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Karel Naiman, Gaëlle Philippin, Robert P Fuchs, Vincent Pagès. Chronology in lesion tolerance gives priority to genetic variability.. Proceedings of the National Academy of Sciences of the United States of America, 2014, 111 (15), pp.5526–5531. 10.1073/pnas.1321008111 . hal-01446624

HAL Id: hal-01446624

<https://hal.science/hal-01446624>

Submitted on 8 Mar 2022

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Chronology in lesion tolerance gives priority to genetic variability

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Edited by Stephen C. Kowalczykowski, University of California, Davis, CA, and approved March 4, 2014 (received for review November 8, 2013)

The encounter of a replication fork with a blocking DNA lesion is a common event that cells need to address properly to preserve genome integrity. Cells possess two main strategies to tolerate unrepaired lesions: potentially mutagenic translesion synthesis (TLS) and nonmutagenic damage avoidance (DA). Little is known about the partitioning between these two strategies. Because genes involved in DA mechanisms (i.e., *recA*) are expressed early and genes involved in TLS (i.e., Pol V) are expressed late during the bacterial SOS response, it has long been thought that TLS was the last recourse to bypass DNA lesions when repair and nonmutagenic DA mechanisms have failed. By using a recently described methodology, we followed the fate of a single replication-blocking lesion introduced in the *Escherichia coli* genome during acute genotoxic stress. We show that lesion tolerance events (i) only occur when the SOS response is fully induced and (ii) are executed in chronological order, with TLS coming first, followed by DA. Therefore, in response to genotoxic stress, bacterial cells give priority to TLS, a minor pathway able to generate genetic diversity before implementing the major nonmutagenic pathway that ensures survival.

DNA repair | mutagenesis

Genomes of living cells are constantly subject to DNA damage. Despite efficient repair mechanisms, some lesions may escape these pathways and therefore be encountered by the replication machinery during DNA replication. Cells possess two major strategies to tolerate residual lesions: (i) translesion synthesis (TLS), where specialized DNA polymerases insert a few nucleotides opposite the lesion, with the possibility of introducing a mutation; and (ii) damage avoidance (DA; also named strand switch, copy choice, or homology-dependent repair), where the cell uses the information of the sister chromatid to circumvent the lesion, ensuring survival in a nonmutagenic manner. The balance between these two strategies is very important because it defines the level of mutagenesis during lesion bypass. Although mutagenesis is essential for evolution (1, 2), it has nevertheless been kept to a low level to prevent excessive genome instability (3). Thus far, little is known about the partitioning between TLS and DA in living cells. In *Escherichia coli*, genes involved both in TLS and in DA mechanisms are regulated by the SOS response. Because genes involved in DA mechanisms (i.e., *recA*) are expressed early and genes involved in TLS (i.e., Pol V) are expressed late during the SOS response, it has long been thought that TLS was the last recourse to bypass DNA lesions when repair and nonmutagenic DA mechanisms have failed (4). In the present paper, we report experimental evidence that invalidates this model and show that even though TLS polymerases are expressed late during the SOS response, TLS occurs before DA mechanisms, allowing cells to generate mutagenesis even for a mild genotoxic stress. This strategy constitutes an adjustable mutator switch that prioritizes genetic variability.

Results

To assess the capacity of a cell to tolerate a single lesion in its genome, we used a recently described methodology by which we integrate, site specifically, a single lesion in the genome of a living cell (Fig. S1) (5). We used a typical replication-blocking lesion formed by covalent binding of the chemical carcinogen *N*-2-acetylaminofluorene (AAF) to the C-8 position of guanine (G-AAF). The G-AAF lesion is site-specifically introduced in the *NarI* sequence, a potent mutation hotspot (6). Within the *NarI* sequence context, the G-AAF lesion can be tolerated via one of three pathways: TLS0 (nonmutagenic) mediated by Pol V, TLS-2 (frameshift -2) by Pol II (Fig. S2) (7), or via DA mainly mediated by RecA (5). Modulation of the respective levels of Pol II and Pol V lets us investigate the potential interplay between TLS and DA pathways. In a strain deficient for nucleotide excision repair and mismatch repair (to avoid repair of the lesion and of the local sequence heterology) (Table S1) expressing Pol II and Pol V from chromosomal *polB* and *umuDC* loci, respectively, the major tolerance pathway is DA (>95%); both TLS0 and TLS-2 represent a total of only ~3% (Fig. 1). As expected, TLS events are fully suppressed upon inactivation of the *polB* and *umuDC* loci (Fig. 1). Induction of SOS to a high level in a *lexA*(Def) strain, where Pol II and Pol V expression is increased by inactivation of the LexA repressor, leads to a distinct increase in both TLS0 and TLS-2 events, reaching about 20% together. In the *lexA*(Def) strain, although the SOS genes are de-repressed, the active form of Pol V (UmuD'₂UmuC) is absent because of the lack of UmuD' (8). Our results suggest that the single patch of single-stranded DNA that results from replication fork uncoupling (9) is enough to generate a RecA filament that in turn mediates UmuD

Significance

When undergoing genotoxic stress, bacteria respond by the induction of their SOS system that promotes the overexpression of several genes. Among these genes are the specialized translesion synthesis (TLS) DNA polymerases that are able to replicate through a DNA lesion with the risk of inserting a mutation. Because these enzymes are expressed late during the SOS response, it has long been believed that TLS was the last recourse used by the cells in response to a genotoxic stress. In this work, we report evidence that TLS occurs before nonmutagenic bypass of a lesion. Thus, by giving priority to mutagenic bypass, cells are able to generate genetic diversity even for mild genotoxic stress.

Author contributions: R.P.F. and V.P. designed research; K.N., G.P., and V.P. performed research; K.N., R.P.F., and V.P. analyzed data; and R.P.F. and V.P. wrote the paper.

The authors declare no conflict of interest.

This article is a PNAS Direct Submission.

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This article contains supporting information online at www.pnas.org/lookup/suppl/doi:10.1073/pnas.1321008111/-DCSupplemental.

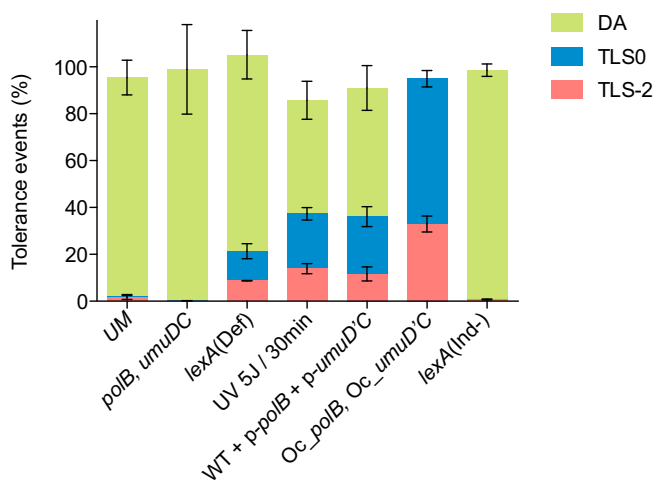


Fig. 1. Modulation of tolerance pathways by the level of specialized polymerases. Tolerance events (%) are determined as the efficiency of site-specific integration of a single G-AAF lesion-containing construct relative to the same amount of damage-free control construct. All strains are deficient for nucleotide excision (*uvrA*) repair and mismatch repair (*mutS*), denoted as the *UM* background. DA, mutagenic (TLS-2), and error-free (TLS0) translesion synthesis events are determined as specified in *Materials and Methods*. In the absence of Pol II and Pol V (*polB*, *umuDC*), no TLS is detected. SOS induction either genetically [*lexA(Def)*], by UV irradiation of the cells with 30 min of expression time, or modulation of the expression of Pol II and Pol V via ectopic expression from a low-copy-number plasmid that expresses *polB* and *umuD'C* from their natural promoters (annotated +*p-polB*, +*p-umuD'C*) or expressed from the chromosomal locus with a constitutive operator (*Oc_polB*, *Oc_umuD'C*) that strongly modulates the relative proportions of TLS and DA. The absence of SOS induction [in a *lexA(Ind-)* strain] does not affect survival.

cleavage and Pol V activation. To bring about a more complete SOS response, we UV irradiated the cells and let them express the SOS genes for 30 min before integrating the single AAF lesion. Under these experimental conditions, at the time the lesion is inserted in the genome, the critical SOS factors including Pol II, Pol V, and up-regulation of dNTP pool (10) are already present in the cell. Under these fully UV-induced conditions, we observe a further twofold increase in TLS, reaching almost 40% of the events (12% of TLS-2 and 24% of TLS0), whereas DA accounts for the remaining ~60% of tolerance events.

Next we used a strain expressing Pol II and Pol V from a low copy number plasmid (four to six copies per cell). This vector (pVP148) carries both *polB* and *umuD'C* genes under the control of their natural promoters. This plasmid strongly promotes both TLS events, reaching about 40% of tolerance events (Fig. 1), similar to the level observed in a UV-irradiated strain. Finally, we used a strain where high expression of both *polB* and *umuD'C* (the activated form of *umuDC*) was achieved by mutation of the promoter region in the chromosomal loci, leading to a constitutive operator. In this particular strain, TLS reaches 100%, fully overriding all DA events (Fig. 1). Finally, in the *lexA(Ind-)* strain that is deficient for SOS induction, we observe ~100% survival, showing that unlike TLS, DA pathways are functional in the absence of SOS induction.

We conclude from these experiments that, under physiological conditions, TLS events are rare compared with DA events even under SOS-induced conditions. TLS level is kept low by limited expression of the TLS polymerases and by the intrinsic low efficiency of TLS pathways (11, 12). Artificial increase in translesion polymerase expression can lead to an increased rate of TLS up to full suppression of all DA events. We would like to stress here that despite the strong induction of RecA protein

when SOS is induced, which may in principle promote DA events, the net effect seen is an increase in TLS events, suggesting an intrinsic priority of TLS over DA events (see below).

Is there competition between TLS and DA pathways? The experiments described above show that an increase in TLS polymerase expression leads to a corresponding decrease in DA. Alternatively, we wondered whether a reduction in the capacity of cells to mediate DA is compensated by a corresponding increase in TLS. To address this point, we compared the levels of TLS achieved by a fixed amount of TLS polymerase in a WT strain and a strain partially defective in DA (*recA*). This critical experiment could be performed for the Pol II-mediated TLS-2 pathway that is *recA* independent (13). For a given amount of Pol II, the extent of Pol II-mediated TLS events is not increased in a *recA* strain compared with the corresponding WT strain (Fig. 2). These data suggest that the relationship between TLS and DA pathways is not governed by reciprocal competition but rather defined in chronological order where TLS is implemented first and followed by DA. The extent of TLS is determined by the amount of available TLS polymerases and not influenced by the extent of DA. Because TLS remains constant whether DA is fully proficient or partially defective, we suggest that TLS occurs within a fixed time frame; once this time frame is over, remaining lesions are tolerated by DA. This experiment could not be implemented for Pol V-dependent TLS0 because Pol V requires RecA-mediated activation to perform TLS (11, 14, 15).

Because our system allows us to insert a specific lesion in the genome, we wanted to follow the fate of that given lesion among lesions distributed randomly over the rest of the chromosome to mimic a natural genotoxic stress situation. For this purpose, we UV irradiated the cells and immediately integrated the vector carrying a single AAF lesion. The kinetics of integration was monitored by quantitative PCR (qPCR) and showed that most of the integration events occur within 5 min after electroporation (Fig. S3). We can therefore assume that the occurrence of the specific G-AAF lesion is concomitant to the occurrence of the UV lesions on the chromosome. Despite the absence of induction of the TLS polymerases (namely Pol II and Pol V) at the time the AAF lesion is inserted, we observe an increase in TLS that is proportional to the level of UV irradiation (Fig. 3A). In fact, for a given level of UV irradiation of 5 J/m², we observe a level of TLS of ~40%, similar to the level reached when cells have been preinduced for SOS (Fig. 1, UV5J/30min). Similar results are observed for the two major UV lesions. Indeed, introduction of either a single cyclobutane pyrimidine dimer

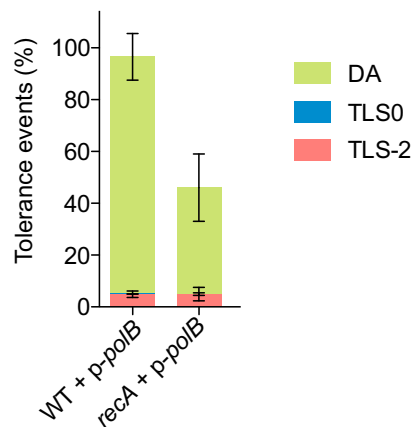


Fig. 2. Level of TLS when DA is reduced. The level of Pol II-mediated TLS-2 events is the same in a WT and a *recA* strain expressing Pol II from a low-copy-number plasmid (annotated +*p-polB*), suggesting that DA and TLS pathways do not compete under these conditions.

(TT-CPD) or a thymine-thymine pyrimidine(6-4)pyrimidone photoproduct [TT(6-4)] lesion concomitant to UV irradiation of the cell leads to a level of Pol V-mediated TLS not significantly different from the level observed when cells were preinduced for SOS (Fig. 3B).

These results are surprising given the kinetics of induction of RecA and Pol V, the two main actors of DA and TLS. Indeed, at the time the lesions appear on the chromosome, the level of RecA protein is already high (~9,000 molecules) and its SOS induction rate is very fast, reaching a sixfold increase within 15 min and up to a 12-fold increase within 30 min (Fig. S4). Conversely, the basal level of Pol V has been reported to be undetectable (4), and its SOS-induced expression rate is quite slow, because it requires both overexpression of UmuC and UmuD and processing of UmuD into UmuD'. Active Pol V only starts to appear 30 min after UV induction and reaches its maximum level after 60 min (Fig. S4) (16, 17).

The extent of TLS across a lesion appears to be the same whether the lesion is processed in cells that are fully SOS induced at the time the lesion is introduced or processed in cells where SOS induction is concomitant to lesion introduction. In the latter scenario, it is surprising to note that during the time needed for the biosynthesis and activation of Pol V, DA does not process lesions despite the fact that DA pathways are functional

in the absence of SOS induction (Fig. 1). These data show that when cells experience a genotoxic stress, lesion tolerance processing is delayed until the SOS response is fully induced. As lesion tolerance events are implemented in chronological order with TLS preceding DA, depending on the intensity of the inflicted stress, a dose-dependent amount of TLS polymerases accumulates in cells, leading to a dose-dependent increase in TLS and in mutagenesis (tunable mutator phenotype). Once TLS has been implemented, the remaining lesions are bypassed by DA pathways, leading to 100% of tolerance in a DA-proficient strain.

Discussion

We show that, following a genotoxic stress, lesion tolerance pathways, TLS and DA, only occur after the induction of SOS is completed. DA does not require any specific SOS function as indicated by the results obtained in the *lexA(Ind-)* strain (Fig. 1). Thus, the observation that DA occurs late, giving time for SOS to be fully induced, is probably not a genetically controlled process and is more likely to result from the intrinsic delay required to implement homologous recombination. This delay is also instrumental to give cells the maximum chance to repair the lesions, because excision repair pathways represent the first line of defense following a genotoxic stress. To focus on lesion tolerance mechanisms, we used cells where nucleotide excision repair (NER) and mismatch repair (MMR) pathways had been inactivated to prevent both lesion removal and sequence heterology correction. To the best of our knowledge, there is no published evidence involving either NER or MMR in lesion tolerance pathways, and thus we believe that the use of strains with a NER^-MMR^- background does not affect the conclusions that are reached. We show that the extent of TLS and of mutagenesis is modulated by the severity of the insult. It appears that cells implement lesion tolerance strategies in chronological order, with TLS coming first, followed by DA. The priority of TLS over DA might simply result from the fact that TLS is a rather simple process that only requires polymerase switches, whereas the process of DA is more complex because it requires the formation of a single-stranded RecA nucleofilament, followed by homology search, strand invasion, and intermolecular DNA synthesis. The extent of TLS is solely determined by the amount of available TLS polymerases that is controlled by the intensity of the genotoxic stress. Under physiological conditions, TLS appears to be used with parsimony due to the intrinsic poor efficiency of the TLS machinery. The molecular clock that determines the "TLS time frame" is presently unknown. We suggest it may be set by the intrinsic stability of the loaded β -clamp that acts as an essential platform for all DNA polymerases (18). Upon encounter with a lesion, the replisome (and therefore the β -clamp) remains stably associated with the DNA, and reinitiation occurs downstream (19). It is known that the stability of the loaded β -clamp is in the range of 1 h (20). We suggest that, although the β -clamp is present at the lesion site, TLS polymerases are recruited, and DA is inhibited. Upon β -clamp dissociation, the blocked primer terminus is no longer available for TLS and irreversibly engages into DA pathways (Fig. 4). In agreement with this model, Dieckman and Washington recently showed that, in eukaryotic cells, a less stable version of proliferating cell nuclear antigen (PCNA) leads to reduced TLS (21). In eukaryotic cells, the processivity clamp also appears to control the chronology of TLS and DA events because PCNA monoubiquitination precedes polyubiquitination, thus suggesting that TLS also occurs before DA because monoubiquitination is required for TLS, whereas polyubiquitination leads to DA (22). Surprisingly, available evidence shows that TLS is actually used to a greater extent in mammalian cells compared with *E. coli* (23, 24).

In addition, this delay to implement DA mechanisms is instrumental under conditions of low genotoxic stress because it allows TLS and thus mutagenesis to occur at a low level with

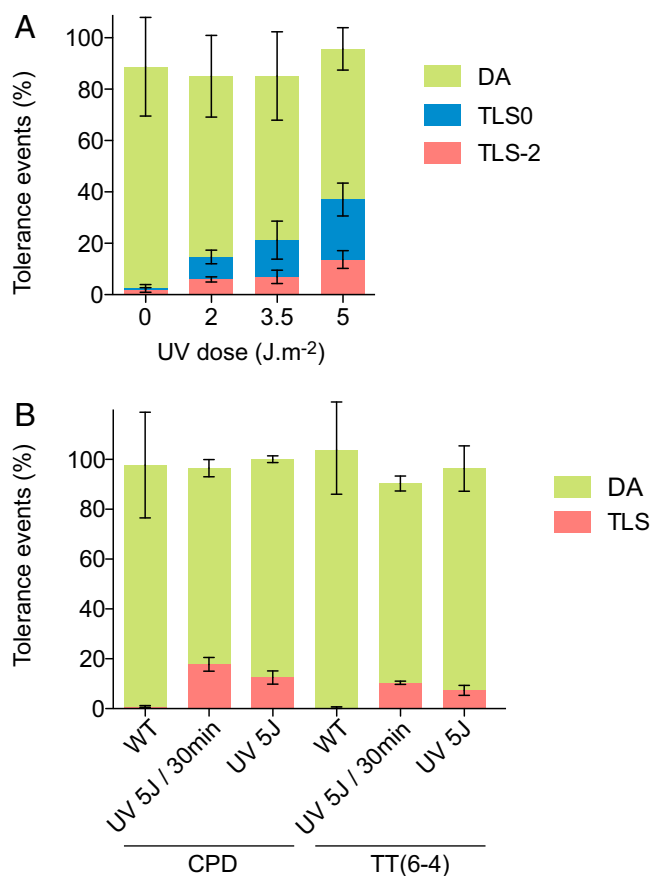


Fig. 3. (A) Fate of a single AAF lesion during an acute genotoxic stress. DA, mutagenic (TLS-2), and error-free (TLS0) TLS events of a single G-AAF lesion inserted immediately after UV irradiation to different doses. Despite the absence of induction of the TLS polymerases at the time the AAF lesion is inserted, we observe an increase in TLS proportional to the level of UV irradiation. (B) TLS and DA events for the two major UV lesions [CPD and TT(6-4)]. TLS at the single lesion increases to the same level after UV irradiation whether there is a 30-min expression time (UV+30min) or not (UV).

a small load of lesions. Otherwise, if lesion processing by DA could occur before SOS has had time to be fully induced, the few lesions could rapidly and efficiently be bypassed by DA before TLS polymerases are induced, depriving cells from the benefit to evolve and adapt by mutation. Hence, as observed (Fig. 3), even for a very low UV dose (2 J/m^2), cells are able to generate mutations, and we observe a gradation in the stress-induced mutations known to participate to adaptive evolution (25–27). In conclusion, the system unraveled here provides cells with a tunable mutator phenotype enabling them to gradually respond to genotoxic stress.

Methods

Plasmids. Vectors carrying a single lesion for integration were constructed as described previously (5) following the gap-duplex method (28). A 13-mer oligonucleotide, 5'-GCAAGTTAACACG, containing no lesion, a TT-CPD lesion, or a TT(6-4) lesion (underlined) was inserted into the gapped-duplex pGP1/2, leading to an in-frame *lacZ* gene. Because the G-AAF lesion can be bypassed by two distinct pathways (7), two vectors were constructed to monitor all TLS events. A 15-mer oligonucleotide containing or not containing a single G-AAF adduct (underlined) in the *NarI* site (ATCACCGCGC-CCACA) was inserted into a gapped-duplex pVP141/142 or pVP143/144, leading, respectively, to an in-frame *lacZ* gene, and a +2 frameshift *lacZ*. Therefore, the construct pVP141/142 *Nar3^{AAF}/Nar+3* monitors TLS0 events, whereas pVP143/144 *Nar3^{AAF}/Nar+3* monitors TLS-2 events.

The pVP147 plasmid expresses the *polB* gene from its own promoter. It was obtained by cloning a *Sall*-*Bam*HI fragment from pYG787 (29) into a pGB2 vector where the spectinomycin resistance gene was replaced by the chloramphenicol resistance gene.

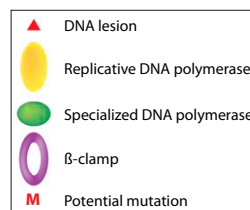
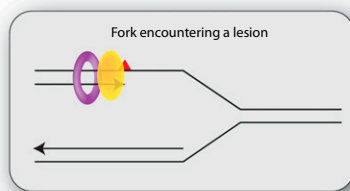
The pVP148 plasmid expresses both *polB* and *umuD'*C loci from their own promoters. It is derived from pRW134 (30), a pGB2 vector that carries *umuD'*C, in which the spectinomycin resistance gene was replaced by the chloramphenicol resistance gene. The *polB* gene was added by subcloning of a *Sall*-*Bam*HI fragment from pYG787 (29). *UmuD'* is the cleaved version of *UmuD* that forms the heterotrimeric complex ($\text{UmuD}'_2\text{UmuC}$, i.e., Pol V).

Strains. All strains used in the present study for site-specific recombination are derivative of strain FBG152 (5, 31). Gene disruptions in *recA*, *mutS*, *uvrA*, *polB*, *umuDC*, *sulA*, *lexA*, and *phrB* were achieved by the one-step PCR method (32). The following FBG152-derived strains were constructed by P1 transduction: EVP23 (FBG152 *uvrA::frrt mutS::frrt*), EVP108 (FBG152 *uvrA::frrt mutS::frrt polB::frrt umuDC::frrt*), EVP113 (FBG152 *uvrA::frrt mutS::frrt sulA::frrt lexA::frrt*), EVP123 (FBG152 *uvrA::frrt mutS::frrt recA::frrt*), EVP184 (FBG152 *uvrA::frrt mutS::frrt phrB::frrt*), and EVP414 (FBG152 *uvrA::frrt mutS::frrt lexA1* (*Ind*-)*malB::Tn9*).

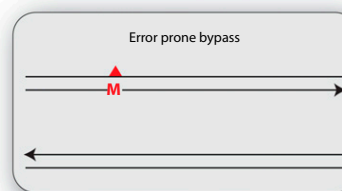
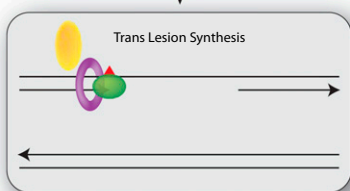
A strain expressing *umuD'*C from a constitutive promoter was constructed as follows: a single mutation in the SOS box (CTGT → CTAT) was introduced by site-directed mutagenesis on plasmid pRW134 (30). This mutation was reported to inactivate the SOS box (33). We inserted the *Kan*/*frrt* cassette from pKD13 (32) upstream of the promoter region into the *PmlI* restriction site. The *Scal*-*EcoRI* fragment containing the *Kan*-*Oc-umuD'*C region was introduced into the chromosome, leading to strain EVP272 (MG1655 *kan::Oc-umuD'*C).

Strains expressing *polB* from a constitutive promoter were constructed by site-directed mutagenesis of the SOS box of *polB* (CTGT → ATTC). A PCR-amplified *Kan* cassette from the pKD13 plasmid with primers harboring the mutations in the *polB* SOS box was transferred into the chromosome by one-step inactivation of chromosomal genes (32), leading to strain EVP207 (MG1655 *kan::Oc-polB*).

Lesion processing is postponed until SOS induction

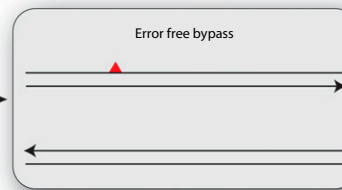
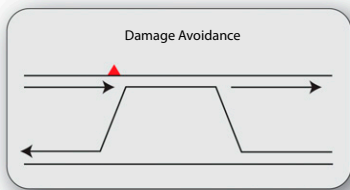


TLS occurs first. It is a minor pathway limited by low amount and the intrinsic low efficiency of TLS polymerases. It is only permitted as long as the β -clamp is present.



Genetic diversity
Adaptation to
the environment

Damage avoidance occurs next, and represent the major pathway.



Survival

Fig. 4. Chronology model for lesion tolerance pathways. Our results suggest that, upon encounter of a replication fork with a blocking lesion, lesion processing is postponed until SOS is induced. Lesion tolerance pathways are then executed in chronological order, with TLS events coming first, followed by DA. The extent of TLS is proportional to the amount of available TLS polymerases and thus to the strength of the SOS signal. We suggest that TLS events occur during a time window defined by the presence of the loaded β -clamp. Following dissociation of the β -clamp from the template, the blocked primer terminus is no longer a template for TLS events and engages into RecA-dependent DA pathways the major pathway that ensures survival in a nonmutagenic manner.

The two constructions were combined by P1 transduction, leading to strain EVP294 (FBG152 *uvrA::frt mutS::frt frt::Oc-polB frt::Oc-umuD*'C).

All strains carry plasmid pVP135, which allows the expression of the integrase-excisionase under the control of isopropyl β -D-1-thiogalactopyranoside (IPTG). Following the site-specific recombination reaction, the lesions [G-AAF, TT-CPD, or TT(6-4)] are located in the leading strand of strain FBG152.

Preparation of Competent Cells. Competent cells with expression time after UV.

One hundred milliliters of lysogeny broth (LB) containing kanamycin to maintain plasmid pVP135 that expresses the *int-xis* operon and 200 μ M of IPTG (to induce the expression of *int-xis*) were inoculated with 500 μ L of an overnight starter. When the culture reached $OD_{600} \sim 0.3$, cells were resuspended in 50 mL of water and UV irradiated. Fifty milliliters of 2 \times LB was added, and cells were grown at 37 $^{\circ}$ C for 30 min. Cells were washed twice in water and once in 10% (vol/vol) glycerol and were finally resuspended in 200 μ L of 10% (vol/vol) glycerol and frozen in 40- μ L aliquots.

Competent cells without expression time after UV. One hundred milliliters of LB containing kanamycin to maintain plasmid pVP135 that expresses the *int-xis* operon and 200 μ M of IPTG were inoculated with 500 μ L of an overnight starter. When the culture reached $OD_{600} \sim 0.5$, cells were resuspended in 100 mL of water and UV irradiated. Cells were washed once more in water and once in 10% glycerol and were finally resuspended in 200 μ L of 10% glycerol and frozen in 40- μ L aliquots.

Integration Protocol—Measurement of Lesion Tolerance Pathways: TLS and DA.

To the 40- μ L aliquot of cells, 1 ng of the lesion-carrying vector mixed with 1 ng of the internal standard (pVP146) was added and electroporated (in a GenePulser Xcell from BioRad, 2.5 kV, 25 μ F, 200 Ω). One milliliter of super optimal broth with catabolic repressor containing 200 μ M IPTG was then added, and cells were incubated for 1 h at 37 $^{\circ}$ C. Part of the cells were plated on LB + 10 μ g/mL tetracycline to measure the transformation efficiency of plasmid pVP146, and the rest were plated on LB + 50 μ g/mL ampicillin and 80 μ g/mL X-gal to select for transformants (Amp) and TLS events (blue colonies). Cells were diluted and plated using the automatic serial diluter and plater EasySpiral Dilute (Interscience). Colonies were counted using the Scan 1200 automatic colony counter (Interscience). The integration rate is about 2,000 clones per picogram of vector for a WT strain.

To measure TLS0, TLS-2, and DA at the G-AAF lesion in a given strain, four integration experiments were performed with the following heteroduplex vectors: pVP141/142Nar0/Nar+3, pVP141/142Nar3^{AAf}/Nar+3, pVP143/144Nar0/Nar+3, and pVP143/144Nar3^{AAf}/Nar+3. Following the integration of pVP141/142Nar3^{AAf}/Nar+3, blue colonies represented TLS0 events, whereas white colonies represented the sum of TLS-2 and DA events.

Similarly, the integration of pVP143/144Nar0/Nar+3 allows one to score TLS-2 events as blue colonies, whereas white colonies reflect the sum of TLS0 and DA events (Fig. S5). The integration efficiencies of damaged vectors compared with their nondamaged homologs, and normalized by the transformation efficiency of the pVP146 plasmid in the same electroporation experiment, gave the overall rate of tolerance of the lesion. DA was scored as the overall lesion tolerance rate from which the TLS0 and TLS-2 rates were subtracted. Each experiment was repeated three to five times with at least two different batches of competent cells.

qPCR. To measure the kinetics of integration, we quantified the ratio of integrated vs. total gDNA by qPCR. After electroporation of the vector in the strain EVP23 expressing the integrase from plasmid pVP135, cells were diluted in LB and incubated at 37 $^{\circ}$ C. Aliquots were taken at different time points, cells were washed twice in 0.5 M NaCl, and total gDNA was extracted using GenElute Bacterial Genomic DNA Kits from Sigma. DNA was quantified by qPCR using the Takara Bio SYBR Premix Ex Taq. Total genomic DNA was quantified using primers VP38/VP39 (CGACTACCTGGTGATCTCG/CGACAT-TGATCTGGCTATCTTG). Integration events were quantified using primers VP161/VP36 (TTACGCGCCGGAGAAAACC/TCAACCACCGCAGATAGAG).

Immunoblots. Strain EVP23 was grown to exponential phase, resuspended in water, UV irradiated at 5 J/m², diluted in an equal volume of 2 \times LB, and incubated at 37 $^{\circ}$ C. Aliquots were taken at different time points. Strain EVP113 was grown to the exponential phase. Cell samples were washed in water resuspended in 1 \times high urea buffer (60 mM Tris-HCl, pH = 6.8; 5% glycerol; 2% SDS; 100 mM DTT; 160 mM urea; 20 mM β -mercaptoethanol; and bromophenol blue), boiled for 2 min, and sonicated.

RecA immunoblot. The equivalent of 0.004 OD_{600} was loaded on a MiniProtein TGX strain-free 15% gel (BioRad). Anti-RecA antibody from abcam (ab63797) was used.

UmuD immunoblot. The equivalent of 0.12 OD_{600} was loaded on a MiniProtein TGX strain-free 4–20% gel (BioRad). Anti-UmuD antibody from abcam (ab91343) and purified anti-UmuD antibody provided by R. Woodgate (Laboratory of Genomic Integrity, National Institute of Child Health and Human Development, National Institutes of Health, Bethesda) were used.

ACKNOWLEDGMENTS. We thank Luisa Laureti and Shingo Fujii for critical reading of the manuscript. We thank Dr. Roger Woodgate (National Institutes of Health, Bethesda, MD) for the anti-UmuD antibodies. This work was funded by Équipe Labellisée Ligue Contre le Cancer and Agence Nationale de la Recherche (ANR) Grant ForkRepair ANR 11 BSV8 017 01. K.N. was supported by a fellowship from the Fondation ARC pour la Recherche sur le Cancer.

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Supporting Information

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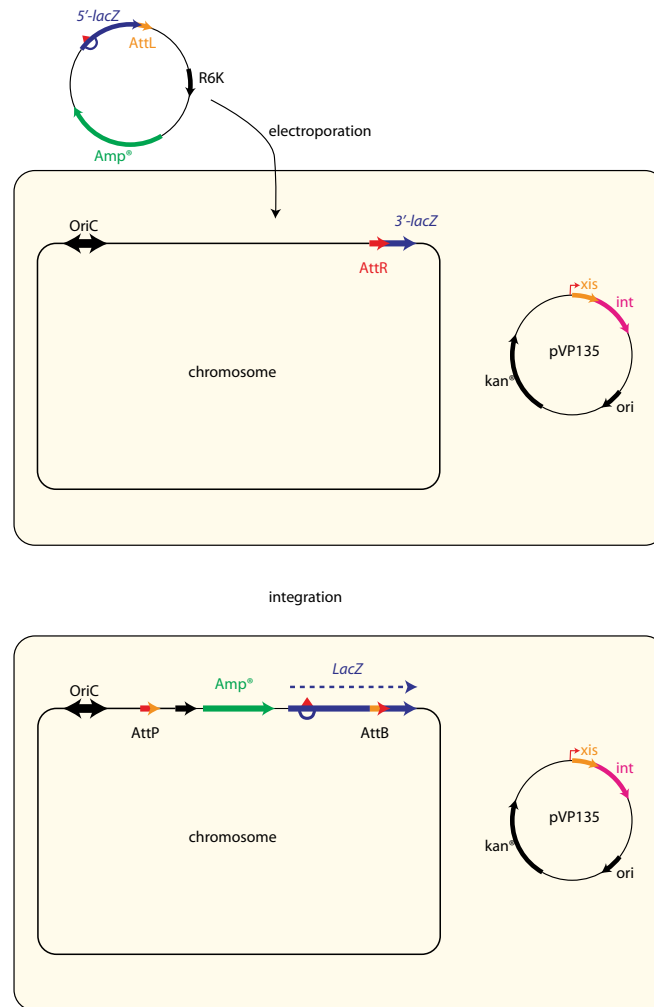


Fig. S1. Integration of a single lesion into the chromosome. The recipient strain contains a single attR integration site in fusion with the 3' end of the *lacZ* gene at minute 17 in the *Escherichia coli* chromosome. Following ectopic expression of phage lambda integrase and excisionase, the lesion-carrying construct is introduced by electroporation. Its attL site will recombine with the chromosomal attR, leading to integration of the entire lesion-containing construct. Integration events are selected on the basis of their resistance to ampicillin. Integration at nucleotide level resolution restores a functional *lacZ* gene, allowing these events to be monitored on X-gal indicator plates.

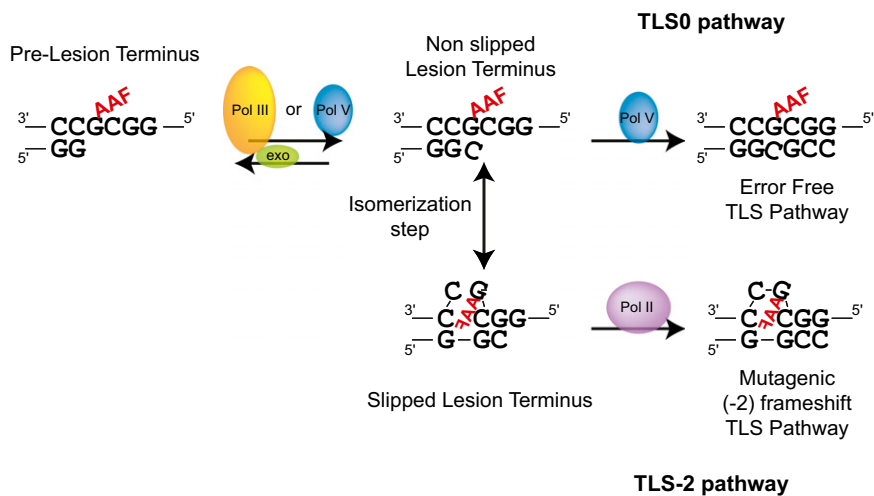


Fig. S2. Translesion synthesis (TLS) pathways across a single G-AAF adduct in the NarI mutation hot spot. Within this sequence context, a single G-AAF adduct can be bypassed via two distinct pathways: the TLS0 pathway is mediated by Pol V and is error free; and the TLS-2 pathway produces -2 frameshift mutations and depends on Pol II (1–3). Insertion of C across the G-AAF lesion can be performed by the replicative DNA Pol III or by Pol V, generating a key replication intermediate referred to as the lesion terminus. Given the local sequence context (i.e., the NarI site contains a 5'-GpC dinucleotide repeat), the lesion terminus can exist in two conformations: nonslipped or slipped. Pol V efficiently extends the nonslipped conformation yielding an error-free replication product (TLS0 pathway). In contrast, Pol II very efficiently elongates the slipped intermediate, thus generating a -2 frameshift mutation (1, 4).

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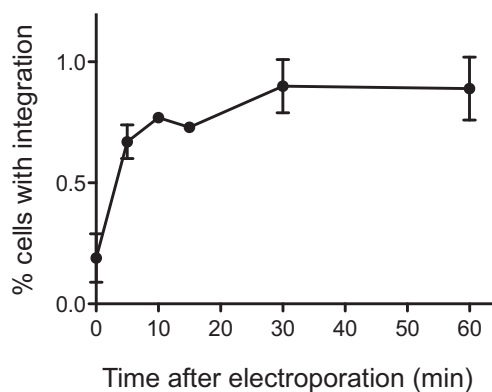


Fig. S3. Kinetics of integration of the vector followed by quantitative PCR. The graph represent the molar ratio of integrated DNA/total DNA as a function of the time after electroporation of the integrating vector.

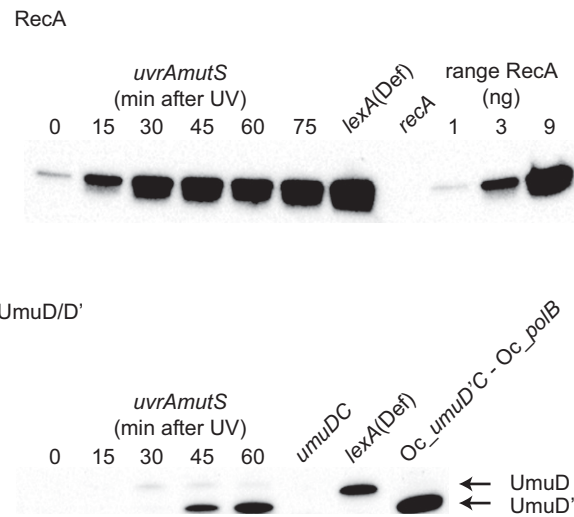


Fig. S4. Kinetics of expression of RecA, UmuD, and UmuD' after UV irradiation. Immunoblots showing the induction of RecA, UmuD, and UmuD' proteins at various time after UV irradiation (5 J/m^2) and in a *lexA(Def)* strain.

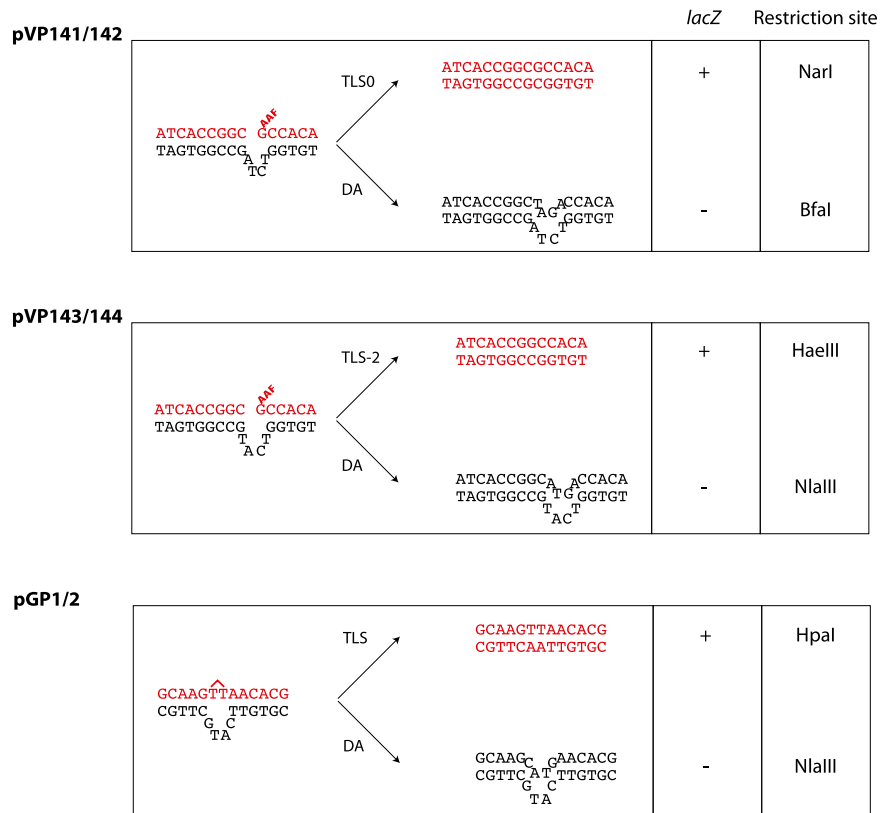


Fig. S5. Molecular structure of the constructs in the vicinity of the lesion, allowing TLS and damage avoidance (DA) pathways to be monitored. For the G-AAF lesion, pVP141/142 monitors TLS0 and DA, whereas pVP143/144 monitors TLS-2 and DA. For the two UV lesions [TT (6-4) and CPD], pGP1/2 monitors TLS0 and DA.

Table S1. Strains used in this study

Name	Short name	Genotype
EVP23	WT	FBG152 <i>uvrA</i> :: <i>frt mutS</i> :: <i>frt</i>
EVP108	<i>polB-umuDC</i>	FBG152 <i>uvrA</i> :: <i>frt mutS</i> :: <i>frt polB</i> :: <i>frt umuDC</i> :: <i>frt</i>
EVP113	<i>lexA(Def)</i>	FBG152 <i>uvrA</i> :: <i>frt mutS</i> :: <i>frt sulA</i> :: <i>frt lexA</i> :: <i>frt</i>
EVP123	<i>recA</i>	FBG152 <i>uvrA</i> :: <i>frt mutS</i> :: <i>frt recA</i> :: <i>frt</i>
EVP184	<i>phrB</i>	FBG152 <i>uvrA</i> :: <i>frt mutS</i> :: <i>frt phrB</i> :: <i>frt</i>
EVP294	<i>Oc-polB, Oc-umuD'C</i>	FBG152 <i>uvrA</i> :: <i>frt mutS</i> :: <i>frt frt</i> :: <i>Oc-polB frt</i> :: <i>Oc-umuD'C</i>
EVP414	<i>lexA(ind-)</i>	FBG152 <i>uvrA</i> :: <i>frt mutS</i> :: <i>frt lexA1(ind-)</i> <i>malB</i> ::Tn9