATPase and helicase activities of DnaB? (ii) Which of these residues participate directly in the functional modulation of the DnaB activity by DnaG? (iii) Which of these residues participate in the functional modulation of the DnaG activity by DnaB? (iv) Are the P16 domain of DnaG and amino-terminal domain of DnaB functionally interchangeable? (v) 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 carboxyl-terminal domains, respectively (Frick and Richardson, 2001; Patel and Pitcha, 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 bi-functional primase-helicase gene followed by divergence with one gene retaining the primase and the other the helicase activity. The striking structural homology between the carboxyl-terminal domain of DnaG and the amino-terminal domain of DnaB support a slightly different scenario whereby the gene duplication applies only to the linker region connecting the primase and helicase activities in the ancestral bi-functional 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 (Fig. 4). Early on in evolution some bacteriophages like T7 and T3 would have assimilated the ancestral bi-functional 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 bi-functional one component complexes. The latter has an obvious 1:1 stoichiometry for the two linked activities whereas the former has mainly a $DnaB_6$-$DnaG_3$ stoichiometry (Bird et al., 2000; Mitkova et al., 2003), with a minority of stoichiometrically different complexes ($DnaB_6$-$DnaG_2$ and $DnaB_6$-$DnaG_1$) also present in the *B. stearothermophilus* system (Thirlway et al., 2004). Separation of the helicase domain of the bi-functional T7 gp4 protein resulted in a helicase that crystallised as a ring hexamer (Sawaya et al., 1999; Singleton et al., 2000) whereas the full length bi-functional T7 gp4 protein crystallised 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 utilised for different functions during DNA replication. More juxtaposed primases relative to the associated helicase may simply increase, whilst less 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 interfere 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 *S. 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 analogues and also small molecules that target the primase activity or primase-helicase interaction have been reported (Moore et al., 2002; Hedge 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, 2001; Cochran, 2000; 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-terminal 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 carboxyl-terminal domain of the primase. New lines of investigation are now obvious to test this notion and could also re-focus rational drug development approaches that target this ubiquitous and essential bacterial interaction.

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Figures

Figure 1

a. A schematic representation of the two domain structure of the DnaB helicase and the solution (pdb: 1JWE) and crystal (pdb: 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: 1D0Q) and central polymerisation (pdb: 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 carboxyl-terminal P16 domain from the E. coli and B. stearothermophilus DnaG proteins have been solved recently (see Fig. 2) and are the subject of this minireview.

Figure 2

a. The P16 structures of the *B. stearothermophilus* and *E. coli* (pdb: 1T3W) DnaG proteins consist of two subdomains: a carboxyl-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_3 ring of the DnaB helicase. P16 binds to the linker region that joins the two domains of DnaB via its carboxyl-terminal hairpin and the amino-terminal subdomain of P16 displaces the structurally/functionally similar P17 domain of DnaB thus maintaining the C_3 ring conformation.

c. A view of the C_3 DnaB ring along the three-fold symmetry axis. The ring adopts a trimer of dimers conformation with three amino-terminal domains (2N, 4N and 6N) making contacts with neighbouring carboxyl-terminal domains (1H, 3H and 5H) as indicated by red asterisks (Yang at el., 2002).

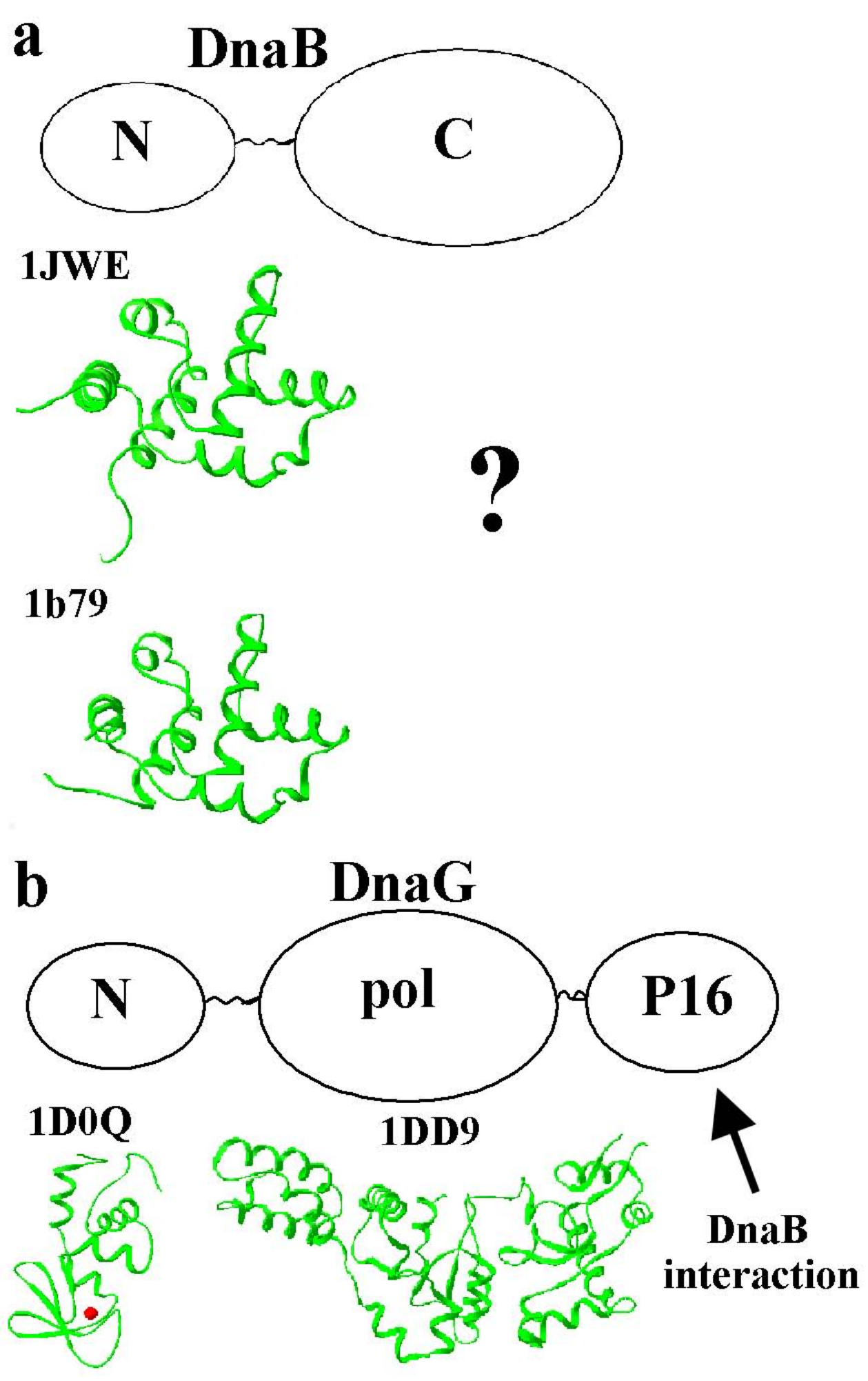
Figure 3

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 panel **a**) are indicated by ν and similar residues by +.
b. The identical residues (ν) from panel **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 ν whereas non-conserved residues are reported.

Figure 4

Schematic diagram showing the evolution of two separate primase and helicase activities from a single ancestral gene. The ancestral gene encoded a bi-functional 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.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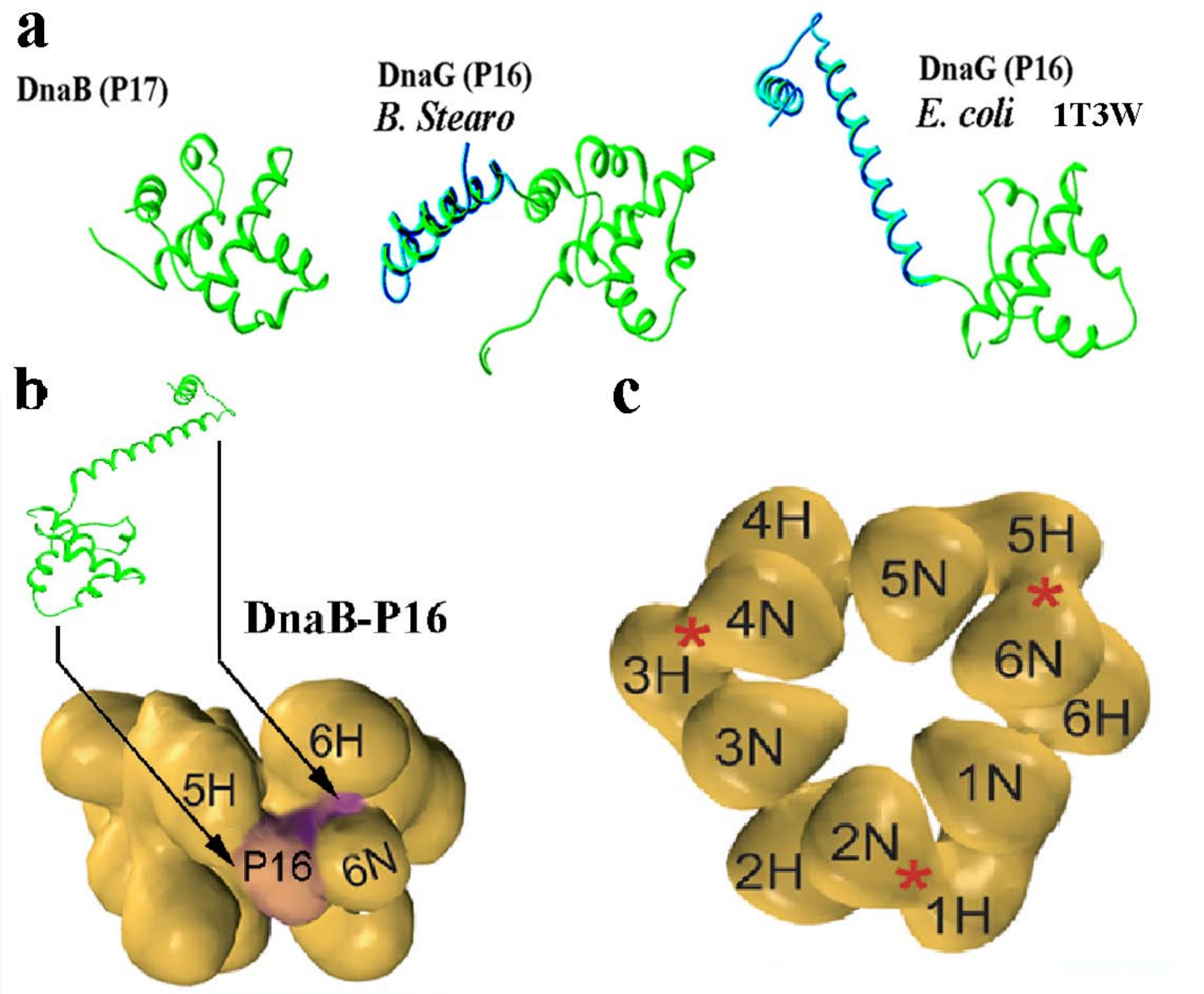
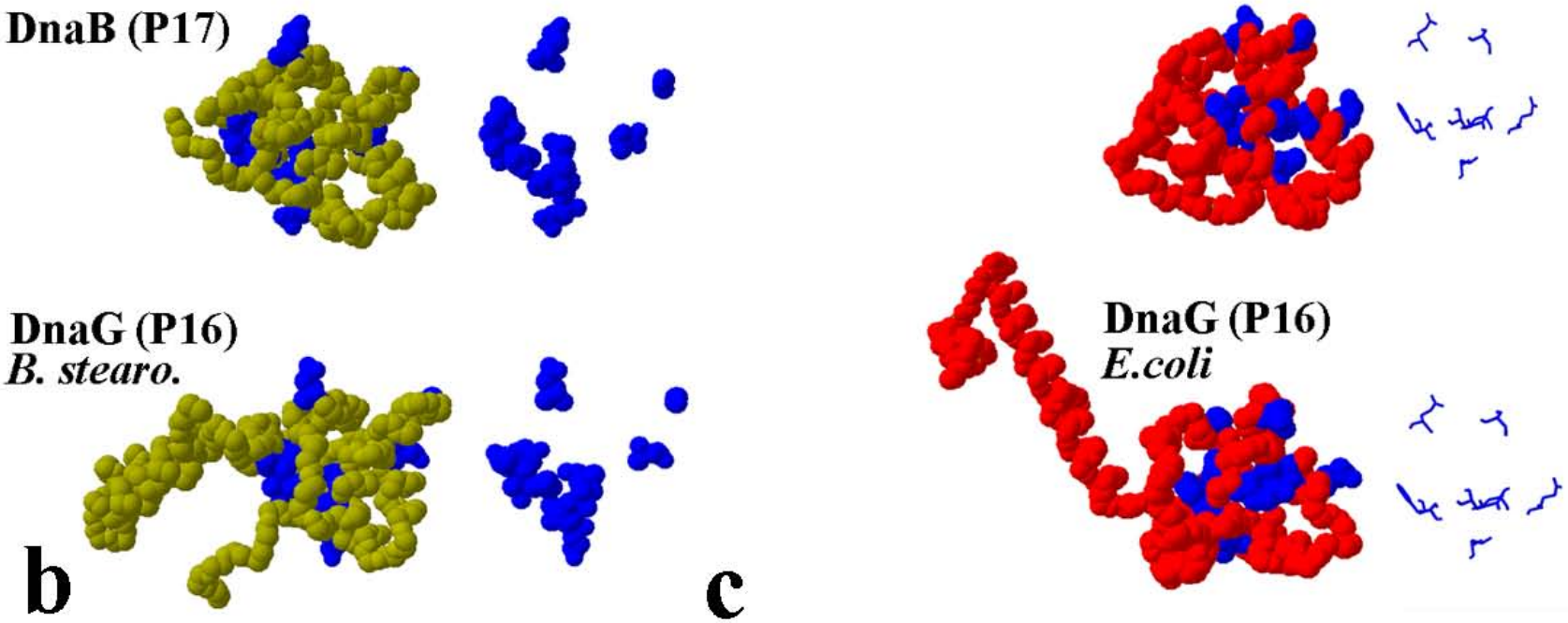
N
pol
P16
1D0Q
1DD9
DnaB
interaction


a
DnaB (P17)
DnaG (P16)
B. Stearo
DnaG (P16)
E. coli 1T3W
b
DnaB-P16
6H
5H
P16
6N
c
4H
5N
5H
4N
6N
3H
6H
3N
1N
2N
2H
1H

a

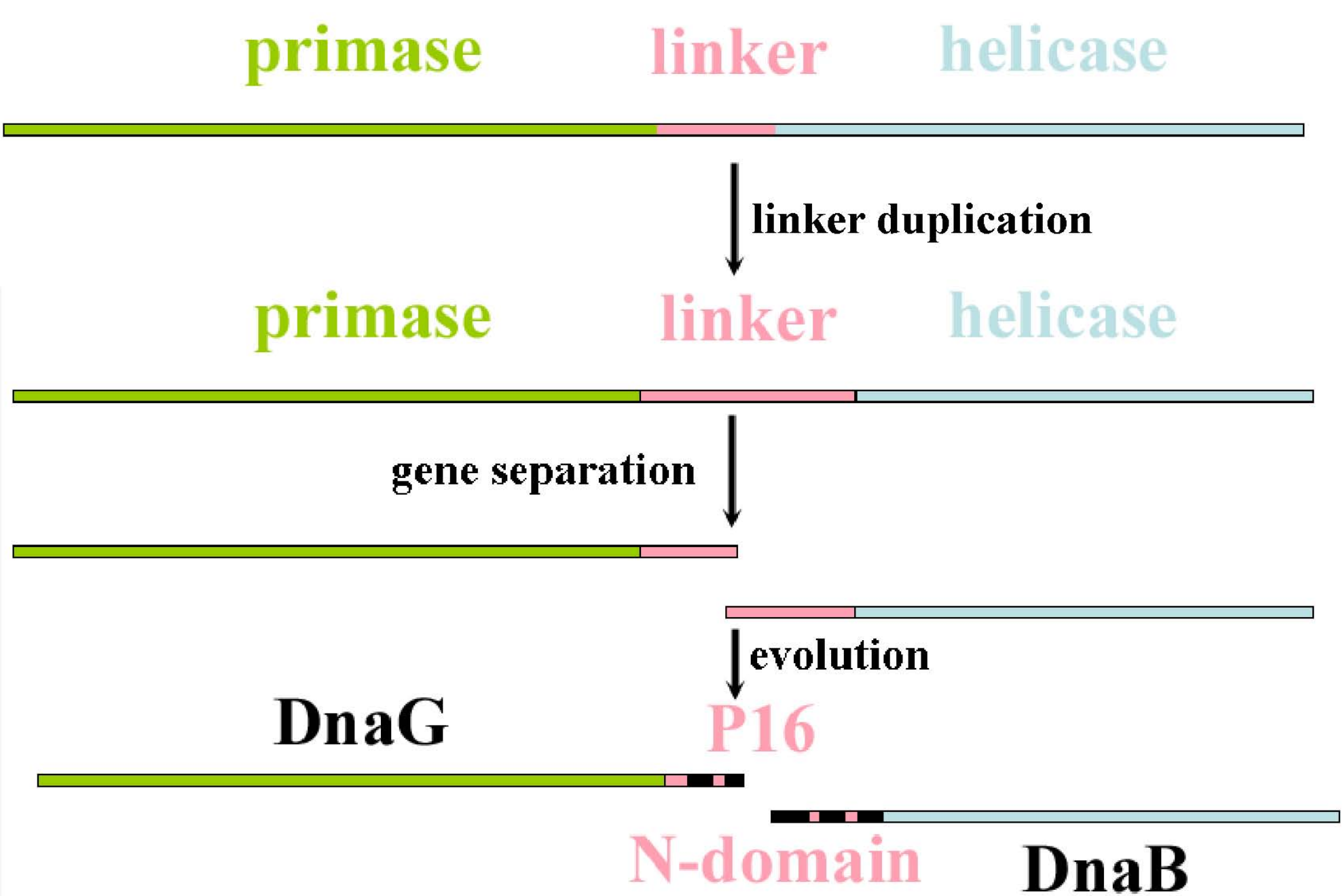


b

P16 B.stearo.	P17 E. coli	P17 B. stearo.		
Ala12	Ala32	Ala16	ν	
Glu13	Glu33	Glu18	ν	
Leu17	Leu37	Leu22	ν	
Met21	Met41	Phe26		
Val30	Val50	Ala35		+
Glu32	Glu52	Glu37	ν	
Arg33	Arg53	Iso38		
His43	His64	His48	ν	
Arg44	Arg65	Gln49		+
Glu55	Glu76	Asp60		+
Gly57	Gly78	Gly62	ν	
Asp61	Asp82	Asp66	ν	
Leu65	Leu86	Val70		+
Glu79	Glu107	Glu91	ν	
Leu80	Leu108	Leu92	ν	
Ser81	Ser109	Ala93		
Tyr97	Tyr121	Tyr105	ν	
Val101	Val125	Val109	ν	
Arg104	Arg128	Lys112		+

c

E. coli	*Sty*	*Hin*	*Bsu*	*Mle*	*Mtu*	*Rma*	*Tpa*	*Ssp*	*Ctr*	*Osi*	*Bbu*	*Scl*	*Aae*	*Hpy*
A32	ν	ν	ν	ν	ν	ν	ν	ν	S	ν	ν	ν	P	Q
E33	ν	ν	ν	ν	ν	ν	ν	ν	ν	ν	ν	ν	ν	ν
L37	ν	ν	ν	ν	ν	ν	ν	ν	ν	ν	I	ν	ν	ν
E52	ν	ν	ν	ν	ν	ν	ν	D	N	R	F	S	ν	S
H64	ν	ν	ν	ν	ν	ν	ν	ν	ν	ν	ν	Y	ν	N
G78	ν	Q	ν	ν	ν	N	ν	S	D	N	R	ν	ν	D
D82	ν	ν	ν	ν	ν	ν	ν	ν	ν	ν	ν	ν	ν	ν
E107	ν	ν	D	T	T	ν	S	Q	T	ν	S	A	ν	-
L108	ν	ν	I	ν	ν	ν	ν	ν	ν	ν	ν	ν	ν	-
Y121	ν	ν	ν	ν	ν	H	ν	F	ν	ν	ν	ν	V	ν
V125	ν	ν	ν	ν	ν	I	ν	I	I	I	ν	ν	ν	I


primase
linker
helicase
linker duplication
primase
linker
helicase
gene separation
evolution
DnaG
P16
N-domain
DnaB