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Specialised DNA polymerases in Escherichia coli: roles within multiple pathways

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Abstract
In many bacterial species, DNA damage triggers the SOS response; a pathway that regulates the production of DNA repair and damage tolerance proteins, including error-prone DNA polymerases. These specialised polymerases are capable of bypassing lesions in the template DNA, a process known as translesion synthesis (TLS). Specificity for lesion types varies considerably between the different types of TLS polymerases. TLS polymerases are mainly described as working in the context of replisomes that are stalled at lesions or in lesion-containing gaps left behind the replisome. Recently, a series of single-molecule fluorescence microscopy studies have revealed that two TLS polymerases, pol IV and pol V, rarely colocalise with replisomes in *Escherichia coli* cells, suggesting that most TLS activity happens in a non-replisomal context. In this review, we re-visit the evidence for the involvement of TLS polymerases in other pathways. A series of genetic and biochemical studies indicates that TLS polymerases could participate in nucleotide excision repair, homologous recombination and transcription. In addition, oxidation of the nucleotide pool, which is known to be induced by multiple stressors, including many antibiotics, appears to favour TLS polymerase activity and thus increases mutation rates. Ultimately, participation of TLS polymerases within non-replisomal pathways may represent a major source of mutations in bacterial cells and calls for more extensive investigation.

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Specialised DNA polymerases in *Escherichia coli*: roles within multiple pathways

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Abstract:

In many bacterial species, DNA damage triggers the SOS response; a pathway that regulates the production of DNA repair and damage tolerance proteins, including error-prone DNA polymerases. These specialised polymerases are capable of bypassing lesions in the template DNA, a process known as translesion synthesis (TLS). Specificity for lesion types varies considerably between the different types of TLS polymerases. TLS polymerases are mainly described as working in the context of replisomes that are stalled at lesions or in lesion-containing gaps left behind the replisome. Recently, a series of single-molecule fluorescence microscopy studies have revealed that two TLS polymerases, pol IV and pol V, rarely colocalise with replisomes in *Escherichia coli* cells, suggesting that most TLS activity happens in a non-replisomal context. In this review we re-visit the evidence for the involvement of TLS polymerases in other pathways. A series of genetic and biochemical

studies indicates that TLS polymerases could participate in nucleotide excision repair, homologous recombination and transcription. In addition, oxidation of the nucleotide pool, which is known to be induced by multiple stressors, including many antibiotics, appears to favours TLS polymerase activity and thus increases mutation rates. Ultimately, participation of TLS polymerase within non-replisomal pathways may represent a major source of mutations in bacterial cells and calls for more extensive investigation.

Keywords: DNA repair; mutagenesis; DNA replication; recombination; reactive oxygen species

Main text:

Replication of the *Escherichia coli* genome is a fast and accurate process. On undamaged DNA, the primary polymerase, DNA polymerase III, inserts close to 1000 nucleotides per second, with an error rate of only one in one billion (Drake 1991; Lewis et al. 2016). Damaged DNA templates, however, lead to replication problems as the primary polymerase is inhibited by the presence of lesions in the template DNA (Goodman and Woodgate 2013). Since cells are frequently exposed to endogenous and exogenous sources of DNA damage, they have evolved error-free repair pathways to remove and replace DNA lesions (Friedberg et al. 1995). Some lesions, however, escape these pathways and are encountered by replication forks. Depending on conditions, this leads to either replication fork arrest or re-priming and continued synthesis downstream of the lesion (known as lesion skipping) (Friedberg et al. 1995; Goodman 2002; Simmons et al. 2008; Waters et al. 2009; Yeeles and Marians 2011, 2013; Fuchs and Fujii 2013; Goodman and Woodgate 2013; Gabbai et al. 2014; Fuchs 2016). Both pathways lead to the accumulation of single-stranded DNA (ssDNA) gaps which are either repaired or processed into double-strand breaks (DSBs) (Heltzel et al. 2012; Fuchs and Fujii 2013; Yeeles and Marians 2013; Gabbai et al. 2014; Scotland et al. 2015). DSBs are particularly toxic to cells (Friedberg et al. 1995). As an overall consequence of DNA damage, the SOS response is triggered (Henestrosa et al. 2000). The SOS response increases the expression levels of many proteins involved in DNA repair mechanisms (Henestrosa et al. 2000; Fuchs and Fujii 2013). The earliest SOS genes to be induced participate in non-mutagenic DNA repair pathways. If damage is not resolved during this stage, mutagenic pathways are initiated (Goodman 2002; Foster 2007). Mutagenesis arises from the upregulation of specialised DNA polymerases that are able to bypass lesions, a process known as translesion synthesis (TLS) (Friedberg et al. 1995; Napolitano et al. 2000; Yeiser et al. 2002; Goodman 2002; Simmons et al. 2008; Waters et al. 2009; Yeeles and

Marians 2011, 2013; Fuchs and Fujii 2013; Goodman and Woodgate 2013; Gabbai et al. 2014; Fuchs 2016; Michel and Sandler 2017).

TLS polymerases generate mutations. In contrast to the primary polymerase, TLS polymerases are capable of efficient lesion bypass. This activity is made possible by the architecture of their template-binding sites, which are more open than that of the primary polymerase (Yang and Gao 2018). This also, however, makes TLS polymerases highly error-prone as they are less likely to discriminate between correct and incorrect nucleotides which can lead to misincorporations. Insertion of the incorrect base can lead to a mutation being established during subsequent rounds of replication (Friedberg et al. 1995).

Mutations caused by TLS polymerases acting on undamaged portions of DNA are called untargeted mutations (Kim et al. 2001). Overexpression of TLS polymerases often leads to increased mutation rates in the absence of damage, suggesting that a drastic increase in TLS polymerase concentration tilts the balance towards TLS activity. For instance, *E. coli* DNA polymerase IV, encoded by the *dinB* gene, is an error-prone polymerase and induces -1 frameshift mutations when highly overexpressed (Kim et al. 1997; Kuban et al. 2005). Similarly, overexpression of *Bacillus subtilis* DNA polymerase Pol Y1, encoded by *yqjH*, results in increased mutagenesis in a rifampicin resistance assay (Sung et al. 2003; Duigou et al. 2004). TLS polymerases increase the genetic diversity of bacterial populations growing in the absence of external damage (Corzett et al. 2013), implying that TLS polymerases may produce untargeted mutations at a low, but significant, rate.

TLS polymerases are specialised because they can extend primed lesion-containing templates (Goodman and Woodgate 2013; Vaisman and Woodgate 2017). The incorporation of an incorrect base opposite the lesion can lead to mutation. This type of mutation is called a targeted mutation (Tang et al. 2000; Kim et al. 2001). TLS polymerases carry out a variety of error-free and mutagenic TLS activities (detailed below). It is important to note that in most

cases the biological context(s) for lesion bypass (stalled replisomes, ssDNA gaps, recombination intermediates etc.) remains poorly understood.

TLS polymerases copy a variety of lesion-containing templates. DNA lesions originate from endogenous or exogenous sources, for instance some antibiotics, other DNA damaging compounds (e.g. methyl methanesulfonate), or ultraviolet light (UV light)(Goodman 2002; Goodman and Woodgate 2013). Lesions can include chemically altered nucleo-bases or changes in the sugar-phosphodiester backbone. Common lesions include abasic sites, alkylated bases, oxidised bases and adducts to the N^2 position of guanines (Fuchs 2016). Certain DNA lesions are only bypassed by a particular TLS polymerase, indicating that the active site of each TLS polymerase differently accommodates different lesion types (Yang and Gao 2018).

Ultraviolet light generates covalently cross-linked pairs of thymidine bases, most commonly forming cyclobutane pyrimidine dimers (CPDs) and single cross-linked (6–4) photoproducts (Friedberg et al. 1995; Tang et al. 2000). In *E. coli*, UV lesions are bypassed by DNA polymerase V (pol V) (Krishna et al. 2007; Patel et al. 2010). This TLS polymerase is encoded by *umuDC* and belongs to the Y-family polymerases (UmuC subfamily). Pol V is a highly error-prone polymerase that is responsible for almost all UV-induced mutagenesis. When carrying out TLS at CPDs, pol V frequently inserts the sequence GA opposite the TT-CPD lesion, rather than the canonical AA (Banerjee et al. 1988, 1990; Timms et al. 1999; Goodman 2002). A second polymerase in *E. coli*, pol II (encoded by *polB*), plays a role in restarting replication in UV-irradiated cells; cells lacking pol II show delayed recovery of DNA synthesis after irradiation (Rangarajan et al. 1999; Goodman 2002; Wang and Yang 2009). The biochemical nature of this activity remains unclear. In *B. subtilis* Pol Y2 is essential for UV-induced mutagenesis, whereas Pol Y1 is not (Duigou et al. 2004). Polymerases of the UmuC subfamily appear to be generally necessary for UV-induced

mutagenesis (Woodgate et al. 1989; Thomas et al. 1990; Hauser et al. 1992; Szekeres Jr. et al. 1996; Woodgate and Levine 1996). While deletion of *E. coli dinB* (encoding pol IV) does not yield effects on UV survival, UV-induced mutagenesis or replication restart after UV arrest (Courcelle et al. 2005), biochemical measurements indicate that pol IV is capable of error-free bypass of CPD lesions (Gabbai et al. 2014).

Alkylating agents, such as methyl methanesulfonate (MMS) modify nucleo-bases in DNA, producing both cytotoxic and mutagenic effects (Bjedov et al. 2007). In *E. coli* pol IV contributes to survival upon MMS treatment (Bjedov et al. 2007). This activity appears to stem from error-free bypass of MMS lesions. Pol V is involved in error-prone bypass of the MMS-induced lesions N^1-methyl-deoxyadenosine (1meA) and N^3-methyl-deoxycytosine (3meC) (Sikora et al. 2010).

Certain compounds generate bulky adducts to the N^2 position of deoxyguanosine (N^2-dG), for instance benzo[*a*]pyrene, nitrofurazone (NFZ), 4-nitroquionoline 1-oxide (4-NQO) and 2-acetylaminofluorene (Kim et al. 2001; Pagès and Fuchs 2002; Jarosz et al. 2006). TLS polymerases do not contribute equally to survival of N^2 modifications. *Escherichia coli* pol V contributes to mutagenesis upon *N*-ethyl-*N*-nitrosourea treatment(Fix 1993), whereas, pols II and IV contribute greatly to 4-NQO survival (Kim et al. 2001; Sanders et al. 2006; Williams et al. 2010). N^2acetylaminofluorene guanine adducts (N^2-AAFdG) can be bypassed by pol II, often inducing -2 frameshift mutations (Becherel and Fuchs 2001). In cells carrying benzo[*a*]pyrene lesions, both pols IV and V have been shown to be active using genetics and *in vitro* reconstitution assays, each contributing to both error-free TLS and -1 frameshifts (Lenne-Samuel et al. 2000; Ikeda et al. 2014).

N^2 adducts can also originate from methylglyoxal, a by-product of the glycolysis pathway (Yuan et al. 2008). These N^2-(1-carboxyethyl)-2'-deoxyguanosine adducts (N^2-CEdG) are accurately bypassed by pol IV suggesting that in cells pol IV might frequently

carry out error-free TLS on N^2-dG adducts that arise during normal metabolism. Moreover, bulky N^2-N^2-guanine cross-links are bypassed by pol IV with high fidelity (Kumari et al. 2008).

Modified nucleotide triphosphates may favour TLS polymerase activity. DNA lesions are also induced by reactive oxygen species (ROS). For instance, guanine is often oxidised to 8-oxo-guanine (Sekiguchi and Tsuzuki 2002; Foti et al. 2012). Such oxidised nucleotides form altered DNA base pairs and are commonly mutagenic (Sekiguchi and Tsuzuki 2002; Sakai et al. 2006). The amount of ROS in cells can vary considerably according to several factors, including for example metabolic rates and oxygen concentrations. There is some evidence that in cells growing aerobically, increased ROS levels lead to increased numbers of lesions (Sakai et al. 2006). This, presumably, would cause an increase in TLS activity. In fact, levels of pol V-dependent mutagenesis in *E. coli* appear to be markedly higher in aerobic conditions than in anaerobic conditions (Bhamre et al. 2001). It is not clear, however, whether the extra mutations that arise under aerobic conditions derive from targeted mutagenesis at oxidised base pairs or whether the conditions favour untargeted mutagenesis. It has been directly demonstrated that pol IV incorporates 8-oxo-dGs into the DNA (Foti et al. 2012). Whether pol V is similarly capable of incorporating oxidised nucleotides requires further investigation.

Cellular stress is also known to increase ROS levels. For instance, ROS increasingly accumulate in response to treatment with several antibiotics or in the case of thymine starvation and in both cases strongly contribute to killing (Hong et al. 2017; van Acker and Coenye 2017). The killing mechanism appears to depend on ROS-induced conversion of ssDNA regions into toxic DSBs (Hong et al. 2017; van Acker and Coenye 2017). Stress-induced increases in ROS also increase mutation rates and TLS polymerases are involved (Foti et al. 2012; Hong et al. 2017; Moore et al. 2017). It remains unclear, however, if this

involvement relates to incorporation of oxidised nucleotides into the DNA, mutagenic TLS at sites of oxidised bases already present in the DNA, error-prone synthesis by TLS polymerases during break repair, or some combination of the three. In general, the incorporation of non-canonical dNTPs into the DNA by DNA polymerases is an important area that remains under-investigated.

Replicative vs post-replicative translesion synthesis. Two models have been proposed for TLS activity upon encounters of replisomes with lesions on the leading strand (see **Fig. 1A**). In the most cited model, known as replicative TLS, TLS polymerases assist stalled replisomes by exchanging for the arrested pol III and bypassing the lesion (Heltzel et al. 2012; Fuchs and Fujii 2013; Scotland et al. 2015). Following TLS, the polymerases exchange back, allowing pol III to resume replication. This model was primarily built upon the results of *in vitro* reconstitution assays and led to the proposal of molecular mechanisms invoking polymerase switching on the β clamp (Wagner et al. 2000; Becherel and Fuchs 2002; Lenne-Samuel et al. 2002; Furukohri et al. 2008; Kath et al. 2014). In the other model, TLS polymerases are involved in post-replicative translesion synthesis. Here the replisome is proposed to skip over lesions (by re-priming downstream), creating lesion-containing gaps behind the replisome (Yeeles and Marians 2013; Gabbai et al. 2014). These gaps are templates for TLS polymerases, which bypass lesions and thus allow the gaps to be filled (Waters and Walker 2006; Indiani and O'Donnell 2013; Fuchs 2016).

Studies conducted *in vitro* have concluded that skipping of lagging strand lesions is an inherent property of the replisome (see **Fig. 1B**) (Higuchi et al. 2003; McInerney and O'Donnell 2004). In light of new observations that demonstrate that Pol III* (three Pol III cores plus clamp loader complex, i.e. $[\alpha\varepsilon\theta]_3\tau_3\delta\delta'\chi\psi$) exchanges readily at replication forks (Beattie et al. 2017; Lewis et al. 2017), the conclusions of these studies may need to be revisited. The Higuchi and McInerney studies demonstrated that lagging strand lesions did

not block the progress of the replisome in bulk-level biochemical assays. From this they each concluded that the replisome simply skips over lagging lesions. In the absence of exogenous DNA damage, Pol III* exchanges readily *in vivo* (Beattie et al. 2017; Lewis et al. 2017). This opens an alternative explanation for the Higuchi and McInerney data: the lagging strand polymerase actually stalled at the lesion, but the stalled Pol III* was replaced by another molecule from the bulk. There are only ~20 molecules of Pol III* available in each cell (Beattie et al. 2017; Lewis et al. 2017), thus exchange could easily become limiting in the presence of damage. It would be of interest to examine the capacity of the replisome to skip lagging strand lesions under dilute conditions, or in pre-assembled single-molecule assays, where exchange of Pol III* would be limited.

TLS polymerases are involved in other DNA repair pathways. Historically, error-prone polymerases have mainly been examined in the context of the replisome. Several studies, however, implicate the TLS polymerases are also involved in other DNA repair mechanisms, for instance, transcription coupled repair (Cohen et al. 2009, 2010; Cohen and Walker 2011), nucleotide excision repair (Courcelle et al. 2005; Williams et al. 2010) and homologous recombination (Ponder et al. 2005; Shee et al. 2011; Pomerantz et al. 2013b; Mallik et al. 2015). Additionally, TLS polymerases play a role in adaptive mutagenesis (Cairns and Foster 1991; Wagner et al. 1999; McKenzie et al. 2001; Rosenberg 2001).

In addition to DNA replication, DNA damage is also a hindrance to transcription. Lesion-containing gaps on the template strand result in RNA polymerase stalling. Work by Cohen *et al.* revealed that RNA polymerases stalled at gaps may recruit TLS polymerases to close the gap and allow transcription to continue (see **Fig. 2A**). The group found that the *E. coli* transcription modulator NusA genetically interacts with both Y-family polymerases pol IV and pol V (Cohen et al. 2010; Cohen and Walker 2011). NusA physically interacts with pol IV (Cohen et al. 2009). NusA functions in both termination and antitermination of

transcription and in both cases is bound to the RNA polymerase (RNAP). In the Cohen transcription-coupled TLS model, NusA recruits TLS polymerases to RNAPs stalled at gaps generated when the replisome encounters a lesion in the nontranscribed strand (Cohen et al. 2010). TLS polymerases could then fill the gap in the template strand and rescue the stalled RNAP. In contrast, RNAPs stalled at lesions on the transcribed strain would be resolved by transcription-coupled repair (Cohen et al. 2010).

Nucleotide excision repair (NER) can remove a variety of bulky DNA lesions, leaving behind ssDNA gaps which, in principle, could be substrates for TLS polymerases (see **Fig. 2B**). In fact, *E. coli* pol IV is involved in both NER-dependent and -independent pathways in cells treated with 4-NQO (Williams et al. 2010). Pol IV and NER are also proposed to work cooperatively on N^2-N^2-guanine interstrand DNA cross-links (ICLs) (Kumari et al. 2008). In another study, ICLs induced by exposure of cells to nitrogen mustard were proposed to be repaired by pol II in concert with NER (Berardini et al. 1999). The role of pol IV in processing of nitrogen mustard ICLs has not yet been investigated.

Aside from NER, another major determinant of NQO survival is homologous recombination (Williams et al. 2010). Although homologous recombination has been described as an error-free repair pathways, several studies have proposed that TLS polymerases can participate in homologous recombination and make the process error-prone (see **Fig. 2C**) (Lovett 2006; Moore et al. 2017). *In vitro* experiments demonstrated that *E. coli* pol IV can proficiently extend D-loops (Pomerantz et al. 2013b). Interestingly, synthesis at D-loops has markedly lower fidelity than at standard primed-template structures. At D-loops, pol II appears to be proficient in correcting errors introduced by pol IV, presumably due to its exonuclease function. Consequently, pol II is proposed to supresses error-prone recombination (Pomerantz et al. 2013b). Similar to pol IV, DNA polymerase I (pol I) is less accurate at RecA-mediated recombination intermediates (Pomerantz et al. 2013a). This

suggests that certain polymerases might generally be error-prone at these unstable recombination intermediates which might be driving error-prone recombination and, conceivably, could represent a major determinant in the development of antibiotic resistance through mutation (Pomerantz et al. 2013a).

Single-molecule microscopy reveals that TLS polymerases mainly act away from replisomes. Considering TLS polymerases being involved in several DNA repair pathways, we investigated if TLS polymerases predominantly act in the vicinity of replisomes using single-molecule imaging in live *E. coli* cells (Robinson et al. 2015; Henrikus et al. 2018). Single molecule microscopy allows TLS polymerase activity to be observed as individual TLS polymerase molecules bind to DNA or replisomes and dissociate.

Using the SOS-inducing agents ciprofloxacin, UV light and MMS, we showed that the concentration of pol IV increases upon damage induction (Henrikus et al. 2018). The increase in concentration was correlated with cell filamentation rate and increased pol IV binding activity at DNA. In contrast to the textbook model, we found that pol IV mainly binds away from replisomes suggesting that the majority of pol IV activity could be non-replisomal. Furthermore, pol IV molecules bound in the vicinity of replisomes were often close to, rather than at, replisomes. These results, and those of others (Thrall et al. 2017), support the model of post-replicative TLS, although do not completely exclude the possibility that pol IV is involved in replicative TLS. Since pol IV mostly binds away from replisomes, pol IV might predominantly work in other pathways such as transcription (Cohen et al. 2010), nucleotide excision repair (Courcelle et al. 2005; Williams et al. 2010) and homologous recombination (Ponder et al. 2005; Shee et al. 2011; Pomerantz et al. 2013a; Mallik et al. 2015) as proposed in several studies. In a microscopy study in which cells were treated with NQO or nalidixic acid, pol IV foci were been shown to colocalise with certain RecA structures and also with DSBs, supporting the idea that pol IV is involved in DSB repair

(Mallik et al. 2015). It is important to note, however, that in this study pol IV was expressed at somewhat higher levels than in wild-type cells. The pol IV colocalisation with RecA agglomerates was observed at a relatively late stage of the DNA damage response, around 180 min after damage induction. It would be of considerable interest to repeat these measurements with higher time resolution, to determine if pol IV acts at RecA structures earlier in the SOS response.

We have also investigated the regulation of pol V and its role in replicative translesion synthesis upon UV damage (Robinson et al. 2015). Pol V is a highly error-prone polymerase and thus underlies several stages of temporal and spatial regulation. After activation, pol V has little activity at replisomes and rather binds away from replisomes, similar to pol IV. However, in a *recA*(E38K) mutant, where pol V is constitutively activated in the absence of damage, many pol V molecules are bound at replisomes. In *recA*(E38K) UV irradiation however, additional binding sites away from replisomes open for pol V. Since pol IV binds at RecA structures upon SOS induction, it would be of interest to determine whether also works on recombination intermediates.

The third TLS polymerase, pol II, is different to pol IV and V in that it has an exonuclease function. Pol II has been shown to suppress the error-prone activity of pol IV at recombination intermediates, presumably due to pol II proof-reading errors introduced by pol IV. To date, live cell single-molecule studies on pol II have not yet been published. It would be interesting to know whether pol II shows a different behaviour to pol IV and V especially because of pol II's exonuclease activity.

Conclusions. Single-molecule live cell imaging reveals that 90% of pol IV foci and 95% of pol V foci form at sites on the DNA that are spatially distinct from replisomes (Robinson et al. 2015; Henrikus et al. 2018). Of the remaining 5–10% of foci, many appear close to replisomes rather than at replisomes. The data appear to indicate that TLS

polymerases frequently participate in mechanisms other than replicative TLS. Based on other studies, these extra-replisomal activities could include post-replicative TLS, incorporation of oxidised dNTPs, rescue of stalled RNA polymerase complexes or participation in NER or homologous recombination.

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Figure 1

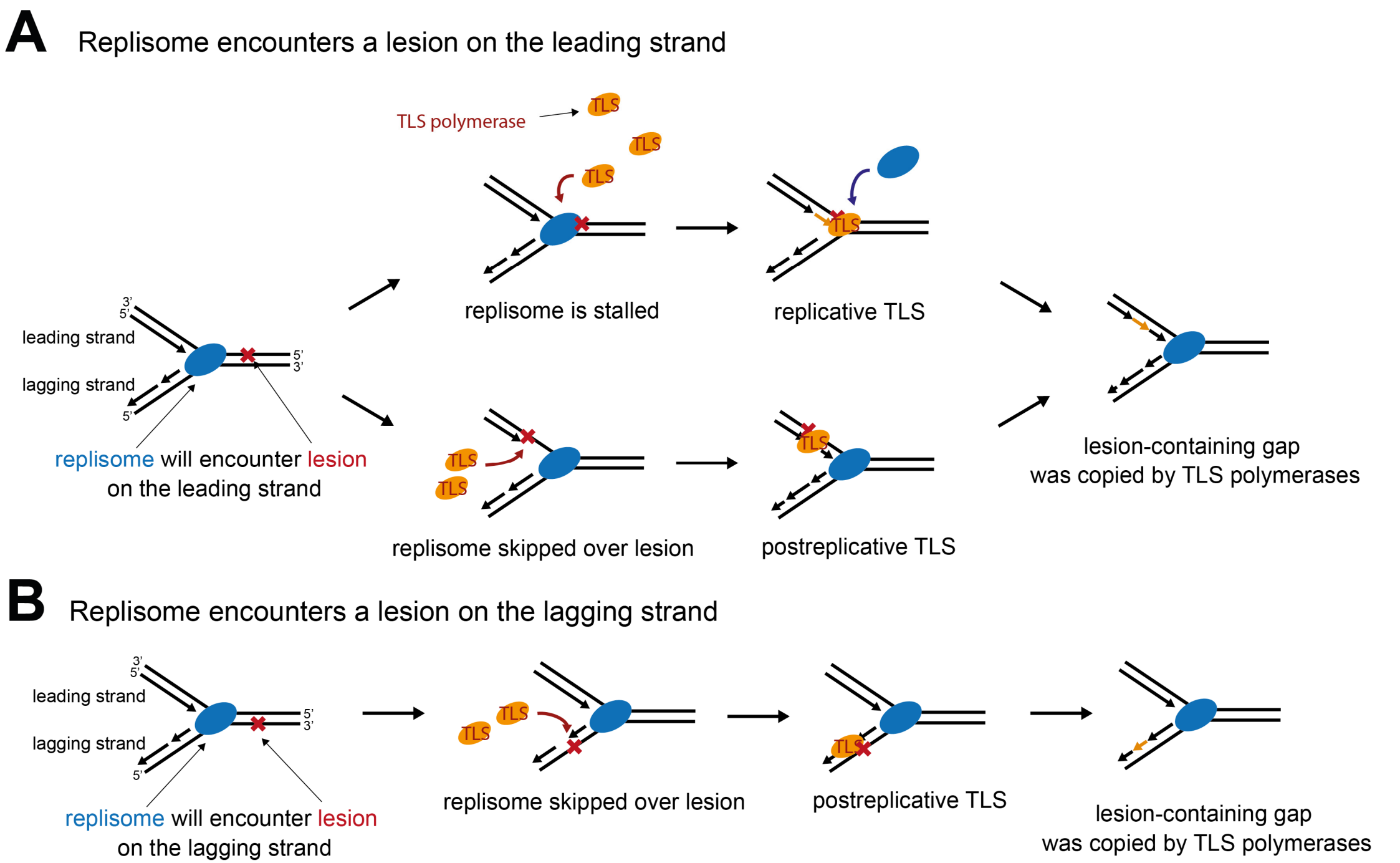
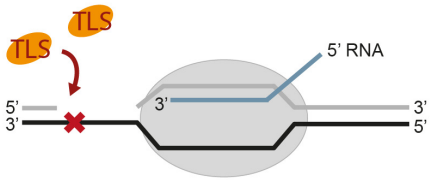

A
Replisome encounters a lesion on the leading strand
TLS polymerase
TLS
TLS
TLS
TLS
3'
5'
leading strand
lagging strand
5'
5'
3'
replisome will encounter lesion
on the leading strand
replisome is stalled
replicative TLS
TLS
TLS
TLS
replisome skipped over lesion
postreplicative TLS
TLS
lesion-containing gap
was copied by TLS polymerases
B
Replisome encounters a lesion on the lagging strand
3'
5'
leading strand
lagging strand
5'
5'
3'
replisome will encounter lesion
on the lagging strand
TLS
TLS
replisome skipped over lesion
postreplicative TLS
TLS
lesion-containing gap
was copied by TLS polymerases

Figure 2

A TLS polymerases rescue stalled RNAPs



Transcription-coupled TLS

B TLS polymerases in nucleotide excision repair

TLS polymerase activity at NER intermediates

C TLS polymerases in homologous recombination

Error-prone recombination