Y-family DNA polymerases in Escherichia coli

The MIT Faculty has made this article openly available. Please share how this access benefits you. Your story matters.

<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>As Published</td>
<td><a href="http://dx.doi.org/10.1016/j.tim.2006.12.004">http://dx.doi.org/10.1016/j.tim.2006.12.004</a></td>
</tr>
<tr>
<td>Publisher</td>
<td>Elsevier</td>
</tr>
<tr>
<td>Version</td>
<td>Final published version</td>
</tr>
<tr>
<td>Accessed</td>
<td>Thu Mar 30 22:30:49 EDT 2017</td>
</tr>
<tr>
<td>Citable Link</td>
<td><a href="http://hdl.handle.net/1721.1/70041">http://hdl.handle.net/1721.1/70041</a></td>
</tr>
<tr>
<td>Terms of Use</td>
<td>Article is made available in accordance with the publisher's policy and may be subject to US copyright law. Please refer to the publisher's site for terms of use.</td>
</tr>
<tr>
<td>Detailed Terms</td>
<td></td>
</tr>
</tbody>
</table>
Y-family DNA polymerases in Escherichia coli

Daniel F. Jarosz 1, Penny J. Beuning 2,3, Susan E. Cohen 2 and Graham C. Walker 2

1 Department of Chemistry, Massachusetts Institute of Technology, Cambridge, MA 02139, USA
2 Department of Biology, Massachusetts Institute of Technology, Cambridge, MA 02139, USA
3 Department of Chemistry and Chemical Biology, Northeastern University, Boston, MA 02115, USA

The observation that mutations in the Escherichia coli genes umuC* and umud* abolish mutagenesis induced by UV light strongly supported the counterintuitive notion that such mutagenesis is an active rather than passive process. Genetic and biochemical studies have revealed that umuC* and its homolog dinB* encode novel DNA polymerases with the ability to catalyze synthesis past DNA lesions that otherwise stall replication – a process termed translesion synthesis (TLS). Similar polymerases have been identified in nearly all organisms, constituting a new enzyme superfamily. Although typically viewed as unfaithful copiers of DNA, recent studies suggest that certain TLS polymerases can perform proficient and moderately accurate bypass of particular types of DNA damage. Moreover, various cellular factors can modulate their activity and mutagenic potential.

SOS transcriptional regulation

The SOS response to DNA damage was the first inducible response to genotoxic stress to be characterized. Many molecular details of this response are now well understood (Figure 1) [1]. Transcription of genes induced as part of the SOS response are typically repressed by the product of the lexA+ gene. When replication is stalled by DNA damage or another mechanism, the recA+ gene product binds to single-stranded DNA (ssDNA) produced at the replication fork, forming a nucleoprotein filament in the presence of nucleoside triphosphates. This filament stimulates a latent autoproteolytic activity causing autocleavage, and to peptide hydrolases that employ a Ser–Lys catalytic diad in their mechanism [1]. Both LexA and RecA* are also SOS-regulated [1]. However, recent results have indicated that this simple view of the SOS response is far from complete. Agents that do not damage DNA, such as β-lactam antibiotics, can induce the SOS response [2] through the two-component signal transduction system dpiBA, presumably in an attempt to mitigate antimicrobial lethality by inhibiting cell division, and induce the expression of the dinB gene in particular through a lexA-independent mechanism [3]. This observation raises the possibility that crosstalk between the SOS response and other cellular signaling pathways could be more extensive than previously realized. Maximal transcription of dinB in stationary phase requires a functional rpoS gene, an effect that is also lexA-independent [4]. This might have particularly important implications for bacteria living under conditions of nutrient starvation. The SOS response also seems to be oscillatory at the single-cell level, and this oscillation is dependent on the umuDC genes [5]. Finally, the SOS response is one component of a broader cellular response to DNA damage. Exposure of Escherichia coli to the DNA-damaging agent mitomycin C (MMC) results in expression changes of >1000 genes [6].

Several of the genes regulated by the SOS response were initially identified using randomly generated Mu d1-generated transcriptional fusions to the lac operon. Mu d1 is a derivative of bacteriophage Mu that has been engineered to create such transcriptional fusions when it inserts into the chromosome. A collection of E. coli strains bearing these fusions was treated with MMC and examined for expression of β-galactosidase. Some of these fusions exhibited inducible expression of β-galactosidase, which was dependent on recA+ and lexA+; thus, they were named din (for damage-inducible) [7]. Many of these genes and their gene products have still not been characterized in detail. Although dinB [which encodes the translesion synthesis (TLS) polymerase (pol), DNA pol IV] was identified in this experiment, deletion of the gene did not initially show any marked phenotypes – this was in striking contrast to umuD and umuC (see later). Both umuD and umuC were subsequently shown to be transcriptionally induced as part of the SOS response using Mu d1-lac operon fusions [1]. This review will focus on the two SOS-regulated Y-family DNA polymerases found in E. coli, DinB (DNA pol IV) and UmuD,Y,C (DNA pol V), and their effects on the fidelity of replication.

Mutagenic function of umuD–C* and dinB*

Early studies of mutagenesis induced by UV irradiation indicated that mutation of either the recA* or lexA* genes could result in a nonmutable phenotype [1]. A screen for additional nonmutable mutants identified the umuD* and umuC* genes [8]. Loss-of-function mutants of each of these umu genes also show modest sensitivity to UV irradiation [1]. UmuD and LexA are structurally related to the lambda repressor, which undergoes RecA-nucleoprotein activated autocleavage, and to peptide hydrolases that employ a Ser–Lys catalytic diad in their mechanism [1]. Both LexA and UmuD form homodimers in solution and, similarly to LexA, interaction of UmuD,Y with the RecA nucleoprotein filament induces a latent autoproteolytic activity causing...
UmuD\textsubscript{2} to remove its N-terminal 24 amino acids to form UmuD\textsubscript{0}. It is UmuD\textsubscript{0} that is active in UV-induced mutagenesis and associates with UmuC to form DNA pol V (UmuD\textsubscript{0}C) [1,9].

In contrast to the marked phenotypes displayed by mutants of umuD and umuC, mutants of dinB show more enigmatic phenotypes [10]. Although deletion of the dinB\textsuperscript{+} gene has almost no discernable effect on spontaneous mutagenesis [11], the dinB\textsuperscript{+} gene is required for targeted mutagenesis of the F plasmid in E. coli [12]. The mutation spectrum observed is distributed between base substitution mutations and frameshift events with a strong preference for mutation at G:C base pairs [1].

Overproduction of dinB leads to an increase in spontaneous and 4-nitroquinoline 1-oxide (4-NQO)-induced base –1 frameshift and, to a lesser extent, spontaneous base substitution mutagenesis [18,19]. Curiously, a preference is observed for spontaneous mutagenesis on the lagging strand and this seems to result from extension of terminal mismatches [20]. Moreover, a considerable fraction of the lagging-strand-directed mutator phenotype of a constitutively SOS-induced recA730 strain requires dinB\textsuperscript{+} [21]. It has recently been shown that dinB strains of E. coli display increased sensitivity to the DNA damaging agents nitrofurazone (NFZ) and 4-NQO [22]. Despite this marked sensitivity to both NFZ and 4-NQO, deletion of the dinB\textsuperscript{+} gene does not reduce mutagenesis induced by either agent [22]. These data suggest that the dinB\textsuperscript{+} gene product is able to contend with DNA damage produced by at least some DNA damaging agents with comparable fidelity to other repair processes available to the E. coli cell.

Biochemical activities of DinB and UmuD\textsubscript{0}C
Although decades of genetic characterization clearly established their roles in spontaneous and induced mutagenesis, the biochemical function of the umuD\textsuperscript{+}C and dinB\textsuperscript{+} gene products remained elusive for many years. Early clues came when UmuD\textsubscript{0}C was shown to bind to DNA [23] and the eukaryotic Y-family member REV1 was
DNA polymerases are divided among six families based on sequence homology. Y-family polymerases usually participate in lesion bypass but X- and B-family polymerases can also be involved. *Escherichia coli* has five DNA polymerases: DNA pol I (A family) and DNA pol III (C family) are high-fidelity polymerases that replicate the majority of the genome; DNA pol II is a relatively accurate X-family polymerase that is involved in translesion synthesis; DNA pol IV/DinB (Y family) and DNA pol V/UmuD/C (Y family) are translesion polymerases that lack exonuclease activity and are, therefore, relatively error-prone [1]. Important eukaryotic Y-family polymerases that participate in translesion synthesis include pol α (DinB ortholog), pol γ, pol γXP-V (UmuC functional ortholog) and Rev1. Y-family DNA polymerases are found in all domains of life and are characterized by their ability to replicate damaged DNA, that is, to perform translesion synthesis [9]. The family was named in 2001 [27], although the catalytic activity of at least some members had been known since 1996 [24]. They typically Exhibit 10–1000-fold lower fidelity than replicative DNA polymerases when replicating undamaged DNA [1]. Thus, translesion synthesis by Y-family polymerases comes at a considerable mutagenic potential.

Crystal structures of Y-family DNA polymerases show that these enzymes adopt a similar right-hand fold to that of replicative DNA polymerases [1,37,38], which is striking considering that they bear relatively little sequence homology to replicative polymerases. Y-family polymerases also have an additional domain, referred to as the 'little-finger' domain (previously also called the 'wrist' or 'polymerase associated domain' (PAD)) that provides additional DNA-binding contacts [1,39]. These structures reveal an accommodating active site and short, stubby finger domains relative to replicative DNA polymerases. To date, the only crystal structure of a Y-family polymerase from *E. coli* is of the little finger domain of DinB bound to the β-processivity clamp [60]. Future structural studies will be required to understand the specific structural features of Y-family polymerases from *E. coli*.

**Loose grips and open active sites**

Although structures of the Y-family polymerases from *E. coli* have not yet been solved, structural analysis of *Sulfolobus solfataricus* (Dpo4) and *Sulfolobus acidocaldarius* (Dbh) homologs have yielded profound insights into function [37,38]. Whereas these enzymes share no clear sequence homology with replicative polymerases, their structures reveal a similar right-hand fold consisting of a thumb, palm and fingers domain. However, Y-family polymerases have an additional little-finger domain that seems to play an important part in both substrate specificity and processivity [39]. Unlike the tight grip seen in active sites of canonical DNA polymerases [40], Y-family polymerases have open active sites that are relatively solvent-accessible (Figure 2). Moreover, an α-helix responsible for several orders of magnitude of fidelity in canonical DNA polymerases (the O-helix) is entirely absent in Y-family polymerases, providing a structural rationale for their comparatively low fidelity when replicating undamaged DNA.

Structural insight into Y-family polymerases encountering their cognate substrates is considerably more limited. A study of Dpo4 encountering a cyclobutane pyrimidine dimer is the most definitive to date [41]. Such UV-induced damage presents a particular problem for replicative polymerases because their active sites can only accommodate one base at a time. The relative openness of the Dpo4 active site enables the enzyme to fit a covalently linked T^T with respect to UV irradiation and T^T-damaged substrates [35,36]. Additional work will be required to determine whether a hallmark of a cognate substrate for Y-family polymerases is comparable efficiency and/or fidelity on damaged and undamaged templates and, if so, what the endogenous sources of such cognate substrates might be.

**Box 1. DNA polymerases in TLS and DNA repair in E. coli and eukaryotes**

DNA polymerases are divided among six families based on sequence homology. Y-family polymerases usually participate in lesion bypass but X- and B-family polymerases can also be involved. *Escherichia coli* has five DNA polymerases: DNA pol I (A family) and DNA pol III (C family) are high-fidelity polymerases that replicate the majority of the genome; DNA pol II is a relatively accurate X-family polymerase that is involved in translesion synthesis; DNA pol IV/DinB (Y family) and DNA pol V/UmuD/C (Y family) are translesion polymerases that lack exonuclease activity and are, therefore, relatively error-prone [1]. Important eukaryotic Y-family polymerases that participate in translesion synthesis include pol α (DinB ortholog), pol γ, pol γXP-V (UmuC functional ortholog) and Rev1. Y-family DNA polymerases are found in all domains of life and are characterized by their ability to replicate damaged DNA, that is, to perform translesion synthesis [9]. The family was named in 2001 [27], although the catalytic activity of at least some members had been known since 1996 [24]. They typically Exhibit 10–1000-fold lower fidelity than replicative DNA polymerases when replicating undamaged DNA [1]. Thus, translesion synthesis by Y-family polymerases comes at a considerable mutagenic potential.

Crystal structures of Y-family DNA polymerases show that these enzymes adopt a similar right-hand fold to that of replicative DNA polymerases [1,37,38], which is striking considering that they bear relatively little sequence homology to replicative polymerases. Y-family polymerases also have an additional domain, referred to as the 'little-finger' domain (previously also called the 'wrist' or 'polymerase associated domain' (PAD)) that provides additional DNA-binding contacts [1,39]. These structures reveal an accommodating active site and short, stubby finger domains relative to replicative DNA polymerases. To date, the only crystal structure of a Y-family polymerase from *E. coli* is of the little finger domain of DinB bound to the β-processivity clamp [60]. Future structural studies will be required to understand the specific structural features of Y-family polymerases from *E. coli*.

Discovered to encode an enzyme with dCMP transferase activity [24]. Shortly thereafter, UmuD'_2'C and DinB were purified and shown to have bona fide DNA polymerase activity, thereby contributing to the recognition of a new superfamily of DNA polymerases known as the Y family [25–28] (Box 1). Unlike DNA pol III (the replicative DNA polymerase of *E. coli*), DinB and UmuD'_2'C catalyze relatively distributive DNA synthesis that is modestly stimulated by the addition of the β processivity clamp subunit of DNA pol III [29–31]. (The β processivity clamp is a ring shaped protein that encircles the DNA helix.) AP lyase activity has been demonstrated for both DinB and UmuD'_2'C, although genetic studies have not established a relevance for this function in *vivo* [32].

The *in vitro* DNA polymerase activity of UmuD'_2'C and DinB on both damaged and undamaged DNA has been examined in some detail. Their specialized function comes with a mutagenic risk because Y-family polymerases replicate DNA with lower fidelity than their replicative relatives. Although UmuD'_2'C and DinB display poor activity and fidelity on undamaged DNA relative to replicative DNA polymerases, they compare far more favourably on certain types of damaged templates. UmuD'_2'C replicates undamaged templates with an error frequency of 10^{-3}–10^{-4} and has an error frequency of 10^{-2} for T^T cyclobutane dimers [33,34], a common photoproduction resulting from UV irradiation that covalently links two adjacent thymines. DinB replicates both undamaged templates and an apparent cognate substrate, an adduct at the N^2 position of guanine (N^2-dG), with an error frequency of 10^{-3}–10^{-5} [22,33]. The difference between the fidelity of these polymerases when replicating damaged substrates might correlate with the clear UV-induced mutagenic signature of *umuDC* in *vivo* and the comparative lack of dinB'-dependent mutagenesis induced by NFZ or 4-NQO [8,22]. Furthermore, DinB shows an increased catalytic proficiency on an N^2-dG damaged substrate relative to an undamaged control, which is dependent on a single active-site residue [22]. Conceptually similar behavior is also observed for human DNA polymerase η with respect to UV irradiation and T^T-damaged substrates [35,36]. Additional work will be required to determine whether a hallmark of a cognate substrate for Y-family polymerases is comparable efficiency and/or fidelity on damaged and undamaged templates and, if so, what the endogenous sources of such cognate substrates might be.
An induced conformational change between an open and substrate-bound closed form is a hallmark of A and B family DNA polymerases [43]. Indeed, this conformational change is believed to contribute substantially to the exquisite fidelity of replicative polymerases [44,45]. Although such a change has not been observed crystallographically for a Y-family polymerase, several studies have indicated that such a conformational change might have a crucial role in translesion synthesis [22,46,47]. These observations provide further evidence that Y-family polymerases catalyze translesion synthesis in an orchestrated fashion rather than exclusively by virtue of an open active site.

**Modulation of function by protein–protein interactions**

Genetic characterization over the past 30 years has underscored the importance of the recA and umuD gene products in regulation of umuC-dependent mutagenesis [1]. Recent studies have recapitulated these results with purified components and identified the pivotal role of the β processivity clamp in dictating UmuD’2C function. Initial reports of UmuD’2C polymerase activity invoked a requirement for UmuD’2, RecA, ssDNA binding protein (SSB) and, in one case, various components of the polymerase III holoenzyme for UmuC activity [31]. The demonstration of polymerase activity of UmuD’2C established UmuD’2 as a subunit of DNA pol V.

X-ray and NMR structures of the polymerase V subunit UmuD’2 have yielded considerable insight into its function [48,49]. Additionally, distance constraints derived from electron paramagnetic resonance spectroscopy have been used to model the structure of full-length UmuD2 [50]. In the X-ray structure of UmuD’2, the catalytic serine and
lysine required for autoproteolysis are located within hydrogen-bonding distance of each other and the N terminus containing the scissile bond is located >50 Å from the active site [48]. By contrast, the UmuD<sub>2</sub> model suggests that the N terminus of the molecule curls upon itself to bring the scissile bond in proximity to the active site [50]. Such structural plasticity might be especially important given the relatively large number of proteins with which UmuD<sub>2</sub> and UmuD<sub>2</sub> interact [51]. A heterodimeric form of the umuD gene products, UmuD<sub>2</sub>C, is the most thermodynamically stable form of the protein and targets it for ClpXP-mediated proteolysis [1]. A structural model of UmuD<sub>2</sub>C has been constructed based upon NMR analysis [36].

Aside from activating UmuD<sub>2</sub>C, RecA has numerous cellular roles. The recA gene is required not only for induction of the SOS response but also for homologous recombination [1]. Biochemical studies differ to some extent on the mode of RecA activation of UmuD<sub>2</sub>C and on the role of ATP in the process [31]. Recent studies have suggested that RecA binds to UmuC as a subunit of the UmuD<sub>2</sub>C holoenzyme and that another molecule of ATP-associated RecA binds to UmuD<sub>2</sub>, thereby stimulating the affinity of the holoenzyme for the primer terminus [52]. It was originally assumed that RecA is bound to the ssDNA template in this activating role, but it has now been proposed that stimulation of UmuD<sub>2</sub>C activity by the RecA-nucleoprotein filament occurs in trans [53]. This has important implications for models of UmuD<sub>2</sub>C action given that the most proficient transactivating RecA nucleoprotein filament is one formed on gapped DNA. These observations foreshadow what seems to be remarkably complex regulation of Y-family polymerases through protein–protein interactions. Initial studies of UmuD<sub>2</sub>C activity also reported an enhancement of activity provided by SSB [31]. This effect, observed at substoichiometric quantities of SSB, has now been attributed to increased formation of dynamic RecA filaments on short ssDNA templates in the presence of DNA [31].

Protein regulators of DinB function have been comparatively less well characterized. A recent report has implicated certain forms of the umuD gene products in regulation of a novel function of DinB [54], and the chaperone GroEL–GroES affects DinB levels, perhaps indirectly [15]. The recent identification of an additional phenotype for ΔdinB E. coli strains [22] should enable knowledge of DinB regulation to expand considerably over the coming years.

**Management role of the processivity clamp**

Interactions with replicative processivity clamps are crucial for regulating Y-family polymerase activity and dictating their access to DNA. Although they are characterized by low processivity on undamaged DNA, Y-family polymerases exhibit an increased processivity in the presence of the β clamp. Indeed, DinB processivity is enhanced 300-fold by the β clamp [29], whereas that of UmuC is stimulated between 5- and 100-fold [30,31]. In either case, the processivity enhancement as a result of β is far less than that of polymerase III (~10<sup>3</sup>-fold) [55]. Mutation or deletion of the β interaction motif in either UmuC or DinB causes a loss of translesion synthesis in vivo [56]. Most prokaryotic proteins that interact with the β processivity clamp do so through a conserved interaction motif: QL[S/D]LF [57], which bears similarity to the conserved eukaryotic proliferating cell nuclear antigen (PCNA) interaction motif, QXXLXXFF [58].

Recent structural studies have shown that proteins as diverse as the δ subunit of the clamp loader and DinB, which interact with β through the conserved interaction motif, bind to the same hydrophobic channel on β at the interface between β domains II and III [59–61]. Thus, mutations in β near this hydrophobic channel can regulate specific DNA polymerase usage [62,63]. A co-crystal structure of the C-terminal little-finger domain of DinB with the β clamp illustrates that, in addition to the conserved β-binding motif interaction, DinB also interacts with β at its dimer interface through a hydrophobic loop in the little-finger domain [60]. When the structure of full-length *S. solfataricus* Dpo4 was superimposed on the DinB little finger in this structure, the active site of Dpo4 was surprisingly far from the DNA that is expected to be running through the center of the β clamp, leading the authors to speculate that this orientation of DinB could represent a recruited-but-inactive state (Figure 3) [60].

What is the role of the β clamp in managing multiple DNA polymerases? Notably, all DNA polymerases in *E. coli* interact with β at the same site [64]. The co-crystal structure of the DinB little finger and the β clamp suggests that it might be possible for β to bind two DNA polymerases simultaneously, with one polymerase in an inactive but still recruited conformation. Indeed, both DinB and the α catalytic subunit of polymerase III were found to bind to β simultaneously [65]. Thus, switching polymerase access to
the primer terminus could occur with two DNA polymerases bound to the β clamp [65]. The hierarchy of affinities of DNA polymerases in E. coli for the processivity clamp has been investigated genetically [62,66,67]. Upon UV irradiation, polymerase III seems to have the greatest affinity followed by pol IV, pol V and pol II [62], whereas during conjugal replication, the hierarchy seems to be pol III first, then pol II, pol IV and finally pol V [67]. Further work will be required to analyze competition among polymerases for access to the β clamp under various conditions.

The β clamp also interacts with UmuD2 and UmuD2C. Moreover, UmuD2 interacts with β more strongly than UmuD2C does, possibly indicating a role in umuDC-dependent replication pausing [51]. UmuD binds to β in the vicinity of the same hydrophobic channel where other β-binding proteins interact [68]. Curiously, the N-terminal region of UmuD contains a cryptic β-binding motif (15TLPLF18) that by itself is insufficient to bind to β [57]. UmuD variants containing mutations in this motif bind to β with essentially the same affinity as wild-type UmuD [69] but with a strikingly different tryptophan fluorescence emission spectrum of β [69], indicating that although this motif itself is not responsible for the interaction, it is important for determining the nature of the complex.

In eukaryotes, polymerase management is even more complex. The processivity clamp PCNA is subject to several different post-translational modifications that dictate its roles in replication, DNA repair and DNA damage tolerance mediated by Y-family DNA polymerases [70,71]. Additionally, the alternative processivity clamp in eukaryotes (Rad9-Rad1-Hus1) is important for modulating the activity of Y-family polymerases [70].

**Novel phenomena involving dinB and umuDC**

In addition to the well-known function of Y-family polymerases in TLS, other functions of umuDC and dinB include UmuD2C-dependent cold sensitivity, involvement in a primitive DNA damage ‘checkpoint’, enhanced survival in response to DNA-damage-independent replication stalling, and replication arrest-stimulated recombination [1,54,72–74].

Overexpression of the umuDC gene products leads to inhibition of growth at 30 °C, known as umuDC-mediated cold sensitivity. The umuDC genes are the only SOS regulated genes required for the manifestation of cold sensitivity and the degree of cold sensitivity is proportional to the amount of expression. This phenomenon is associated with the rapid and reversible inhibition of DNA synthesis and sulA-independent filamentation [1]. Strikingly, the genetic requirements for cold sensitivity are different from those needed for TLS [1]. Namely, neither RecA nor the catalytic activity of UmuC is needed and UmuD (but not UmuD2C) is required. Cold sensitivity seems to result from an exaggeration of a DNA-damage-induced ‘checkpoint’ in which UmuD2C delays the resumption of DNA synthesis after DNA damage, perhaps through interaction with the β clamp, to enable error-free repair processes to occur [73,75]. The response is temporally regulated by the cleavage of UmuD to UmuD2C.

Both E. coli Y-family polymerases have been implicated in enhancing cellular survival under conditions of depleted deoxyribonucleotide pools, such as after the addition of hydroxyurea (HU). Strains carrying a umuC122::Tn5 allele, resulting in a truncated protein that retains an intact polymerase domain but is deficient for induced mutagenesis, are strikingly resistant to HU [54]. Although seemingly unrelated, cold sensitivity and resistance to HU share a genetic requirement for umuD. HU resistance requires the catalytic activity of UmuC122 and DinB and certain forms of the umuD gene products. Moreover, this resistance might, at least in part, be because of failed communication with the toxin–antitoxin pairs MazEF and RelBE that would normally lead to cell death [54]. The increased mutation frequency observed in a umuC122::Tn5 strain upon HU treatment could imply that under conditions of deoxyribonucleotide limitation, DinB and UmuD2C take over a considerable fraction of DNA replication. Furthermore, recent studies have shown that Y-family DNA polymerases participate in oxidation-induced mutagenesis by virtue of their ability to incorporate oxidized nucleotides during replication [76,77]. Taken together, these results suggest that Y-family polymerases might have a larger role in DNA replication when the deoxyribonucleotide pool is substantially perturbed, such as under conditions of HU treatment or oxidative stress.

Interestingly, dinB has also been implicated in replication-arrest-stimulated recombination [74]. Deletions of tetA fragments that are set in tandem repeats are elevated at the permissive temperature in a strain background bearing a temperature-sensitive mutant of the replicative DNA helicase (dnaB107). This type of mutagenesis is reduced in a dnaB107 dinB strain, contributing to a model in which RadA, RecG and RuvAB can stabilize a D-loop/recombination intermediate that enables DinB to extend the invading 3’ strand and promote continued replication [74].

**Concluding remarks and future perspectives**

Recent developments have greatly enhanced the understanding of Y-family polymerases and, particularly, their role in DNA damage tolerance and mutagenesis. Whether the paradigm for understanding their function should be that of unfaithful copiers or specialized polymerases is still a subject of some debate (Box 2). The picture is likely to be considerably more nuanced than either extreme. In the case of E. coli, DinB seems to be a specialized polymerase under many circumstances. However, UmuD2C seems to function with both lower fidelity and broader substrate specificity. The observation of novel ‘checkpoint’ functions associated with both umuDC and dinB have also greatly

---

**Box 2. Major questions regarding the function and regulation of Y-family polymerases in E. coli**

1. How do Y-family polymerases gain access to an appropriate primer terminus and how is their action coordinated with that of replicative polymerases?
2. How do protein–protein interactions regulate the activity of Y-family polymerases?
3. Are there families of cognate lesions for each different Y-family polymerase?
4. Can mutations introduced by Y-family polymerases be corrected by exonucleolytic proofreading *in trans*?
expanded our understanding of the multifaceted roles of these genes in E. coli. However, Y-family polymerases are not enzymes that function in isolation and considerable effort needs to be directed towards understanding their function in the context of a living cell. The protein regulators of UmuD'2C have been studied in some detail whereas those of DinB are largely unknown.

E. coli delays the mutagenic function of UmuD'2C by timing the cleavage of UmuD2 to UmuD'. This temporal separation of more accurate DNA repair and error-prone DNA damage tolerance might be echoed in eukaryotes, in which the Y-family member Rev1 is not maximally expressed until the G2/M transition of the cell cycle [78]. Indeed, the exquisite regulation of Y-family polymerases could be particularly important in eukaryotes, which, according to some estimates, rely on translesion synthesis 50-fold more than prokaryotes [79].

Acknowledgements
This work was supported by a National Institutes of Health grant (CA021615) to G.C.W and a National Institute of Environmental Health Sciences grant (P30 ES002109) to the Massachusetts Institute of Technology Center for Environmental Health Sciences. G.C.W. is an American Cancer Society Professor.

References
1 Friedberg, E.C. et al. (2006) DNA Repair and Mutagenesis, ASM Press
29 Wagner, J. et al. (2000) The β clamp targets DNA polymerase IV to DNA and strongly increases its processivity. EMBO Rep. 1, 484–488
32 Shen, X. et al. (2009) Lyase activities intrinsic to Escherichia coli polymerases IV and V. DNA Repair (Amst.) 4, 1368–1373
AGORA initiative provides free agriculture journals to developing countries

The Health Internetwork Access to Research Initiative (HINARI) of the WHO has launched a new community scheme with the UN Food and Agriculture Organization.

As part of this enterprise, Elsevier has given hundreds of journals to Access to Global Online Research in Agriculture (AGORA). More than 100 institutions are now registered for the scheme, which aims to provide developing countries with free access to vital research that will ultimately help increase crop yields and encourage agricultural self-sufficiency.

According to the Africa University in Zimbabwe, AGORA has been welcomed by both students and staff. “It has brought a wealth of information to our fingertips”, says Vimbai Hungwe. “The information made available goes a long way in helping the learning, teaching and research activities within the University. Given the economic hardships we are going through, it couldn’t have come at a better time.”

For more information, visit www.aginternetwork.org