

Breaking the rules: bacteria that use several DNA polymerase IIIs

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Studies using *Escherichia coli* DNA polymerase (Pol) III as the prototype for bacterial DNA replication have suggested that—in contrast to eukaryotes—one replicase performs all of the main functions at the replication fork. However, recent studies have revealed that replication in other bacteria requires two forms of Pol III, one of which seems to extend RNA primers by only a few nucleotides before transferring the product to the other polymerase—an arrangement analogous to that in eukaryotes. Yet another group of bacteria encode a second Pol III (ImuC), which apparently replaces a Pol Y-type polymerase (Pol V) that is required for induced mutagenesis in *E. coli*. A complete understanding of complex bacterial replicases will allow the simultaneous biochemical screening of all their components and, thus, the identification of new antibacterial compounds.

Keywords: *Bacillus subtilis*; DNA replication; DNA polymerase III; error-prone polymerase; *Pseudomonas aeruginosa*
EMBO reports (2011) 12, 408–414. doi:10.1038/embor.2011.51

See Glossary for abbreviations used in this article.

Introduction

Cells encode many polymerases that have roles in processes including replication, DNA repair, error avoidance and error fixation. A subset of these polymerases is devoted to most of the chromosomal DNA replication and, together with their accessory proteins, are known as replicases. Cellular chromosomal replicases from all branches of life have three parts (Kornberg & Baker, 1992): a polymerase—Pol III in bacteria, and Pol δ and ϵ in eukaryotes, a sliding-clamp processivity factor and a clamp loader. Alone, replicative polymerases are not different from other polymerases, but in association with the sliding clamp and clamp loader, they become highly processive (Fay *et al.*, 1981).

Before DNA elongation begins, the Pol III holoenzyme forms an initiation complex through an ATP-dependent reaction (Johanson & McHenry, 1981, 1982; Wickner, 1976; Wickner & Kornberg, 1973). In cells, elongation probably occurs in a tightly coupled series of reactions in which Pol III is chaperoned onto the newly loaded sliding clamp by τ -containing clamp loaders (Downey & McHenry, 2010). Other reviews describe the details of replication by the *Escherichia coli* Pol III holoenzyme (Bloom, 2009; Johnson &

O'Donnell, 2005), which is not discussed here. Bacteria are replicated by family-C DNA polymerases (Pol III holoenzymes), whereas family-B polymerases replicate eukaryotic chromosomes (Braithwaite & Ito, 1993). Although eukaryotic and prokaryotic replicases use almost identical sliding clamps and clamp loaders, there are mechanistic differences between the two systems. In eukaryotes, the clamp loader does not seem to interact with the polymerase, whereas in *E. coli* it interacts with both Pol III and the replicative helicase, presumably maintaining them in a position in which the replicase can be readily assembled on newly synthesized primers. The *E. coli* clamp loader binds tightly to Pol III—with a K_D of 70 pM (Kim & McHenry, 1996a)—through the carboxy-terminal domain of its τ subunit. As τ is oligomeric, the leading- and lagging-strand polymerases are maintained in one coupled complex (McHenry, 2003). By contrast, Gram-positive clamp loaders bind extremely weakly to their cognate polymerases (Bruck *et al.*, 2005; Bruck & O'Donnell, 2000), suggesting a transient interaction that has greater similarity with the eukaryotic system. However, the τ subunit of Gram-positive bacteria interacts with the replicative helicase (Haroniti *et al.*, 2004)—which is similar to the case in *E. coli* (Gao & McHenry, 2001; Kim *et al.*, 1996)—and this interaction might increase the concentration of the clamp loader at the replication fork, facilitating τ -subunit interaction with Pol IIIs and coordinating their trafficking at the replication fork.

Another important distinction exists in the number and function of polymerases at the replication fork: in *E. coli*, the Pol III holoenzyme is the only replicase, whereas there are three replicases in eukaryotes. Pol ϵ is the eukaryotic leading-strand replicase, Pol δ is the lagging-strand replicase and eukaryotic Pol α is part of the priming apparatus; it elongates nascent primers with dNTPs for a short distance before transferring them to Pol δ (Dua *et al.*, 1999; Nethanel & Kaufmann, 1990; Nick McElhinny *et al.*, 2008; Pursell *et al.*, 2007). In low-GC Gram-positive bacteria, however, two forms of DNA polymerase III are required for chromosomal replication. In addition, other bacteria require a second DNA polymerase III for induced mutagenesis, replacing the error-prone Pol V that was first identified in *E. coli*.

Hence, the prototype provided by the widely studied *E. coli* is not universally followed in bacteria. Here, I critically evaluate the emerging knowledge about bacterial chromosomal replication and highlight areas that require further investigation.

Gram-positive bacteria use two forms of DNA polymerase III

Low-GC Gram-positive bacteria have two forms of Pol III: PolC and DnaE (Koonin & Bork, 1996). They are homologous, but PolC has

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Received 27 January 2011; accepted 16 March 2011; published online 8 April 2011

Glossary

dNTP	deoxyribonucleotide triphosphate
nt	nucleotide
Pol	DNA polymerase
SSB	single-stranded DNA-binding protein
UV	ultraviolet

some domains rearranged and an endogenous Mg^{2+} -dependent proofreading activity (Table 1). DnaE is more closely related to *E. coli* Pol III in sequence and domain organization. Genetic and cell physiology studies have indicated that DnaE makes a unique contribution to lagging-strand synthesis in *Bacillus subtilis* (Dervyn *et al*, 2001). On the basis of this observation, PolC was proposed to be the leading-strand polymerase and DnaE the lagging-strand polymerase (Dervyn *et al*, 2001). However, DnaE has low fidelity *in vitro* (Bruck *et al*, 2003; Le Chatelier *et al*, 2004), although its overproduction *in vivo* does not increase mutation rates (Le Chatelier *et al*, 2004). These observations argue against a replicative role for this polymerase.

We reconstituted a rolling circle replication system, using 13 purified *B. subtilis* replication proteins (Sanders *et al*, 2010) that had been predicted to be required by previous genetic or biochemical investigations (Bruand *et al*, 1995, 2001; Bruck & O'Donnell, 2000; Dervyn *et al*, 2001; Polard *et al*, 2002; Velten *et al*, 2003). This system seems to mimic accurately the reaction at the replication fork of a Gram-positive bacterium, in terms of both its correspondence with genetic requirements and the replication-fork rate *in vivo* (500 nt/s at 30°C; Wang *et al*, 2007). Leading-strand replication requires 11 proteins, including the Pol III encoded by *polC*. The second Pol III encoded by *dnaE* cannot substitute its function. In addition to these 11 proteins, lagging-strand replication requires DnaE and primase (Sanders *et al*, 2010), which is consistent with the proposed lagging-strand role for DnaE (Dervyn *et al*, 2001). However, the elongation rate of DnaE is too slow (approximately 25 nt/s) to keep up with the replication fork. By contrast, PolC supports a physiologically relevant elongation rate (approximately 500 nt/s). PolC discriminates against RNA primers; DnaE uses RNA primers efficiently (Sanders *et al*, 2010). These characteristics suggest a role for *B. subtilis* DnaE analogous to that of eukaryotic Pol α , which extends RNA primers and transfers them to a replicase.

The reinterpretation of the structure of a Gram-positive PolC might reveal the mechanism by which it discriminates against an RNA primer. Primer-template binding by PolC differs from DnaE and uses two β -strands of the Thumb domain to interact with the minor groove of the template-primer duplex (Evans *et al*, 2008). The wider diameter of the A form RNA-DNA duplex does not seem to fit well into the primer-template binding channel of PolC. Thus, an RNA-DNA duplex might not bind strongly, or the conformational changes that are coupled to primer-template binding might not occur completely, leading to improper formation of the catalytic site. These predictions should be tested experimentally.

Consistent with the role of Pol α in eukaryotes, RNA-primed single-stranded DNA is used inefficiently by PolC, whereas the addition of low levels of DnaE stimulates a higher level of synthesis than that obtained with DnaE alone (Sanders *et al*, 2010). However, in the absence of PolC, DnaE can catalyse extensive synthesis, which makes it difficult to determine the position at which the extended primer is transferred from DnaE to PolC. This issue was addressed by

Table 1 | Three classes of DNA polymerase III

Model organism	Name (or proposed name)	Former names	Function
<i>Escherichia coli</i>	α , DnaE	PolC	Extension of RNA primers Rapid, processive chromosomal replication
<i>Bacillus subtilis</i>	PolC	DnaF	Rapid, processive chromosomal replication
	DnaE		Extension of RNA primers
<i>Pseudomonas aeruginosa</i>	DnaE		Extension of RNA primers Rapid, processive chromosomal replication
	ImuC	DnaE2	Error-prone replication, induced mutagenesis

using a specific PolC inhibitor (HB-EMAU) developed by George Wright, Neal Brown and colleagues (Tarantino *et al*, 1999). This class of inhibitor probably acts as a dGTP analogue, forming a ternary complex with template-primer and PolC and trapping the enzyme in a dead-end complex (Low *et al*, 1974). When HB-EMAU was included in cooperative RNA primer extension reactions containing DnaE and PolC, synthesis was markedly inhibited. This suggests that the transfer to PolC occurs early in the reaction (Sanders *et al*, 2010), although the precise timing remains to be elucidated. Preliminary experiments suggest that the processivity of DnaE is low, but increases to more than the length of an Okazaki fragment in the presence of *B. subtilis* SSB, the sliding clamp and known components of the *B. subtilis* clamp loader (τ , δ , δ' ; J.C. Zinder & C.S. McHenry, unpublished data). This suggests that the transfer of the nascent product from DnaE to PolC is probably active, rather than the result of passive 'falling off' of DnaE and replacement by PolC (Fig 1). The transfer mechanism might be conceptually similar to that of error-prone polymerase exchange at the replication fork.

Benkovic and colleagues have observed dynamic processivity in the T4 system, in which one replicative polymerase can replace another without interrupting elongation (Yang *et al*, 2004). This polymerase replacement—discovered using a catalytically inactive mutant—requires that the invading polymerase interact with the T4 sliding-clamp protein gp45. In *E. coli*, Pol II, Pol IV and Pol V can invade the elongating Pol III holoenzyme and gain access to the primer terminus under appropriate conditions (Friedberg *et al*, 2005; Patel *et al*, 2010). A tool-belt model was initially proposed, in which multiple polymerases might bind to multiple sites in an oligomeric sliding-clamp processivity factor and switch out at the replication fork (Pages & Fuchs, 2002). However, the interactions of polymerases with sliding clamps are weak when the polymerase cannot access the primer terminus to acquire binding energy, and the off-rates are fast (Kim & McHenry, 1996b). Therefore, if the sliding clamp (β_2) is the initial contact with an exogenous polymerase (which remains to be demonstrated), other interactions and contacts must be present to drive, and perhaps regulate, the exchange process. Sutton and colleagues have provided evidence that would limit polymerase interaction to one set of adjacent sites on one half of the β_2 sliding clamp (Heltzel *et al*, 2009), further decreasing the likelihood of a reservoir of polymerases stably associated with β_2 . In addition to the known *E. coli* Pol IV- β_2 interaction, other contacts between Pol IV and a component of *E. coli* Pol III* (Pol III+ τ -containing clamp loader)

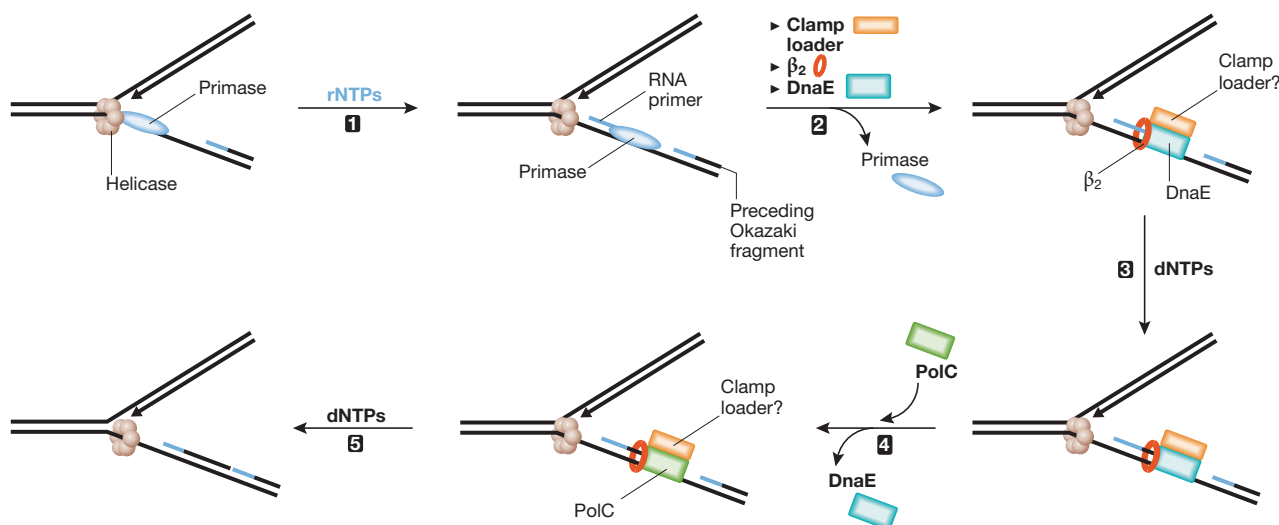


Fig 1 | Model for polymerase transfer at the replication fork of low-GC Gram-positive organisms. Primase is brought to the replication fork through interaction with the replicative helicase and (1) synthesizes a short RNA primer that is (2) transferred to DnaE with the assistance of the clamp loader and β_2 . It is unclear whether the clamp loader remains part of the resulting initiation and elongation complexes. If present, it is probably stabilized by interacting with helicase (Haroniti *et al*, 2004). (3) The RNA primer is extended by a few nucleotides by DnaE and then (4) transferred to PolC, which (5) catalyzes extensive DNA replication and completion of an Okazaki fragment. The molecular interactions that are responsible for DnaE–PolC exchange are unknown. dNTP, deoxyribonucleotide triphosphate; rNTP, ribonucleotide triphosphate.

Sidebar A | ImuC might be a therapeutic target to decrease the frequency of antibiotic resistance

In a seminal study, cystic fibrosis patients were shown to be colonized early in life with a few *Pseudomonas aeruginosa* strains that remain for the lifetime of the patient and adapt to the environment by mutation (Oliver *et al*, 2000), a factor that contributes to drug resistance and treatment failure. These strains become progressively hypermutable, often due to mutation of mismatch repair genes as the disease progresses (Ciofu *et al*, 2005; Hassett *et al*, 2010; Hogardt *et al*, 2007; Oliver *et al*, 2000). In *Escherichia coli*, induction of SOS mutagenesis has been shown to be important for the evolution of drug resistance, even in the presence of mismatch repair deficiencies (Cirz & Romesberg, 2006). In *Mycobacterium tuberculosis*, persistence and evolution of drug resistance in animal models is diminished in ImuC knockouts (Boshoff *et al*, 2003). As the development of drug resistance affects treatment outcome in patients with cystic fibrosis, the ImuC system therefore represents a target for chemotherapy (Smith & Romesberg, 2007).

trigger Pol III* release (Furukohri *et al*, 2008). Future research in this area should address the role of the clamp loader in the polymerase-exchange mechanism. It is probably involved in this process, given its role in chaperoning a polymerase onto a newly loaded β_2 (Downey & McHenry, 2010) and its possible role in escorting Pol III away from DNA during lagging-strand cycling (P.R. Dohrmann & C.S. McHenry, unpublished data).

Many bacteria can use a mutagenic Pol III

In *E. coli*, a specialized class of Pol Y polymerases produces induced mutagenesis and stress-induced adaptive modifications (Foster, 2005; McKenzie & Rosenberg, 2001; Pages & Fuchs, 2002; Tippin *et al*, 2004; Walker, 2005). From the sequencing of many bacterial genomes, it has become apparent that bacteria belonging to several phyla encode two forms of *E. coli*-like *dnaE* (not including the PolC–DnaE combi-

nations). The second of these apparently replaces Pol V, the polymerase mainly responsible for induced mutagenesis in *E. coli*. These two forms of Pol III have sometimes been designated DnaE1 and DnaE2. Two early elegant studies in *Mycobacterium tuberculosis* and *Caulobacter crescentus* (Boshoff *et al*, 2003; Galhardo *et al*, 2005) associated *dnaE2* with a role in induced mutagenesis. In *M. tuberculosis*, *dnaE2* knockout resulted in loss of the enhancement of mutation that accompanies UV irradiation (Boshoff *et al*, 2003), although overproduction of DnaE2 did not result in mutagenesis, a characteristic of single-subunit Pol Y polymerases in other organisms (Boshoff *et al*, 2003; Kim *et al*, 1997). In *C. crescentus*, *dnaE2* knockout reduced the stimulation of mutagenesis following UV irradiation or mitomycin C treatment (Galhardo *et al*, 2005). Furthermore, DNA polymerase IV (DinB) was not induced by UV irradiation or treatment with mitomycin C, analogous to the situation in *M. tuberculosis*. In *C. crescentus*, *dnaE2* is the distal gene in an operon preceded by a small gene (*imuA*) that has weak similarity to *E. coli* *sulA* and *recA*, and a gene similar to Pol Y-like genes (*imuB*). Knockouts of *imuA* or *imuB* ablate induced mutagenesis and are epistatic to *dnaE2*. Knockout of the Pol IV structural gene *dinB* does not result in a diminution in UV or mitomycin-C-induced mutation (Galhardo *et al*, 2005). *M. tuberculosis* has ImuA and ImuB homologues that are required for DnaE2 function (Warner *et al*, 2010); I therefore propose to name DnaE2 as ImuC, for clarity (Table I). This is a logical extension of the nomenclature of Menck and co-workers (Galhardo *et al*, 2005) and will help to distinguish this polymerase from DnaEs that coexist with PolCs, with which they are often confused in the literature.

In the most advanced studies so far, *M. tuberculosis* ImuB has been shown to interact with the replicative DnaE, ImuC and ImuA in a yeast two-hybrid assay (Warner *et al*, 2010). ImuB, despite being homologous to Pol Y error-prone polymerases, does not contain the triad of conserved Pol III catalytic acidic residues (Warner *et al*,

2010) and must therefore be inactive. Mutation of the predicted catalytic aspartic acid residues of ImuC ablates induced mutagenesis (Warner *et al.*, 2010). Thus, ImuC is the error-prone polymerase in *M. tuberculosis* and presumably other organisms that contain ImuA/B/C and lack Pol V homologues. However, ImuB interacts with β_2 , but ImuC does not. Thus, it seems that ImuB has an important regulatory role, interacting with β_2 , the replicative DnaE and the error-prone polymerase ImuC.

What could be the role of an inactive Pol Y polymerase? A mechanism must exist to give Pol Y polymerase access to the replication fork, in order to replace the normal cellular replicase when blocking lesions are encountered. Perhaps ImuB preserves these functions and ImuC catalyses translesion replication. Such a system would be useful to study the non-catalytic activities of Pol Y polymerases. A eukaryotic translesion polymerase with limited activity, Rev1, interacts with other translesion polymerases including Pol κ , Pol ζ , Pol ι and Pol η (Acharya *et al.*, 2005; Guo *et al.*, 2003; Ohashi *et al.*, 2004; Tissier *et al.*, 2004). This could be a functional parallel to the ImuB–ImuC interaction.

In *Pseudomonas aeruginosa*, an *imuC* gene is preceded by SulA/RecA-like *imuA* and Pol Y-like *imuB* structural genes in an apparent operon, suggesting that it is regulated by the SOS response. Mutation of ImuC abolishes UV-induced mutagenesis (Sanders *et al.*, 2006) and knocking out *imuB* also abolishes mutagenesis (Sidebar A; R.C. Pope, J.C. Lindow, P.R. Dohrmann & C.S. McHenry, unpublished data). These results are in agreement with the *M. tuberculosis* and *C. crescentus* systems, but in contrast to a report about another pseudomonad (Koorits *et al.*, 2007). *ImuA*, *B* and *C* are induced by agents that also induce the SOS response and by ciprofloxacin treatment (Blazquez *et al.*, 2006; Brazas & Hancock, 2005; Cirz *et al.*, 2006). No studies have addressed the role of ImuA in ImuC-dependent induced mutagenesis, but given its homology to RecA, it might be involved in the protein–protein interactions that form the nucleoprotein ‘mutasome complex’, as described for the *E. coli* Pol V-dependent induced mutagenesis pathway (Jiang *et al.*, 2009).

There has been confusion about the relationship between DnaE-like polymerases that coexist in PolC-containing strains and those that coexist with replicative DnaEs. To distinguish between them, I compared the consensus sequences resulting from a comprehensive ImuC alignment with an alignment of DnaEs that exist alone or with PolCs. The latter are similar, and distinguishable from ImuC. There are only a few instances of absolutely conserved residues in ImuC that differ from DnaE, but several elements are absent in ImuC, such as an obvious β_2 -binding loop (Warner *et al.*, 2010; Fig 2). These differences are near the three catalytic aspartic acids and at the C-terminal end of the Fingers domain. Mapping the differences onto the DNA–dNTP–Taq Pol III α structure shows that these residues line the active site and the end of the DNA-binding channel near the primer terminus. These changes could relax substrate binding in a way that diminishes fidelity and allows bypass, which would be consistent with the error-prone function attributed to ImuC. However, this hypothesis needs to be tested experimentally.

Chemical biology of DNA replication

DNA replication is an essential process for the proliferation of all pathogens and an unexplored target for the development of new antibacterials. Therapeutically useful inhibitors have been developed

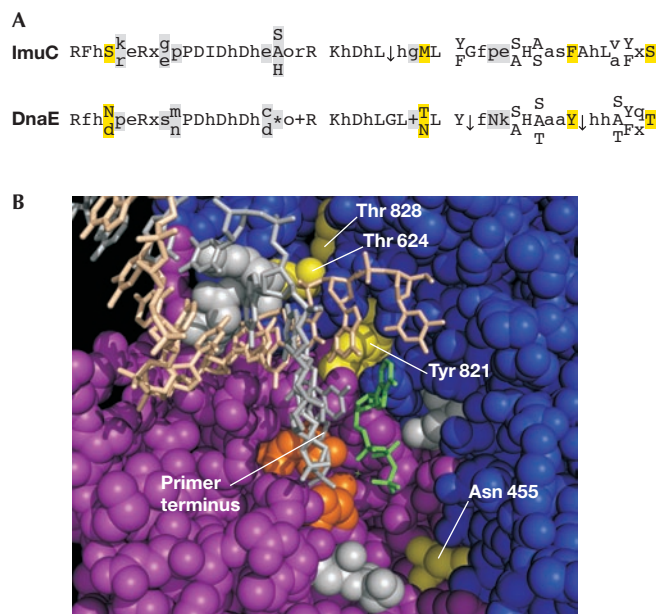


Fig 2 | Conserved residues in ImuC that differ from other DnaE polymerases.

(A) Residues conserved in ImuC that differ from DnaE are highlighted in yellow. Other differences are shown in grey. The first two sequence blocks show residues surrounding the three catalytic aspartic acids (PDID and hDh) in the ImuC line. The third block shows sequences at the carboxy-terminal end of the Fingers domain. (B) The active site of Taq Pol III α . Residues that differ from those conserved in ImuC are shown in grey or yellow, as in (A). The three catalytic aspartic acid residues are shown in orange. The incoming dNTP is green, and the primer and template strands are shown in stick form in grey and beige, respectively. The figure was prepared in PyMOL using Protein Data Bank 3E0D. Only the Palm and Fingers domains are shown. Gly 666, Arg 767 and Lys 771 are hidden for clarity. dNTP, deoxyribonucleotide triphosphate; h, hydrophobic amino acid; o, polar amino acid; asterisk, basic amino acid; x, no consensus; arrow, small amino acid.

that inhibit processes and molecules upstream and downstream from DNA replication, such as nucleotide precursor biosynthesis (Hawser *et al.*, 2006) and DNA gyrase (Mitscher, 2005), respectively. Most of the subunits of the bacterial DNA replication apparatus are essential, suggesting that their inhibition should lead to a block in cell proliferation or death (Kornberg & Baker, 1992). This has been shown by a class of compounds, 6-anilinoouracils, that are targeted to the polymerase subunit of the Gram-positive replicase PolC. These compounds are not only potent biochemical inhibitors, but also specific blockers of DNA replication in Gram-positive bacteria (Daly *et al.*, 2000). Although screens targeting individual replicase subunits have been described (Butler & Wright, 2008; Georgescu *et al.*, 2008; Shapiro *et al.*, 2005; Yang *et al.*, 2002), complete bacterial replicases have only recently been explored by chemical genetic approaches (Dallmann *et al.*, 2010).

The ten subunits of *E. coli* DNA Pol III holoenzyme interact to form a complex protein machine (Jeruzalmi *et al.*, 2001; Kim & McHenry, 1996b; Kim *et al.*, 1996; McHenry, 2003; Williams *et al.*, 2003). Protein interactions change at the various steps of the replicative reaction. Taking into account all of the individual protein components and their interactions with other subunits and

Sidebar B | In need of answers

- (i) What are the interactions between and relative roles of PolC and DnaE in low-GC Gram-positive organisms?
- (ii) How does PolC transfer extended lagging-strand primers from DnaE?
- (iii) What additional replicase components exist in Gram-positive organisms, beyond those identified so far by homology searches and genetics?
- (iv) What is the mechanism used by ImuA, ImuB and the Pol III-like ImuC to catalyse error-prone replication and generate mutations?

substrates, it has been estimated that more than 100 essential targets are potentially useful for the development of antibacterial agents (Dallmann *et al*, 2010). Given the impracticality of running 100 specific screening assays, a biochemical high-throughput screen was developed in which inhibition of any of the essential targets could be detected through a common endpoint (Dallmann *et al*, 2010). In a trial screen with a small (20,000-compound) library against full replication systems derived from model Gram-negative and Gram-positive organisms, it was possible to distinguish compounds that inhibited the replicase of a single species from compounds that exhibited broad-spectrum potential. Counterscreens against non-orthologous enzymes with related activities identified the compounds that are most likely to be target-specific (Dallmann *et al*, 2010).

Bacteriophages express inhibitory peptides against several cellular processes, including DNA replication. This is another source of *in vitro* inhibitors that enable mechanistic studies and allow the identification of potential therapeutic targets. For example, some *Staphylococcus aureus* phages produce peptides that bind to and inhibit the β_2 sliding clamp and DnaI helicase loader (Belley *et al*, 2006; Liu *et al*, 2004), and coliphage N4 produces a peptide inhibitor of the *E. coli* clamp loader that interacts with the δ subunit (Yano & Rothman-Denes, 2011). The crystallization of complexes of such peptides and their targets should provide data that could support library design and the development of small-molecule inhibitors.

Further developments in this area will hopefully provide compounds that facilitate biochemical investigation. Those that specifically inhibit Gram-positive DnaE or *P. aeruginosa* ImuC will be useful tools to distinguish the cellular and biochemical functions that are different to those of the main replicative Pol III. Stage-specific inhibitors will probably be useful to stop reactions, so that transient intermediates can be isolated and studied, as is the case with inhibitors of stages of nucleotide biosynthesis (Santi & McHenry, 1972; Sintchak *et al*, 1996) and enzymes involved in other aspects of nucleic acid metabolism (Classen *et al*, 2003; Ho *et al*, 2009). Such compounds should be useful *in vivo*—in combination with classical genetics—for example, to identify resistant mutants for target identification or verification. Chemical genetics provides a route to extend standard genetics. For example, the addition of a compound gives an immediate response, allowing the measurement of the effects of the kinetics of a block, which can also be rapidly reversed. Broad-spectrum compounds can be used to modulate responses in many cell types, including genetically intractable ones.

Conclusions

For decades, *E. coli* has been the main prototype for bacterial DNA replication, through which the central principles of DNA replication have been established. However, several key studies have now

shown that there are important differences between *E. coli* and other bacteria in DNA replication. Among them are bacteria that use two forms of Pol III for chromosomal replication, subdividing the tasks performed by one *E. coli* Pol III holoenzyme. In *B. subtilis* and other low-GC Gram-positive bacteria, PolC, together with a sliding clamp and clamp loader, is the main replicase. However, it is unable to extend the RNA primers for Okazaki fragment synthesis on the lagging strand. A second Pol III, DnaE, assumes the primer extension role and transfers the extended primer to PolC. This reaction is reminiscent of lagging-strand replication in eukaryotes, in which an RNA primer extended by eukaryotic Pol α is handed off to Pol δ . Thus, a reaction that was previously thought to be ‘eukaryotic’ occurs in an important class of bacteria, which include important human pathogens.

In diverse classes of bacteria, the prototypical Pol V that is required for induced mutagenesis in *E. coli* is replaced by a new Pol III, ImuC, which functions with ImuB, a homologue of standard Pol Y family polymerases that lacks a competent polymerase site. This system also requires a regulated mechanism for the transfer of a primer terminus between two Pol IIIs. Defining the molecular interactions and mechanism for polymerase-transfer reactions is an important challenge in the enzymology of several important processes (Sidebar B). Progress has been hampered, in part, by the inefficiency of the transfer mechanisms previously studied. In *B. subtilis*, transfer must occur rapidly (in less than 1 s) during the synthesis of each Okazaki fragment. Thus, it might constitute a useful system in which to study this important problem and, perhaps, reveal a universally shared mechanism.

The availability of well-defined biochemical assays that include all proteins required for complex replicative transactions has provided an opportunity to discover small-molecule inhibitors that block conformational changes in proteins, macromolecular interactions or freeze complexes at specific reaction stages. These will provide important tools for studying DNA-replicative reactions. A subset of these compounds might lead to the development of antibacterials that specifically block DNA replication and associated processes.

ACKNOWLEDGEMENTS

I thank Diane Hager for preparation of the figures and assembly of the bibliography and Melissa Stauffer, PhD, of Scientific Editing Solutions, for editing the manuscript. Our work with *B. subtilis* was supported by US National Science Foundation grant MCB-0919961.

CONFLICT OF INTEREST

The author declares that he has no conflict of interest.

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