

THE SOS RESPONSE: Recent Insights into *umuDC*-Dependent Mutagenesis and DNA Damage Tolerance

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■ **Abstract** Be they prokaryotic or eukaryotic, organisms are exposed to a multitude of deoxyribonucleic acid (DNA) damaging agents ranging from ultraviolet (UV) light to fungal metabolites, like Aflatoxin B₁. Furthermore, DNA damaging agents, such as reactive oxygen species, can be produced by cells themselves as metabolic byproducts and intermediates. Together, these agents pose a constant threat to an organism's genome. As a result, organisms have evolved a number of vitally important mechanisms to repair DNA damage in a high fidelity manner. They have also evolved systems (cell cycle checkpoints) that delay the resumption of the cell cycle after DNA damage to allow more time for these accurate processes to occur. If a cell cannot repair DNA damage accurately, a mutagenic event may occur.

Most bacteria, including *Escherichia coli*, have evolved a coordinated response to these challenges to the integrity of their genomes. In *E. coli*, this inducible system is termed the SOS response, and it controls both accurate and potentially mutagenic DNA repair functions [reviewed comprehensively in (25) and also in (78, 94)]. Recent advances have focused attention on the *umuD*⁺*C*⁺-dependent, translesion DNA synthesis (TLS) process that is responsible for SOS mutagenesis (70, 86). Here we discuss the SOS response of *E. coli* and concentrate in particular on the roles of the *umuD*⁺*C*⁺ gene products in promoting cell survival after DNA damage via TLS and a primitive DNA damage checkpoint.

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THE SOS RESPONSE

The SOS Regulon

In the *E. coli* SOS response, the expression of approximately 30 unlinked genes is induced after the cell is exposed to DNA damaging agents (19, 25). Many of these gene products are involved in DNA damage tolerance and repair (*e.g.* *recA*, *lexA*, *umuDC*, *polB*, *sulA*, and *uvrA*). The SOS response to DNA damage requires the *recA*⁺ (56) and *lexA*⁺ (16) gene products. LexA is a repressor protein that binds to a site (the "SOS box") located near the promoters of the SOS response genes and interferes with the binding of RNA polymerase (3, 46). The *recA*⁺ gene product is required not only for the regulation of the SOS response (Figure 1) but also for homologous recombination and for varied DNA repair and damage tolerance pathways including the repair of double-strand breaks, SOS mutagenesis (25), and for the restart of stalled replication forks (14, 18, 36, 92). When it binds to single-stranded DNA (ssDNA), RecA forms a helical, multimeric nucleoprotein filament that is central to its activities in the cell (25) (Figure 1).

Following DNA damage, RecA becomes activated for its role in SOS induction when it forms a nucleoprotein filament by binding to ssDNA generated by the cell's failed attempts to replicate damaged DNA (15, 74). The RecA/ssDNA nucleoprotein filament then functions as a coprotease that mediates LexA cleavage by stimulating the latent ability of the LexA repressor to cleave itself in two via a proteolytic autodigestion mechanism (44, 45). The resulting decrease in the cellular pool of LexA results in the induction of the SOS regulon. Additional regulation of SOS induction may be provided by the competition between LexA cleavage and homologous recombination, both of which are mediated by the RecA/ssDNA nucleoprotein filament. Structural studies have revealed that LexA interacts with a region of the RecA nucleoprotein filament that may also be the binding site for a double-stranded DNA (dsDNA) molecule (80, 98). This hypothesis is supported by biochemical experiments indicating that the LexA cleavage and DNA strand exchange functions of the RecA nucleoprotein filament are competitive reactions (28, 69).

In spite of the intensive study of the SOS response over the years, recent work has revealed two new factors that may influence the induction of the SOS response.

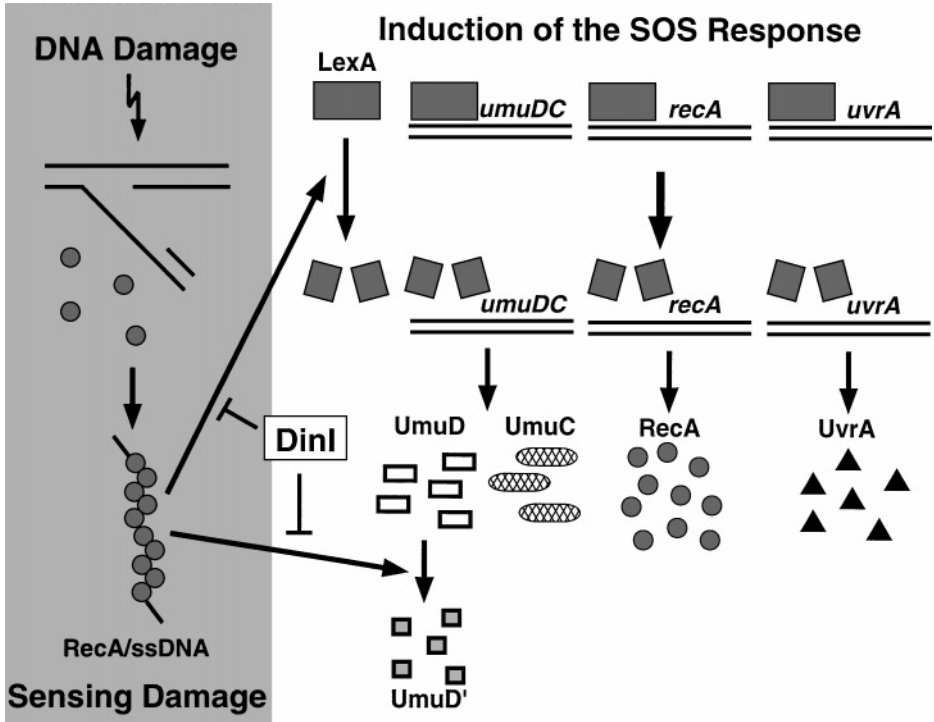


Figure 1 Induction of the SOS response and of UmuD to UmuD' processing by DNA damage. See text for details.

First, the DinI protein, a protein that is induced as part of the SOS response, can inhibit the coprotease activity of the RecA/ssDNA nucleoprotein filament (Figure 1). In this way, DinI modulates the induction of the SOS response by inhibiting the autodigestion of LexA (and also of UmuD, see below) (96).

The second factor was suggested by a study in which the induction of the SOS response was fully reconstituted *in vitro* using double-strand DNA breaks to promote the inducing signal (1). The authors of this report found that transcription from a LexA-repressed promoter was induced *in vitro* by the addition of linear dsDNA to a reaction containing the promoter construct, LexA, RecA, RecBCD (a multifunctional enzyme that unwinds and degrades DNA from the end of a linear dsDNA molecule), SSB, RNA polymerase, and nucleotides. Interestingly, the addition of linear dsDNA containing a Chi (χ) site enhanced the rate of derepression. The Chi site is an octameric sequence involved in recombination that is overrepresented in the *E. coli* chromosome. It was suggested that the effect of Chi sites on SOS induction may have evolved to enhance the SOS response to damaged "self" chromosomal DNA as opposed to "non-self" viral and plasmid DNA that lack Chi sites (1). The *in vivo* significance of this Chi-dependent modulation of the rate of SOS induction by double strand DNA breaks remains to be determined.

The present model for the regulation of the SOS response can be summarized as follows (Figure 1): When ssDNA (the cell's internal signal that it has suffered DNA damage) is present, free RecA molecules polymerize on ssDNA to form a nucleoprotein filament. This nucleoprotein filament, modulated by DinI, stimulates the cleavage of LexA, thus inducing the SOS response and, therefore, the increased synthesis of RecA, which remains in the activated nucleoprotein filament as long as the inducing signal persists. Although less is known about what happens when the cell recovers from the DNA damage, the amount of ssDNA is presumed to decrease, thereby reducing the amount of RecA/ssDNA present in the cell. This results in the reaccumulation of intact LexA, which represses the entire SOS regulon, thereby shutting down the response. Further modulation of the response is provided by the differential affinity of LexA for the promoters of SOS response genes, which allows some genes to be fully induced at a lower level of DNA damage than others (25).

Translesion DNA Synthesis and SOS Mutagenesis

Translesion DNA synthesis, the mechanistic basis of SOS mutagenesis, endows the cell with an increased capacity to recover from DNA damage by allowing it to replicate past lesions that would normally block continued polymerization by *E. coli*'s replicative polymerase (DNA Pol III) (25). In exchange for increased survival, the cell pays the cost of an elevated mutation rate resulting from translesion DNA synthesis. This process requires the products of the SOS-regulated *recA* gene and the similarly regulated *umuDC* operon, which was originally identified by screening for *E. coli* mutants that were not mutable by UV light and other agents (33, 79). TLS requires not the full-length UmuD protein, but rather a post-translationally processed form called UmuD' (25, 63). The biochemical nature of this processing is similar to that of LexA autodigestion (Figure 1): Interaction of UmuD with the RecA/ssDNA nucleoprotein filament stimulates a latent ability of UmuD to autodigest, resulting in the removal of the amino-terminal 24 amino acids (8, 63, 77). As with LexA autodigestion, the DinI protein acts to inhibit the RecA/ssDNA-mediated autodigestion of UmuD to UmuD' in vitro. In vivo, the induced mutation frequency in a *dinI*⁻ strain is higher than that of a *dinI*⁺ strain, presumably due to the increased rate of UmuD to UmuD' processing (96).

DNA POLYMERASE ACTIVITY OF MEMBERS OF THE UmuC/DinB SUPERFAMILY

The UmuC/DinB Superfamily

It has very recently become apparent that UmuC is the founding member of a superfamily of novel DNA polymerases that can replicate over lesions or operate on particular classes of imperfect DNA templates (Table 1). *E. coli* has two members of this superfamily; UmuC, when complexed with UmuD'₂, can replicate

TABLE 1 The UmuC/DinB superfamily of DNA polymerases

Name	Alternate name	Organism	References
Pol V	UmuD' ₂ C/UmuC	<i>E. coli</i>	(70, 71, 84–86)
Pol IV	DinB	<i>E. coli</i>	(85, 88)
Pol { η }	Rad30	<i>S. cerevisiae</i>	(30, 31)
XP-V	Pol η /Rad30A	Humans	(29, 51, 52)
Pol ι	Rad30B	Humans/mouse	(54)
Pol κ	DinB1	Humans/mouse	(26, 64)
Rev1	Deoxycytidil transferase	<i>S. cerevisiae</i> /human	(43, 61)
Pol ζ	Rev3-Rev7	<i>S. cerevisiae</i>	(62)

Names of members of the UmuC/DinB superfamily of DNA polymerases and the organisms that they have been identified in are indicated. Pol ζ is not a member of the UmuC/DinB superfamily, but does serve a role in TLS in *S. cerevisiae* (39, 42, 62) and humans (27, 58). See text for details.

over abasic sites (70, 71, 84, 86), thymine-thymine cyclobutane dimers, and [6-4] photoproducts (85), whereas DinB can replicate templates with a bulged-out nucleotide in the template strand, thereby causing -1 frameshift mutations (88). *Saccharomyces cerevisiae* has two members of this superfamily; Rad30 appears to carry out relatively accurate postreplication repair by inserting two As when it polymerizes across a thymine-thymine cyclobutane dimer (30, 31), whereas Rev1 plays a role in induced mutagenesis and predominantly inserts C across from an abasic site (61). As of the writing of this review, humans have been found to possess at least four members of this superfamily: XP-V (Rad30A) (29, 51, 52), hRev1 (43), hRad30B (54), and hDinB1 (26, 64). Homologs of these are also present in mice (26, 54, 64). The XP-V protein, which is absent in individuals with the cancer-prone hereditary disease *Xeroderma pigmentosum* variant, resembles yeast Rad30 in inserting two As when it polymerizes across a thymine-thymine cyclobutane dimer (51, 52) (Table 1).

UmuD⁺*C*⁺-Encoded DNA Polymerase Activity Is Necessary for DNA Synthesis in Vitro

Recently, the Goodman (84, 86) and Livneh (70, 71) groups have independently reconstituted TLS in vitro using purified *E. coli* proteins. Collectively, these studies have indicated that the DNA polymerase activity of the *umuDC* gene products is required for TLS in vitro. Furthermore, both groups have demonstrated that *umuDC*-dependent DNA synthesis is error-prone in vitro relative to that observed for DNA Pol III under similar conditions. This *umuDC*-encoded polymerase has been termed DNA Pol V (70, 86). However, presumably due to differences in experimental design, the Livneh and Goodman groups reported different biochemical requirements for in vitro TLS. These similarities and differences in experimental

design have been discussed in detail elsewhere (89). Here, we summarize the major conclusions from both groups.

The major roadblock to the development of an in vitro reconstituted TLS system was purification of biologically active UmuC protein (95). Livneh and colleagues solved this problem by purifying UmuC in the form of an N-terminal fusion to the maltose-binding protein (MBP-UmuC) (71), whereas Goodman's group, in collaboration with others, purified a soluble UmuD'₂C complex (7). Consequently, while Livneh's group compared the effects of pairing UmuC with UmuD versus UmuD', Goodman's group studied only UmuD'₂C. On the other hand, the Goodman group used the natural UmuC protein in their experiments whereas the Livneh group used a chimeric MBP-UmuC protein. In addition to these differences with respect to the UmuC preparations, the Livneh and Goodman groups also used different types of DNA templates. Although both employed a site-specific, synthetic tetrahydrofuran moiety to mimic an abasic site, Livneh and colleagues located it within a defined 350-nucleotide ssDNA gap contained in a covalently closed, circular dsDNA, thus somewhat mimicking the in vivo DNA substrate (71). By contrast, Goodman and collaborators used a ~7.2-kilobase linear M13-derived single-stranded DNA containing the model abasic site 50 nucleotides away from the 5' end with primers located various distances before the lesion (67). The location of the lesion near the end of the very long, linear ssDNA substrate might account for the different requirements for in vitro TLS described by the Goodman and Livneh groups.

Despite these differences, which presumably influenced the biochemical requirements, both the Goodman and Livneh groups found that UmuD'₂C alone was unable to promote TLS in vitro (70, 71, 84, 86). Interestingly, Livneh's group found that, by itself, UmuC is capable of limited DNA synthesis on undamaged DNA templates in vitro (70). This finding confirms that the catalytic activity of the *umuDC* gene products resides within UmuC, as initially suggested by the observations that other members of the UmuC/DinB superfamily have DNA polymerase activity (31, 51, 52, 61, 88). However, although Livneh and colleagues found that UmuC contains the intrinsic DNA polymerase activity, in order to participate in TLS in vitro it also requires UmuD', RecA, and single-strand DNA binding protein (SSB) (70), an observation consistent with the genetic requirements of SOS mutagenesis (25).

Using their system, Goodman and his collaborators observed a more complicated set of requirements for TLS. Not only were UmuD'₂C, RecA, and SSB required, but also the beta subunit (processivity clamp) and the processivity clamp loader (the γ complex) of the replicative DNA polymerase, DNA Pol III (84, 86). However, a subsequent study from Goodman's group reported that the requirement for the beta clamp and the clamp loader could be partially relaxed if RecA/ssDNA nucleoprotein filaments were stabilized by the inclusion of the nonhydrolyzable ATP analog, ATP γ S (85). This result, suggesting that the beta clamp and clamp loader do not play a critical role in TLS when RecA/ssDNA filaments are stabilized, is consistent with the findings of Livneh's group (70).

Although a definitive answer to the question of whether or not the beta processivity clamp and clamp loader complex of DNA Pol III play a physiologically important role in TLS must await further studies, it nevertheless seems clear that *umuDC*-dependent TLS requires RecA protein and SSB. Since RecA protein interacts physically with UmuD (23, 40, 95), UmuD' (23, 95), and UmuC (24), the molecular mechanism of TLS in *E. coli* will likely be found to involve a highly complex series of protein-protein and protein-nucleic acid interactions. Although multiprotein complexes that include UmuD' and UmuC have been referred to as a "mutasome" (67, 84, 86) or as "DNA Pol V holoenzyme" (70), a full understanding of the physiologically important interactions and complexes will require further research. If DNA replication in *E. coli* does in fact take place within a factory, as has been suggested (41), interaction of the *umuDC* gene products with components of the factory might be instrumental for regulating the hand-off of the replication of the damaged DNA from DNA Pol III to DNA Pol V (81).

A Role for the *umuDC* Gene Products in Chromosomal Untargeted Mutagenesis

In addition to the role of the *umuDC* gene products in SOS mutagenesis, they are likewise known to play a role in chromosomal untargeted mutagenesis (UTM) (11, 20, 91, 93). Chromosomal UTM refers to the transient increase in the mutation frequency of chromosomal genes following induction of the SOS response and is characterized by a striking increase in transversions (20, 55, 97), a class of mutations known not to be a result of DNA Pol III alone (66).

Livneh and colleagues observed a pronounced difference in the mutational signature of DNA Pol III relative to their MBP-UmuC as measured using a biological assay subsequent to an *in vitro* gap-filling DNA synthesis reaction employing purified components (50). Most notable was the marked increase in the frequency of MBP-UmuC-dependent transversions generated at a frequency 74-fold higher than that observed by DNA Pol III (50). This finding complements that of the Goodman group who reported a propensity for the *umuDC* gene products to promote nucleotide misinsertion *in vitro* (84, 85). The finding by the Livneh and Goodman groups that *umuDC*-dependent DNA replication of undamaged DNA *in vitro* is inherently less accurate than DNA Pol III, together with the observation that MBP-UmuC generates a mutational signature very similar to that characteristic of chromosomal UTM (50), led Livneh and colleagues to propose that low-fidelity chromosomal replication by the *umuDC*-encoded DNA polymerase is the mechanistic basis for chromosomal UTM (50).

Roles and Properties of Other Members of the UmuC/DinB Superfamily

Presently, UmuC is unique within the UmuC/DinB superfamily in that its polymerase activity is managed by the products of the *umuD* gene (Table 1 and see

below) (63, 81); whether or not the activities of the other family members are similarly regulated is unknown. The *E. coli dinB* gene, which is a member of the SOS regulon (35, 37), encodes a DNA polymerase (DNA Pol IV) (88); homologs of DinB have recently been cloned from human (26, 64) and mouse (64), and hypothetical open reading frames encoding DinB homologs can be found in *Caenorhabditis elegans* (NCBI databases), *Drosophila*, (NCBI databases), *S. pombe* (26) (NCBI databases), and archaea (38). DinB has an ability to elongate a DNA substrate with a bulged-out nucleotide in the template strand, resulting in -1 frameshifts (88). Mutations in the *dinB* gene result in only a modest UV-sensitive phenotype, indicating that it does not play a major role in helping *E. coli* tolerate DNA lesions introduced by UV irradiation (37). However, DinB may play a role in the bypass of DNA lesions caused by other types of DNA damage.

The first physiologically important role for the *dinB* gene product to be identified was in lambda phage UTM (5). This phenomenon is observed when an undamaged lambda phage infects a UV-irradiated host and differs from the chromosomal UTM discussed above. The genetic requirements for lambda UTM, in addition to *dinB*, include the *recA*, *uvrA*, *uvrB*, *mutL*, *mutH*, *mutS*, and *polA* genes and DNA Pol III (5, 6, 9, 10, 37, 48, 49). However, these requirements for genes besides *dinB* for lambda UTM are bypassed when the *dinB* gene dosage is elevated (37). Increasing the copy number of the *dinB* gene by virtue of its presence on a multicopy plasmid was found to increase, among others, the frequency of -1 frameshift mutations in a gene present on an F' plasmid (37). Interestingly, ectopic expression of the mouse *Dinb1* cDNA in cultured murine cell lines likewise led to an almost tenfold increase in the frequency of point mutations, of which roughly 30% were frameshift mutations (64), suggesting that the activity of the eukaryotic DinB homologs resembles that of *E. coli* DinB.

The *REV1*, *REV3*, and *REV7* loci in *S. cerevisiae* were identified in a similar manner to that used to identify the *umuDC* genes in *E. coli* (33, 79), namely by screening for nonmutable mutants (39, 42). The *S. cerevisiae REV1* gene, a member of the UmuC/DinB superfamily, encodes a protein with a remarkably limited DNA polymerase activity in that it incorporates predominantly C opposite an abasic site (61). It has been suggested that the Rev1 protein might act together with DNA Pol ζ , the product of the *REV3* and *REV7* genes (62), to promote TLS in yeast (62). A similar situation appears to pertain to humans since downregulation of human *REV3*, using antisense RNA technology, reduces the mutation frequency in cultured human cell lines (27). Although the specifics of the DNA polymerase activities of *S. cerevisiae REV1* and *E. coli umuC* differ, they can nonetheless be viewed as performing conceptually similar roles in error-prone lesion bypass.

In contrast to *S. cerevisiae REV1*, the *S. cerevisiae RAD30*-encoded DNA Pol η (31) and its human homolog, XP-V or human *RAD30A* (51, 52), despite being highly error-prone on undamaged templates (53, 90), appear to bypass thymine-thymine cyclobutane dimers in a seemingly accurate manner by inserting two As opposite the lesion (31). The ability (and respective fidelity) of DNA Pol η to bypass other types of DNA lesions is unknown. Finally, a second human and mouse homolog of *S. cerevisiae RAD30*, *RAD30B*, of unknown function, is highly

expressed in the testis (54). Given the extremely poor fidelity of the yeast and human *RAD30*-encoded DNA polymerases, it seems certain that their activities will be managed or coordinated with the action of the cells replicative machinery.

Multiple DNA Polymerases Involved in Lesion Bypass

Given the large number of DNA polymerases now known to exist (Table 1), the recent findings that, depending on the DNA lesion, more than one polymerase may be required for lesion bypass is perhaps not surprising. In this context, bypass is defined as both insertion of a nucleotide opposite the DNA lesion or adduct and its subsequent elongation from that 3' end. For example, the *S. cerevisiae* *RAD30*-encoded DNA Pol η inserts a C opposite an aminoflourine-adducted G in vitro. In addition, it inserts predominantly a G, and less frequently an A, opposite an AP site. In both cases, however, further elongation requires DNA Pol ζ (99). Furthermore, bypass of a benzo(a)pyrene adduct in *E. coli* is dependent on both the *umuDC* and *dinB* gene products (R Napolitano, R Janel-Blintz, J Wagner & RPP Fuchs, personal communication). Together, these results indicate that there likely exists a highly coordinated interplay among multiple DNA polymerases during lesion bypass. Furthermore, the notion that multiple polymerases act in concert to negotiate a lesion suggests that they may serve as interchangeable modules that together help constitute part of a replication factory.

UNDERSTANDING THE PHYSIOLOGICAL ROLES OF THE *umuD*⁺*C*⁺ GENE PRODUCTS IN A CELL'S RESPONSE TO DNA DAMAGE

A Role for *umuD*⁺*C*⁺ in Cell-Cycle Regulation

Recent experiments suggest that the *umuDC* gene products may play physiologically important roles besides serving as a DNA polymerase in TLS. Specifically, uncleaved UmuD in combination with UmuC provides protection from DNA damage that is genetically distinct from that provided by UmuD' and UmuC in TLS, probably by functioning in a primitive DNA damage checkpoint (65). This observation is of particular interest because intact UmuD had previously been viewed as a molecule whose primary roles were confined to (i) being an inactive precursor to UmuD', and (ii) helping to shut off SOS mutagenesis as the cell recovered from DNA damage by trapping UmuD' in the form of UmuD-UmuD' heterodimers (2, 25).

If UmuD and UmuC are acting in a noncatalytic fashion to delay resumption of the cell cycle after DNA damage to allow additional time for accurate repair pathways to act, then the checkpoint would not be effective if the cell lacked accurate DNA repair mechanisms. This is precisely what is observed. The protection provided by intact UmuD and UmuC is eliminated in a cell that is deficient for nucleotide excision repair, the primary accurate repair pathway in *E. coli* (65). A possible mechanism for this checkpoint is suggested by the observation that

uncleaved UmuD in combination with UmuC acts in vivo to inhibit the recovery of DNA replication after DNA damage (65). Therefore, the cleavage of UmuD to UmuD' can be viewed as a timed molecular switch controlling the checkpoint and TLS activities of the *umuD*⁺*C*⁺ gene products. Immediately after DNA damage, UmuD and UmuC are produced, and they act to inhibit the recovery of DNA replication, thereby allowing accurate repair pathways more time to act. However, if the DNA damage signal (and therefore the activated RecA/ssDNA nucleoprotein filament) persists, the pool of UmuD molecules will begin to be converted into UmuD' via RecA/ssDNA nucleoprotein filament-mediated autodigestion. The cleavage of UmuD deactivates the checkpoint and activates TLS. In this way, the cell becomes capable of replicating over any remaining lesions in the template DNA that would normally block further elongation. Supporting this model, measurements of the steady-state levels of the UmuD and UmuD' proteins in the cell indicated that in the first 25 minutes after DNA damage by 25 J/m² of UV, the UmuD protein predominates. After that initial phase, the level of UmuD' protein increases dramatically and this processed form of the molecule predominates (65).

UmuD and UmuC also increase a cell's resistance to DNA damage while it is in stationary phase. Physiologically relevant levels of the *umuDC* gene products increase the survival of UV-irradiated stationary-phase cells in a fashion that correlates with a significant *umuDC*-dependent increase in the length of the lag phase upon nutritional upshift (59). This delay in lag phase could increase DNA damage tolerance and help to avoid induced mutations by allowing extra time for accurate repair before the cells attempt to replicate their damaged DNA. This type of DNA damage checkpoint, which appears to involve uncleaved UmuD acting together with UmuC (59), could be important in nature for cells that have accumulated DNA damage while in a quiescent phase and then have a chance to resume replication upon a nutrient upshift. The *umuDC*-dependent mechanism of increasing UV resistance seems to involve, at least in part, counteracting a Fis-dependent activity (59).

A Model for SOS-Regulated Replisome Restart

Work from a number of different laboratories over the past 15 years has shed some light onto the very complicated process by which *E. coli* reestablishes replication of its genome following replication-arresting damage to its DNA [reviewed in (25) and (14)], a process known as replication restart (18) or induced replisome reactivation (36). DNA replication in wild-type *E. coli* is transiently arrested (for ~1–2 min) immediately following a sublethal UV dose (36). Resumption of DNA replication is dependent on *recA* function, although inhibition is not (36), thus underscoring the close relationship between recombination and replication (14, 25). Further evidence that recombination plays an essential role in replication restart was provided by the finding that *recFR* mutants exhibit a delay in the resumption of DNA synthesis after DNA damage (12, 13). RecFR is proposed to act by helping to protect the nascent strands of disrupted replication forks until such time as the lesion is bypassed and processive replication resumes (12).

In addition to the role(s) played by recombination, at least three DNA polymerases appear to play pivotal roles in reestablishing stalled replication forks. Although the specifics remain ambiguous, it is nevertheless clear that DNA Pol II, DNA Pol III, and the *umuDC*-encoded DNA Pol V are essential for the efficient restart of stalled replication forks (36, 65, 68, 92). It will be interesting to see whether these three polymerases are acting in a carefully regulated manner as interchangeable modules that constitute part of the replication factory, or whether they are acting independently of each other. Finally, a role for the *E. coli* primosome in replication restart has been interpreted as implying that the reestablishment of chromosomal replication requires the reloading of DnaB, the replicative DNA helicase (14, 72, 73).

Managing the Activity of the *umuDC* Gene Products

Given that (i) the *umuDC* gene products appear to serve two temporally separate physiological roles, and (ii) UmuD'₂C possesses a relatively low-fidelity DNA polymerase activity, the actions of the *umuDC* gene products very likely must be controlled or regulated in some way. In agreement with this notion was the finding that UmuD and UmuD' interact physically with the alpha (catalytic), beta (processivity), and epsilon (proofreading) subunits of the *E. coli* replicative DNA polymerase, DNA Pol III (81). Furthermore, the fact that UmuD interacts more strongly with beta than with alpha, whereas UmuD' interacts more strongly with alpha than with beta, is consistent with the *umuDC* gene products serving two temporally separate roles and suggests that the UmuD-beta interaction is important for the proper mediation of the checkpoint control while the UmuD'-alpha interaction is critical for enabling TLS (81). Since these interactions between the *umuDC* gene products and components of the replicative DNA polymerase are likely to dictate which physiological role the *umuDC* gene products will play, they are suggested to be part of a higher-order regulatory system of replication fork management that serves to determine which events occur, and in what order, when the replicative DNA polymerase encounters a DNA lesion (81). Such a series of highly regulated protein-protein and protein-nucleic acid interactions among multiple specialized DNA polymerases within the context of a replication factory might well serve as a mechanism to regulate the activities of the various factory components to allow for maximal bypass efficiency while maintaining a minimal mutation frequency (81).

Genetic Studies of the Relationship of DNA Polymerase III to SOS Mutagenesis

Although UmuD'₂C, together with RecA and SSB, is capable of replication over particular types of DNA lesions *in vitro*, an interesting question remains as to what constitutes the protein machine (or factory) required for lesion bypass *in vivo*. Older models describing possible mechanisms for SOS mutagenesis in *E. coli* postulated that the *umuDC* gene products somehow modified the activity of the replicative DNA polymerase, leading to low-fidelity lesion bypass (reviewed

in 25). However, the recent discovery that the *umuDC* gene products encode a protein complex capable of polymerizing over particular types of DNA lesions (70, 71, 84, 86) strongly supports the model that SOS mutagenesis results from *umuDC*-dependent TLS, and that this *umuDC*-dependent lesion bypass gives rise to mutations. What then is the relationship of DNA Pol III to SOS mutagenesis? Does UmuD'₂C simply act at exposed primer termini at lesion sites or is *umuDC*-dependent TLS coordinated with the action of the replisome? The highly specific physical interactions observed in vitro involving the *umuD* gene products and components of the replicative DNA polymerase, DNA Pol III, led Sutton et al to suggest that UmuD'₂C, RecA and DNA Pol III might act in a coordinated fashion to enable TLS (81). In light of these recent findings, it is worthwhile reviewing some of the older observations regarding the genetic requirements for SOS mutagenesis in *E. coli*, with specific emphasis on the possible involvement of the replicative DNA polymerase.

One line of experimentation that strongly suggests a direct involvement of DNA Pol III in TLS was that overexpression of either the beta processivity clamp (83) or the epsilon 3'-to-5' exonuclease proofreading subunit (21, 22) of DNA Pol III led to an inhibition of SOS mutagenesis. In the case of beta, the inhibition of SOS mutagenesis could be overcome by simultaneous overexpression of the *mucAB* gene products (83); *mucAB* are structural and functional homologs of *umuDC* (25). Although the inhibition of SOS mutagenesis by epsilon was later attributed to the ssDNA binding activity of the epsilon protein (32), an effect on SOS mutagenesis of direct interaction between the *umuDC* gene products and epsilon has not yet been ruled out. Indeed, overexpression of either beta or epsilon, as well as alpha, leads to an inhibition of RecA/ssDNA-facilitated self-cleavage of UmuD to yield UmuD' in vivo (81), suggesting that at least part of the inhibition of SOS mutagenesis by overexpression of beta and epsilon may be due to direct and specific protein-protein interactions between the *umuD* gene products and specific replicative polymerase subunits or subassemblies.

Perhaps the most compelling evidence for a direct role for DNA Pol III in TLS in vivo was the finding that the fixation of induced mutations was temperature dependent in an *E. coli* strain bearing a temperature-sensitive DNA Pol III alpha subunit (4). During incubation of the DNA Pol III temperature-sensitive strain at the nonpermissive temperature, DNA synthesis was observed but there was no fixation of induced mutations. Since DNA replication was still observed during incubation at the nonpermissive temperature, this result was interpreted to suggest that DNA Pol III serves a direct role in TLS in vivo.

Biochemical Evidence for a Role for DNA Polymerase III in Translesion DNA Synthesis

Reevaluation of the recent biochemistry regarding the role of *umuDC* gene products in TLS in the context of the results of the older genetic studies also provides

evidence for a direct role for DNA Pol III in TLS, consistent with the direct interaction between UmuD' and the alpha subunit of DNA Pol III (81). The pioneering work by Echol's group nearly a decade ago demonstrating that denatured/renatured UmuC, together with UmuD', RecA, SSB, and DNA Pol III was capable of TLS in vitro, but the same reaction lacking DNA Pol III was not, was initially interpreted as meaning that DNA Pol III was required for TLS in vitro (67). The more recent finding that highly purified MBP-UmuC was similarly active for TLS in vitro in the presence of DNA Pol III but not in its absence (71) seemed to corroborate the conclusion that DNA Pol III served a direct role in TLS. However, the subsequent finding that further purification of the MBP-UmuC by heparin chromatography obviated the DNA Pol III requirement dispelled this notion (70). Reevaluation of these biochemical observations in light of the results of older genetic analyses suggests the possibility that the UmuC preparations that were inactive for in vitro TLS in the absence of DNA Pol III may have been active in the presence of DNA Pol III due to a direct, physical interaction between the two polymerases that served to stabilize the presumably misfolded UmuC protein.

Additional biochemical evidence that the UmuD'-alpha interaction is important for TLS in vivo was the finding that a crude fraction enriched with UmuD'₂C enhanced the polymerase activity of a temperature-sensitive mutant alpha protein in vitro (86). Furthermore, comparison of the in vitro lesion bypass efficiencies of temperature-sensitive DNA Pol III and partially purified UmuD'₂C indicated that, by far, the most efficient bypass was observed when they were combined, even at temperatures nonpermissive for the DNA Pol III mutant (86). Similar results were observed using wild-type alpha in that the efficiency of *umuDC*-dependent TLS was enhanced in the presence of purified alpha (84).

Another intriguing aspect of TLS relates to the role of the DNA Pol III epsilon subunit. Epsilon possesses the 3' to 5' exonuclease activity and serves as the proofreading subunit of the replicative DNA polymerase (34, 75). Although the exonuclease activity of epsilon is markedly enhanced when it is in complex with the polymerase catalytic subunit, epsilon nonetheless exhibits a significant exonuclease activity on its own (76). Consequently, its exonuclease activity must likely be regulated in some way to allow for efficient TLS in vivo, as has been previously suggested [(47, 87) and reviewed in (25)]. Consistent with this proposal, the exonuclease activity of epsilon is negatively regulated by RecA protein in vitro (47). Thus, RecA, in addition to serving critical roles both in activating the *umuDC* gene products for their role in TLS (8, 63, 77) as well as serving a direct role itself in TLS (17, 63, 82), may help to ensure efficient TLS further by also inhibiting the proofreading activity of the epsilon subunit of DNA Pol III. Furthermore, the direct, physical interaction between UmuD' and the alpha subunit of DNA Pol III has been suggested as possibly being important for attenuating the activity of DNA Pol III during TLS such that it becomes less sensitive to non-cognate base pairs during extension of lesion-containing primer-template termini, thus avoiding futile cycling between its polymerase and exonuclease activities (81).

CONCLUDING REMARKS

Recent work has indicated that UmuC and other members of UmuC/DinB superfamily play a variety of roles in DNA damage-tolerance mechanisms by serving as DNA polymerases that can operate on damaged or imperfect DNA templates. In humans, XP-V appears to promote relatively “accurate” bypass of a thymine-thymine cyclobutane dimer, thereby helping cells to avoid mutations. The finding that, in the absence of XP-V, error-prone TLS provides a potential origin for cancer (29, 52) underscores the importance of elucidating how the activities of the various UmuC/DinB superfamily DNA polymerases are regulated and coordinated with the action of their respective replicative DNA polymerases to allow for maximal bypass efficiency while maintaining a minimal mutation frequency. Presently, UmuC is unique within this superfamily in that the products of the *umuD* gene manage its polymerase activity (63, 81). It is still not known whether the activities of the other family members are similarly regulated. Despite the fact that these newly discovered DNA polymerases appear to be highly distributive in vitro, a characteristic that would seemingly be well suited for their roles as potentially mutagenic bypass polymerases, their exact nature in vivo is unknown. Indeed, the catalytic subunit of the *E. coli* replicative polymerase, DNA Pol III, in the absence of its accessory subunits, is a strictly distributive polymerase that exhibits relatively poor fidelity (57). Therefore, accessory subunits capable of modifying the activities of the respective superfamily members may be discovered, leading to a better understanding of their respective roles in the complex cellular pathways that serve to ensure the integrity of the genome. Furthermore, it will be interesting to see whether any of these newly discovered DNA polymerases participate in cell cycle regulation in response to DNA damage as has been proposed for *umuDC* (65). Given the large number of unanswered questions, it seems certain that future research will lead to new and exciting discoveries concerning how this new family of DNA polymerases acts in helping cells respond to DNA damage.

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