Managing DNA polymerases: Coordinating DNA replication, DNA repair, and DNA recombination

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Two important and timely questions with respect to DNA replication, DNA recombination, and DNA repair are: (*i*) what controls which DNA polymerase gains access to a particular primer-terminus, and (*ii*) what determines whether a DNA polymerase hands off its DNA substrate to either a different DNA polymerase or to a different protein(s) for the completion of the specific biological process? These questions have taken on added importance in light of the fact that the number of known template-dependent DNA polymerases in both eukaryotes and in prokaryotes has grown tremendously in the past two years. Most notably, the current list now includes a completely new family of enzymes that are capable of replicating imperfect DNA templates. This UmuC-DinB-Rad30-Rev1 superfamily of DNA polymerases has members in all three kingdoms of life. Members of this family have recently received a great deal of attention due to the roles they play in translesion DNA synthesis (TLS), the potentially mutagenic replication over DNA lesions that act as potent blocks to continued replication catalyzed by replicative DNA polymerases. Here, we have attempted to summarize our current understanding of the regulation of action of DNA polymerases with respect to their roles in DNA replication, TLS, DNA repair, DNA recombination, and cell cycle progression. In particular, we discuss these issues in the context of the Gram-negative bacterium, *Escherichia coli*, that contains a DNA polymerase (Pol V) known to participate in most, if not all, of these processes.

The boundaries that once separated the fields of DNA replication, recombination, and repair have become increasingly blurred in the last few years. Recent advances in each of these three fields have not only illuminated the molecular mechanisms of the individual processes, but have also provided significant insights into their interrelatedness and codependence. For example, recent studies indicate that the *Escherichia coli* RecA protein is not only required for homologous recombination, but is also required for efficient chromosomal DNA replication even under normal growth conditions (1, 2), as well as for the regulation of cellular responses to DNA damage and the replication of damaged DNA (3–5). Furthermore, DNA replication by specialized DNA polymerases, such as the *umuDC*-encoded DNA polymerase V in *E. coli* (6, 7), underlies the molecular mechanism of translesion DNA synthesis, a major source of mutagenesis in living cells (3, 4, 8).

In this report, we have attempted to summarize not only our current understanding of how cells regulate the action of their various DNA polymerases, but also how this regulation may be coordinated with DNA replication, recombination, and repair. Although we discuss these issues as they are currently understood in both eukaryotes and prokaryotes, we pay special attention to how *E. coli* regulates the actions of its five different DNA polymerases, particularly Pol III and Pol V, because it represents the paradigm for the study of DNA replication, recombination, and repair at both the genetic and biochemical levels.

A Superfamily of DNA Polymerases Involved in Replication of Imperfect DNA Templates. Recently, the field of translesion DNA synthesis and induced mutagenesis has generated a great deal of excitement because of the discovery that key gene products required for these processes, in both prokaryotes (9, 10) and in eukaryotes (11, 12), possess an intrinsic DNA polymerase activity (refs. 6, 7, and 13–20 and reviewed in refs. 21–24). A common, defining feature of these DNA polymerases is a remarkable ability to replicate imperfect DNA templates. Depending on the DNA polymerase, these include templates such as those containing a misaligned primer–template junction (13), an abasic site (6, 7), a cyclobutane dimer (15, 16, 25), or a pyrimidine–pyrimidone (6–4) photoproduct (25). These newly discovered DNA polymerases contain highly conserved blocks of amino acid sequences (26) and constitute a new superfamily of novel DNA polymerases termed the UmuC-DinB-Rad30-Rev1 superfamily because the two *E. coli* members, UmuC and DinB, and the two *Saccharomyces cerevisiae* members, Rad30p and Rev1p, define its four subfamilies. For brevity, we will refer to it as the UmuC superfamily because UmuC was its founding member (9, 10, 27, 28).

Humans have at least four members of this superfamily. These include two members of the RAD30 subfamily, Pol η encoded by the *hRAD30A*/XP-V gene (16, 29) and Pol ι encoded by the *hRAD30B* gene (30); Pol κ encoded by the *hDINB1* gene (18, 26); and *hREV1* gene product (31). Pol η is mutated in individuals having the xeroderma pigmentosum-variant (XP-V) defect (32, 33). The xeroderma pigmentosum (XP) genetic disorder is characterized by an unusually high sensitivity to UV light (UV) that results from an inability to cope properly with UV-induced DNA lesions (reviewed in ref. 34). XP-V is unique in that it is the only one of the eight XP genetic complementation groups that is not deficient in nucleotide excision repair of DNA lesions (3). Biochemical characterization of human Pol η indicates that it is able to bypass *cis-syn* cyclobutane dimers in a relatively accurate fashion by inserting two adenines opposite the lesion (16, 29, 35). The current model to describe the molecular events underlying the response of XP-V individuals to UV suggests that, in the absence of a functional Pol η, cyclobutane dimers are bypassed by a different polymerase such as the error-prone polymerase Pol ζ (16, 29, 35). The reduced accuracy of translesion DNA synthesis (TLS) over cyclobutane dimers leads to an increased mutation frequency that contributes to the XP-V disorder.

What Controls Which DNA Polymerase Acts at a Given Primer Terminus? The discovery of the UmuC superfamily of DNA polymerases, taken together with the recent discovery of additional

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Abbreviations: ssDNA, single-stranded DNA; PCNA, proliferating cell nuclear antigen; XP, xeroderma pigmentosum; XP-V, XP-variant.

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eukaryotic DNA polymerases [i.e., Pol λ (36, 37), Pol μ (37, 38), Pol θ (39), and Trf4p (40)], further complicates the already daunting issue of understanding the control systems that govern which DNA polymerase gains access to a given DNA primer terminus, and, subsequently, whether the polymerase hands off the DNA substrate to yet another DNA polymerase or to a different enzyme.

All template-directed DNA polymerases must recognize and bind to the 3′ end of primer termini that occur at nicks or single-strand gaps within double-stranded DNA (41). Consequently, because DNA molecules with such nicks and gaps are also substrates for other metabolic DNA processes, including recombination (3), base excision repair (3), nucleotide excision repair (3), and DNA replication itself (41), a DNA polymerase cannot "know" *a priori* whether a given primer terminus is part of a replication fork, or whether it is intended for a different biological transaction. To some extent, which DNA polymerase binds to a given primer terminus can be controlled by regulating the expression, activity, or localization of DNA polymerases. However, indications that such control systems may not be sufficient can be seen even in *E. coli*, where three different DNA polymerases (Pol II, Pol IV, and Pol V) with different properties are induced by DNA damage (42, 43). The situation is even more complicated in mammalian cells, which are now known to contain at least 14 distinct DNA polymerases (Table 1), as well as numerous template-independent terminal dNTP transferases. A growing body of evidence suggests that an important additional level of control results from DNA polymerases being "coached" as to their correct biological role through interactions with other proteins associated with the particular DNA substrate. We have suggested (44, 45) that such protein–protein interactions constitute a higher-order regulatory system of replication fork management.

Coordination of Multiple DNA Polymerases During Chromosomal DNA Replication in Eukaryotes. In an effort to put the management of DNA polymerase action into perspective, it is worth briefly reviewing what is known about the regulation and coordination of the various DNA polymerases involved in replication of a cell's genetic material. As described below, the coordinated management of the actions of multiple polymerases is an important aspect of DNA replication. Current models to describe the molecular mechanisms of chromosomal DNA replication in eukaryotes and prokaryotes represent the only well studied examples of multiple DNA polymerases being simultaneously involved in a common biological process (41). However, it should be stressed that our understanding of the regulation of the activities of the various replicative DNA polymerases involved in chromosomal replication is still very much in its infancy.

Models to describe chromosomal replication in eukaryotes implicate three distinct DNA polymerases, Pol α, Pol δ, and Pol ε (reviewed in refs. 46–48). Here we will discuss only the events involved in the elongation phase of DNA replication. Pol α, with its associated DNA primase activity (41), is generally accepted as serving roles in both the initiation of DNA replication, as well as in the repeated priming events necessary for replication of the lagging strand (46). Pols δ and ε are thought to serve roles as the major replicative DNA polymerases, with Pol δ acting on the leading strand, and Pol ε (or possibly Pol δ) acting on the lagging strand (46). Given that chromosomal replication in eukaryotes involves the coordinated action of at least three distinct DNA polymerases, there must be some intricate regulation of their respective activities to ensure the efficient duplication of the cell's genetic material.

An important question regarding DNA replication relates to polymerase switching. Polymerase switching refers to the coordinated hand-off of the DNA template from one DNA polymerase to another. There are at least two instances where this must occur during chromosomal replication in eukaryotes. The first is the initial priming event for leading strand fork formation. This process involves priming by Pol α and its associated primase subunit. After primer synthesis, however, Pol α must hand over the DNA template to Pol δ (and or possibly Pol ε). The second instance of polymerase switching is the precisely repeated priming and subsequent elongation of Okazaki fragment synthesis on the lagging strand. Here again Pol α, together with its associated primase activity, must repeatedly prime the lagging strand for subsequent elongation by Pol ε (or possibly Pol δ). Recent work suggests that a series of competitive protein–protein interactions involving Pol α, Pol δ, proliferating cell nuclear antigen (PCNA; the eukaryotic counterpart to the β processivity clamp), and the PCNA loader complex, Replication factor C (RFC), each involving Replication Protein A (RPA), the eukaryotic single strand DNA binding protein, underlie the polymerase switching mechanism (49–51).

Examples of further coordinated hand-offs of the DNA template by a DNA polymerase to another enzyme during DNA replication include the final processing steps of the Okazaki fragments by the actions of FEN1 (52) and DNA ligase I (53), each in association with PCNA. A common feature of many factors involved in lagging strand synthesis is their ability to interact with PCNA (54). Thus, it is perhaps not unexpected that the eukaryotic clamp loader complex, RFC, appears to play a pivotal role in polymerase switching (48, 50).

Coordination of Multiple DNA Polymerases During Chromosomal DNA Replication in Prokaryotes. Chromosomal DNA replication in prokaryotes appears to be somewhat simpler than that in eukaryotic organisms in that only a single origin of replication is used per DNA molecule instead of the many used by eukaryotic organisms, and, under normal circumstances, only two DNA polymerases appear to be involved (41). In *E. coli*, the elongation phase involves the coordinated action of both Pol III and Pol I (41, 55). In contrast to the compact nature of the Pol I enzyme that is fully contained within a single polypeptide chain (41), Pol III is a ten-subunit, 18-polypeptide protein machine termed Pol III holoenzyme (55). This sophisticated protein machine consists of (*i*) a core complex, composed of the α (catalytic), ε (proofreading), and θ (unknown function) subunits; (*ii*) a dimer of the τ subunit, which serves to bridge two core complexes; (*iii*) a processivity clamp, which consists of a homodimer of the β subunit that topologically tethers the core complex to the DNA; and (*iv*) the five-subunit (γ, δ, δ′, ψ, and χ) clamp loading complex termed the γ complex (41, 55). Interestingly, the γ complex serves triple duty; in addition to actively recycling β clamps during replication of the lagging strand by loading a clamp at the start of each Okazaki fragment (55), the χ subunit of the γ complex interacts with single-strand DNA binding protein (SSB; 56–58). This interaction is not only important for DNA replication (56, 58), but also serves a critical role in displacing primase bound to the nascent RNA that serves as primer for each Okazaki fragment, thereby allowing the binding of Pol III for subsequent elongation (57).

In contrast to eukaryotes, priming of lagging strand in *E. coli* is performed by DnaG primase (41). This enzyme is targeted to the replication fork by a direct interaction with the moving replicative helicase, DnaB (59). It then synthesizes a short primer, to which it remains bound (57). The interaction between primase and the nascent primer is stabilized by its association with SSB (57). Interaction of the χ subunit of the γ complex of Pol III with SSB serves to displace primase from the primer–template junction, thereby permitting γ complex to load a β clamp (57). Subsequent translocation of the lagging strand Pol III assembly to the newly primed Okazaki fragment leads to elongation. When Pol III reaches the previously synthesized Okazaki fragment, the process is repeated. Pol I, with its

Table 1. Biochemically documented template-dependent DNA polymerases found in humans or *E. coli*

Name	Alternate designations	Polymerase family	Function (or functions)
Human			
Pol γ		A	Mitochondrial DNA replication and repair
Pol θ	PolH	A	? Repair of DNA cross-links
Pol α		B	Priming DNA synthesis
Pol δ		B	DNA replication; nucleotide and base excision repair
Pol ε		B	DNA replication; nucleotide and base excision repair
Pol ζ	Rev3p	B	TLS
Pol β		X	Base excision repair
Pol λ		X	?
Pol μ		X	?
Trf4p	Pol κ	X	Sister chromatid cohesion
Pol η	Rad30Ap XP-V	UmuC	Relatively accurate TLS past *cis-syn* cyclobutane dimers
Pol ι	Rad30Bp	UmuC	?
Pol κ	DinB1p PolQ Pol θ	UmuC	?
Rev1p	Deoxycytidyl transferase	UmuC	TLS
E. coli			
Pol I		A	Maturation of Okazaki fragments; nucleotide excision repair
Pol II	DinA	B	Induced replisome reactivation; TLS
Pol III		C	DNA replication; nucleotide excision repair
Pol IV	DinB	UmuC	Adaptive mutagenesis, TLS
Pol V	UmuC or UmuD′$_2$C	UmuC	TLS (“SOS mutagenesis”)

See text and ref. 24 for additional information.

associated 5′–3′ FEN1-like activity, then acts together with DNA ligase to carry out the important task of Okazaki fragment maturation (41). However, little is known about the coordination of the actions of Pol I and Pol III during Okazaki fragment maturation. Given the important roles played by PCNA and RFC in Okazaki fragment maturation in eukaryotes, it is not unexpected that the β clamp and γ complex of Pol III serve roles in this process in prokaryotes (60).

Other Physiological Roles of DNA Polymerases Besides Chromosomal DNA Replication. In addition to their roles in chromosomal DNA replication, DNA polymerases participate in numerous DNA repair pathways, including double-strand break repair (61–63), mismatch repair (3, 64, 65), base excision repair (3, 66, 67), and nucleotide excision repair (3). Furthermore, as discussed at this colloquium, at least in prokaryotes RecA-ssDNA-mediated homologous recombination serves to restart stalled or collapsed replication forks that arise during normal DNA replication (1, 68), whereas a specialized mechanism of DNA replication in *E. coli*, termed induced replisome reactivation (IRR) (69) or replication restart (70), occurs in response to DNA damage. The latter process involves the almost immediate arrest of replication in response to DNA damage, followed by a resumption of DNA synthesis and involves the coordinated actions of at least four different DNA polymerases, including Pols I, II, III, and V (a role for Pol IV has not yet been described or precluded) (4, 71).

In addition to these enzymatic roles, certain DNA polymerases undergo other classes of interactions because of the roles they play in checkpoint control systems. These cell cycle surveillance mechanisms ensure that the various processes required for cell cycle progression occur in the correct order, and that each process is appropriately completed before initiation of subsequent events (72, 73). In eukaryotes, both Pol α and Pol ε participate in cell cycle checkpoint controls (reviewed in ref. 48). In the case of Pol α, genetic analyses suggest that its primase component is an important target for the S-phase checkpoint control in *S. cerevisiae* (74). Furthermore, the finding that Cdc45p appears to facilitate the loading of Pol α onto origins and that this ability is down-regulated following activation of the S phase checkpoint has been interpreted as evidence for an additional target for checkpoint regulation (75). In contrast to Pol α, it has been suggested that *S. cerevisiae* Pol ε acts as a sensor of DNA damage during DNA replication, thereby coordinating replication blocks with the appropriate transcriptional and cell cycle responses (76). In *E. coli*, when the UmuC protein is in a different complex (UmuD$_2$C) than that involved in TLS (UmuD′$_2$C), it has been shown to modulate the growth rate as cells exit stationary phase and enter into exponential growth (77). In addition, UmuD$_2$C has also been shown to regulate the rate of DNA replication in response to DNA damage (78, 79). These checkpoint functions of the *umuDC* gene products appear to act to regulate DNA replication following instances in which cells have acquired damage to their DNA [e.g., either after exposure to UV light or prolonged incubation under nondividing (stationary phase) conditions].

***E. coli* DNA Polymerase V (*umuDC*): Lessons Learned from More than 25 Years of Studying a Lesion-Bypass DNA Polymerase.** The *E. coli umuC* gene and its partner *umuD* have been intensively studied for more than 25 years because their functions are required for most UV and chemical mutagenesis in *E. coli*, a process known as SOS mutagenesis (9, 10). As a consequence, the protein–protein interactions that UmuC undergoes while fulfilling its biological roles are better understood at present than for any other member of the UmuC superfamily. In an effort to illustrate some of the general principles that may apply to the management of DNA polymerases, we will summarize lessons that have been learned concerning how the physiologically correct action of the *umuDC*-encoded DNA polymerase is ensured and how it is coordinated with other cellular processes.

***E. coli* DNA Polymerase V (*umuDC*) Is Regulated both Transcriptionally and Posttranslationally.** One straightforward method of managing the action of a DNA polymerase is to confine its presence to the appropriate environmental, cellular, or development context by regulating the expression of the gene or genes encoding the DNA

polymerase. In the case of *E. coli* DNA pol V, the expression of the genes encoding it, *umuDC*, are induced by DNA damage as part of the SOS response (3–5). In this intricately regulated stress response, the expression of more than 40 genes is induced when RecA protein, the main bacterial recombinase (3, 80), binds to single-stranded DNA (ssDNA) generated by the cell's failed attempts to replicate over DNA lesions (3, 81). Although transcription of these genes is normally repressed by the LexA protein in the absence of DNA damage, these RecA-ssDNA nucleoprotein filaments facilitate the latent capacity of LexA to autodigest (82, 83). LexA cleavage inactivates it as a transcriptional repressor, leading to an increased level of transcription for the LexA-regulated genes, including the *umuDC* operon (3). Translation of the *umuDC* mRNA results in the synthesis of the 139-aa UmuD protein and the 422-aa UmuC protein (28).

However, the transcriptional induction of the *umuDC* operon and the synthesis of the UmuC and UmuD proteins do not result in the production of an active lesion bypass DNA polymerase. Instead, UmuD must first be modified posttranslationally by undergoing a RecA-ssDNA facilitated self-cleavage reaction that is mechanistically similar to that undergone by LexA (84–86). This RecA-ssDNA-facilitated autodigestion reaction removes the N-terminal 24 residues of UmuD to yield a derivative termed UmuD′. UmuD and UmuD′ each form homodimers and, in addition, interact with each other to form a heterodimer that is more stable than either of the homodimers (87). All three classes of dimers interact with UmuC (88) and greatly influence its action (7, 44, 78). Despite the fact that UmuC, like other members of the UmuC superfamily, contains an intrinsic catalytic DNA polymerase activity (7), the purified protein has a strict requirement for the presence of UmuD′ to function as a polymerase on damaged DNA (7). Thus, *E. coli* uses both transcriptional and posttranslational control to ensure that DNA Pol V in only present in cells that have suffered DNA damage.

It is interesting to note that the requirement for SOS induction and for UmuD cleavage was discovered before the recognition that UmuC was a member of a superfamily of DNA polymerases. Instead these insights were gained through genetic and physiological studies in which SOS mutagenesis, the ultimate biological consequence of TLS by Pol V, was used as the experimental endpoint. In fact, when UmuC was finally purified in a soluble form, the observed requirement for UmuD′ for TLS *in vitro* provided assurance that the biochemical properties of UmuC being studied were indeed relevant to its biological role in translesion DNA synthesis.

Translesion Synthesis by DNA Pol V also Requires RecA and SSB. Genetic and physiological studies had also established that RecA plays a mechanistic role in SOS mutagenesis beyond its involvement in facilitating LexA and UmuD cleavage (reviewed in ref. 3). When *umuDC*-dependent TLS was subsequently reconstituted by using purified proteins, the test of biological relevancy was again satisfied when it was found that TLS by Pol V had strict requirement for RecA (6, 7, 89). It has been suggested that interactions of the $UmuD'_2C$ complex with the end of RecA-ssDNA filaments position Pol V for its role in the TLS responsible for SOS mutagenesis (90–93); this positioning seems most likely to involve a direct interaction between UmuD′ and RecA (90, 92, 94). It thus appears that this interaction between RecA positioned at a site of DNA damage and UmuD′, a component of Pol V, may represent an example of how one polymerase is selectively directed to a particular class of primer termini despite the simultaneous presence of several other DNA polymerases.

In vitro characterization of Pol V also revealed a strict requirement for SSB (ssDNA binding protein) (6, 7). It seems likely that SSB's role is not only to bind ssDNA, but also to contact UmuC because evidence has been reported that MucB, a homolog of UmuC, can interact directly with SSB and induces a major conformational change in its complex with ssDNA (95). By analogy to current models of polymerase switching in eukaryotes and prokaryotes discussed above, the interaction of UmuC or MucB with SSB may serve an important role in the loading of these lesion bypass DNA polymerases at the replication fork. Although the significance of this additional interaction with UmuC remains to be determined, it is interesting to note that the $UmuD'_2C$ complex binds cooperatively to single-stranded DNA (96). The relationship of this property to UmuC's biological roles remains to be determined. It is also possible that $UmuD'_2C$-dependent TLS is facilitated by the β processivity clamp and γ clamp loader complex of Pol III (6, 25), although this interaction has not been observed in a different assay system (7).

A DNA Polymerase Protein Can Have Additional Biological Roles. The *umuDC* gene products appear to have additional biological roles besides participating in SOS mutagenesis as Pol V. Not only transcriptional and posttranslation regulation, but specific protein–protein contacts as well appear to be important in controlling these other roles. One of these roles has been suggested based on the ability of overexpressed levels of $UmuD'_2C$ to inhibit RecA-mediated homologous recombination (93). Devoret and his colleagues (93, 97) originally suggested that the interaction of $UmuD'_2C$ with RecA not only targets Pol V to the site of DNA lesions, but also acts to influence which DNA repair pathways are operative by simultaneously inhibiting homologous recombination. This inhibition of recombination by $UmuD'_2C$ has been observed *in vitro* by using purified components (98) and the binding of $UmuD'_2C$ to RecA-ssDNA filaments has been visualized by cryo-electron microscopy (92). These findings have led to the suggestion that this modulation by $UmuD'_2C$ promotes a transition from an accurate mode of recombinational repair to one of error-prone DNA replication.

In addition to functioning in SOS mutagenesis, the *umuDC* gene products, in the form of the $UmuD_2C$ complex (but not the $UmuD'_2C$ complex), participate in a DNA damage checkpoint control that appears to help slow down the rate of DNA replication in response to DNA damage, thereby allowing additional time for accurate repair processes, such as nucleotide excision repair, to remove lesions from the DNA before attempts to replicate the genome (78). This DNA damage checkpoint control function of the *umuDC* gene products presumably helps to promote cell survival by preventing more serious types of DNA damage from arising by attempting to replicate damaged DNA. UmuD′ is not able to participate in the checkpoint (78). Measurements of the steady-state levels of the UmuD and UmuD′ proteins in the cell indicate that in the first 25 min after DNA damage by 25 J/m^2 of UV, the UmuD protein predominates (78). After that initial phase, the level of UmuD′ protein increases dramatically and this processed form of the molecule predominates. Thus, the checkpoint role of the *umuDC* gene products is separated from their TLS/recombination-inhibition roles by temporally separating the production of UmuD from that of UmuD′. Factors that contribute to the temporal separation of UmuD and UmuD′ production include not only the slow rate of UmuD cleavage relative to LexA cleavage, but also the susceptibility of UmuD (relative to UmuD′) to degradation by the Lon protease (99, 100), the selective degradation of UmuD′ within UmuD′-UmuD heterodimers by the ClpXP protease (100, 101), and possibly modulation of the rate of UmuD cleavage by the SOS-induced *dinI* gene product (102).

Interactions with Components of the Replicative DNA Polymerase Appear to Play a Role in TLS and Checkpoint Functions of the *umuDC* Gene Products. Both the DNA damage checkpoint function and the TLS function of the *umuDC* gene products involve some

aspect of DNA replication. We therefore reasoned that particular protein–protein interactions between the *umuDC* gene products and components of the *E. coli* replisome might play a role in ensuring that the *umuDC* gene products act in the appropriate manner. This hypothesis was supported by our discovery that both UmuD and UmuD′ interact with the α (catalytic), ε (proofreading), and β (processivity) subunits of Pol III (44) and that they do so in distinctive fashions (44). In a comparison of the affinities of $UmuD_2$ and $UmuD'_2$ for each subunit of pol III, $UmuD_2$ was found to bind most tightly to the β sliding clamp, whereas $UmuD'_2$ was found to interact most strongly with the α catalytic subunit. These findings are consistent with the interpretation that interaction of UmuD with β helps to regulate the rate of DNA replication in response to DNA damage, whereas interaction of UmuD′ with α helps to enable TLS.

The α subunit appears to be particularly important for translesion synthesis by Pol V. By using purified protein to reconstitute TLS *in vitro*, it was found that high concentrations of $UmuD'_2C$ in the absence of the α subunit of pol III cannot promote translesion synthesis as efficiently as low levels of $UmuD'_2C$ with low levels of α (6). These findings agree well with genetic evidence indicating that the α subunit is important for SOS mutagenesis in the living cell (summarized in refs. 103 and 104). A direct interaction between Pol V and the α subunit of Pol III is also indicated by the observation that the presence of $UmuD'_2C$ stabilizes a mutant temperature-sensitive α protein (6). The observation that overexpression of the β clamp inhibits SOS mutagenesis *in vivo* was initially interpreted as meaning that the β clamp served an important role in $UmuD'_2C$-dependent TLS (105). However, the subsequent finding that β interacts more strongly with UmuD than it does with UmuD′ suggests that the interaction is important for the checkpoint function of $UmuD_2C$ (44) [although a stimulatory role for the β clamp in $UmuD'_2C$-dependent TLS *in vitro* has been reported (106)]. Viewed in this way, the $UmuD_2C$-dependent DNA damage checkpoint control has similarities with the proposed p21-dependent regulation of PCNA in humans (107).

Recently, we have further characterized the interactions of the various forms of the *umuD* gene product with the α, ε, and β subunits of Pol III both genetically and biochemically. As part of this effort, we have also further characterized the ability of elevated levels of the *umuDC* gene products to confer a cold-sensitive growth phenotype. This phenomenon was first noted 15 years ago and relates to the inability of an *E. coli* strain expressing elevated levels of the *umuDC* gene products to grow at 30°C (108). This cold sensitivity correlates with a rapid inhibition of DNA synthesis at the nonpermissive temperature, and is particularly pronounced as cells are exiting from stationary phase and making the transition into exponential growth (77, 108). Our recent characterizations of this phenomenon indicate that the cold sensitivity is independent of the catalytic DNA polymerase activity of the UmuC protein (109, 110). In these experiments we took advantage of the *umuDC104* allele, which encodes a UmuC derivative with a D104N substitution in a highly conserved motif found in all members of the UmuC superfamily that abolishes the catalytic DNA polymerase activity *in vitro* (6, 7). We found that the *umuDC104* allele was as proficient as the wild-type *umuDC* allele in conferring cold sensitivity, but was inactive in SOS mutagenesis (110). In contrast, overexpression of the *umuDC125* allele, which encodes a UmuC derivative with an A39V substitution, did not confer cold sensitivity, despite the fact that this allele was largely proficient for SOS mutagenesis (110). All of these findings, taken together with the fact that modest overexpression of the *umuDC* gene products conferred the cold sensitive growth phenotype, whereas similarly elevated levels of the *umuD′C* gene products did not (110), led us to conclude that the cold sensitivity conferred by elevated levels of the *umuDC* gene products is due to a function or functions of $UmuD_2C$ involved in the DNA damage checkpoint control (77, 78, 108–110). Thus, we exploited this phenomenon to identify elements of the checkpoint control system by characterizing the effects on $UmuD_2C$-dependent cold sensitivity of overexpression of the individual subunits of Pol III (111, 112).

Of the ten different Pol III subunits, overexpression of only the ε (proofreading) or β clamp subunits affected the degree of cold sensitivity conferred by elevated levels of the *umuDC* gene products (111, 112). Interestingly, whereas overexpression of ε or deletion of its structural gene (*dnaQ*) suppressed, albeit to varying degrees of efficiency, $UmuD_2C$-dependent cold sensitivity (111), overexpression of β significantly exacerbated the extent of cold sensitivity (112). Our further analyses of the effects of elevated levels of ε on $UmuD_2C$-dependent cold sensitivity indicated that it correlated with a suppression of the inhibition of DNA synthesis that serves as the mechanistic basis for the cold sensitivity (111). These finding, taken together with our observation that UmuD and UmuD′ interact specifically with the C-terminal domain of ε suggested that the *umuDC* gene products help to enable a DNA damage checkpoint control through specific interaction with the ε subunit of Pol III (111, 112). The recent finding that α also interacts with the small C-terminal domain of ε (113, 114) suggests that the $UmuD_2C$-dependent checkpoint might involve the sequestration of ε away from Pol III, thereby affecting the DNA polymerase activity of the replisome leading to the replication arrest observed *in vivo* (111, 112).

Likewise, our finding that overexpression of the β subunit of Pol III exacerbated $UmuD_2C$-dependent cold sensitivity suggested that interaction of $UmuD_2C$ with β helps to enable a DNA damage checkpoint control (112). Because of the extremely strong cold sensitivity conferred by the simultaneous overexpression of β together with either the *umuDC* or the *umuD′C* gene products, we were able to select for mutants of β deficient for this exacerbation (112). This approach resulted in the identification of eight unique mutant β proteins, each resulting from a single amino acid substitution, that were unable to confer a cold sensitive growth phenotype on either a *umuD′C*- or a *umuDC*-expressing strain, but retained at least partial activity for chromosomal replication. Interestingly, seven of these eight mutations map to the same surface of the β clamp that is believed to interact with the clamp loader and the α catalytic subunit of Pol III (112), a finding that echoes our observation that UmuD and UmuD′ also interact with the same domain of ε that has recently been shown to interact with α (111). Our observation that at least one of these eight mutant β proteins is severely affected for interaction with UmuD *in vitro* as measured by solution cross-linking (M.D.S. and G.C.W., manuscript in preparation) indicates that the exacerbation of $UmuD_2C$-dependent cold sensitivity by elevated levels of β *in vivo* is in fact related to the ability of UmuD and β to physically interact (44). These findings, taken together, suggest that the $UmuD_2C$-dependent DNA damage checkpoint control involves the sequestration of both the ε and β subunits of Pol III away from the replisome (111, 112). A satisfying aspect of this model is that both ε and β enhance the processivity of Pol III (55), thus making them ideal targets for a DNA replication checkpoint.

The β Processivity Clamp of Pol III Communicates Directly with Multiple Proteins to Promote DNA Replication and DNA Repair. Our biochemical characterization of the mutant β proteins described above indicates that the same face of the β clamp interacts with at least three different protein complexes, namely (*i*) $UmuD_2C$ (and possibly $UmuD'_2C$ as well), (*ii*) the α catalytic subunit of Pol III, and (*iii*) the clamp loader complex. Given the strong similarities between *E. coli* and eukaryotes with respect to DNA replication and repair (see above), it is likely that β will interact

with additional factors involved in DNA replication, DNA repair, and possibly recombination as well. Consistent with this notion, a report by López de Saro and O'Donnell in this issue (60) discusses interactions involving the β clamp with Pol I and DNA ligase, which play roles in the maturation of Okazaki fragments, as well as with MutS, which acts in methyl-directed mismatch repair. Although one can easily imagine how the association of $UmuD_2C$ with β could effectively block access of the β clamp to the α and γ complex components of Pol III, it prompts the important question of how these various protein–protein interactions are coordinately regulated such that the correct protein complex is formed.

As discussed above, the clamp loader complex of Pol III serves an important role in displacing primase and recycling β clamps during lagging strand replication (57). A poorly understood component of this process, however, relates to the release of the newly loaded β clamp from the clamp loader complex to allow for its association with the α catalytic subunit of Pol III. The factor (or factors) that governs this process clearly serves an important role helping to determine which DNA polymerase will gain access to the replication fork. Given that Pol III and the clamp loader complex can exist as part of the same multiprotein machine, this process is likely to be achieved via a coordinated set of protein–protein and protein–nucleic acid interactions. Our current challenge is to better understand these interactions at a molecular level.

Genetic and Biochemical Evidence Suggesting a Need for Multiple DNA Polymerases for Bypassing Certain DNA Lesions. A growing body of evidence suggests that TLS is even more complicated than initially thought, and can involve multiple DNA polymerases and hence multiple polymerase-switching events. Regardless of how one views TLS, the simplest of models would assume that the replicative polymerase, being unable to bypass a lesion in the DNA, would either "fall off" of the DNA or simply translocate downstream of the lesion to continue replication. This would then allow for the loading of another DNA polymerase capable of replicating over the lesion. Eventually, the replicative polymerase would regain control of the template until such time as replication was completed or the polymerase again encountered a replication-blocking DNA lesion. Even in this overly simplistic model, one must account for at least two events of polymerase switching; first from the replicative polymerase to the TLS polymerase, and then back to the replicative polymerase. Given its distributive nature, Goodman (71) has suggested that after Pol V has bypassed a lesion, it "falls off" of the DNA, thereby allowing the reassociation of Pol III. However, the various interactions between the *umuDC* gene products and Pol III summarized above (44) suggest that a set of protein–protein contacts may guide these switches.

Recently, it has become evident that lesion bypass can be considerably more complicated than the above model would suggest. The notion of multiple DNA polymerases being required for bypass of a single DNA lesion, although in the forefront of discussion today, was first suggested nearly 5 years ago by Nelson *et al.* (14, 115). These two reports represent the first direct biochemical demonstrations of two key aspects of TLS. First, they showed that *S. cerevisiae* Rev1 protein (a member of the UmuC superfamily) contains an intrinsic DNA polymerase activity (14), albeit with remarkably limited abilities [Rev1p contains a template-dependent (but not template-directed) deoxycytidyl transferase activity], and second, they demonstrated that the combined actions of *S. cerevisiae* REV1 and *S. cerevisiae* Pol ζ was more effective at enabling replication past a synthetic abasic site than either enzyme alone (115). As discussed above, it is now generally appreciated that proteins related to *S. cerevisiae* Rev1p also contain an intrinsic DNA polymerase activity, and more recently, that multiple specialized DNA polymerases are involved in bypassing various types of DNA lesions.

Some specific examples of a need for multiple DNA polymerases, in addition to the replicative DNA polymerase, for efficient bypass of certain types of lesions include (*i*) bypass of a synthetic abasic site *in vitro* by the combined actions of human Pol η followed by *S. cerevisiae* Pol ζ (116), (*ii*) bypass of highly distorting or noninstructive DNA lesions *in vitro* by the sequential actions of human Pol ι followed by *S. cerevisiae* Pol ζ (117), and (*iii*) the requirement of both Pol IV and Pol V of *E. coli* for bypass of a benzo[*a*]pyrene adduct *in vivo* (118).

Perspectives and Conclusion. The *E. coli umuDC*-encoded Pol V-dependent SOS response represents the paradigm for the study of TLS and induced mutagenesis. Significant advances have been made in understanding how a set of elaborate regulatory controls and a sophisticated system of protein–protein contacts ensure that the *umuDC* gene products carry out their appropriate biological roles. However, as is so often the case in science, the discoveries of today are posing even more challenging questions for tomorrow. For example, some poorly understood general issues that lie at the heart of not only TLS, but also DNA replication itself, include (*i*) an understanding of the molecular mechanism of polymerase switching, (*ii*) the identity and mode of action of factors involved in coordinating DNA replication with DNA repair, including cell cycle and checkpoint regulation, and determining how they act, and (*iii*) the controls systems governing the relationship between DNA replication and DNA recombination. Furthermore, there is growing evidence that DNA replication takes place in stationary factories (119–122) so that understanding the events such as polymerase-switching or the recombinational initiation of DNA synthesis in the context of these factories will be a challenging but fascinating problem. We anticipate that the lessons learned from studying the coordinated regulation of the actions of the five *E. coli* DNA polymerases, together with their associated accessory factors, in the context of DNA replication, DNA repair, recombination, and cell cycle progression, will help to provide a framework for characterizing similar control networks in eukaryotes, where both the number of DNA polymerases and the level of complexity of the events are far greater.

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