Cells limit mutagenesis by processing DNA lesions behind the replication fork

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Abstract

DNA lesions are a threat to genome stability. In order to cope with these lesions, cells have evolved lesion tolerance mechanisms: Translesion Synthesis (TLS) that allows the cell to insert a nucleotide directly opposite to the lesion, with the risk if introducing a mutation, and error-free Damage Avoidance (DA) that uses homologous recombination to retrieve the genetic information from the sister chromatid.

In this article, we investigate the timing of lesion bypass. Our findings reveal that TLS can occur at the fork immediately after encountering the blocking lesion. However, TLS can also take place behind the fork, at post-replicative gaps that are generated downstream of the lesion after repriming. We show that in this latter situation, TLS compete with the Damage Avoidance pathway. Additionally, our study demonstrates that EXO1 nuclease influences the balance between TLS and DA by modulating the size of the post-replicative gaps.

Introduction

The DNA of every organism is continually subject to damage from various exogenous and endogenous agents. These lesions will frequently block the progression of replicative DNA polymerases, impeding the progression of the replication fork and thereby posing a threat to genome stability. Cells have evolved lesion tolerance mechanisms that allow them to cope with DNA lesions. Translesion synthesis (TLS) is an error-prone process involving specialized DNA polymerases that insert nucleotides directly opposite the lesion. Damage avoidance (DA) is an error-free process relying on homologous recombination (HR) to bypass the damaged site. The balance between error-prone TLS and error-free DA determines the level of mutagenesis during lesion bypass.

Whether cells deal with DNA lesions at the replication fork or post-replicatively has been a long-standing debate. Initial studies by Rupp and Howard-Flanders (Rupp and Howard-Flanders, 1968) proposed that repriming could occur downstream of a lesion, leading to the generation of gaps behind the fork that were later filled in post-replicatively. However, this perspective changed with the discovery of TLS polymerases: at that time the prevailing model suggested that TLS polymerases would transiently replace the replicative DNA polymerase at the fork, without need for repriming or formation of gaps (Pagès and Fuchs, 2002). This model was challenged when gaps were directly observed using electron microscopy in UV-irradiated *S. cerevisiae* (Lopes et al., 2006), and replication restart downstream a lesion was observed *in vitro* in *E. coli* (Heller and Marians, 2006). Following these observations, several studies demonstrated that *S. cerevisiae* deals with a range of DNA lesions in a post-replicative manner (Daigaku et al., 2010) (Karras and Jentsch, 2010) (Wong et al., 2020) (Fumasoni et al., 2015).

The identification of PRIMPOL in mammalian cells strongly supports the repriming model and, consequently, post-replicative lesion bypass (Bianchi et al., 2013; García-Gómez et al., 2013).

While repriming is now generally accepted, there are still some debates about what happens at the replication fork. TLS could theoretically occur both at the replication fork and at a post-replicative gap. Similarly, DA can occur by Homologous Recombination (HR) at a post-replicative gap, but some other kind of HR-related strand exchange could also take place directly at the replication fork through the formation of regressed fork, also known as a "chicken-foot" structure. Such structure could facilitate the use of the sister chromatid as a template without the need of generating post-replicative gaps (Sogo et al., 2002).

In this article, we reconciliate both model by investigating the timing of lesion bypass in *Saccharomyces cerevisiae*. We show that TLS can occur at the fork, rapidly after the encounter with the blocking lesion. We show that TLS can also occur behind the fork, at post-replicative gaps that are generated downstream of the lesion after repriming has occurred. We show that in this latter situation, TLS is reduced because it is in competition with the DA pathway. We also showed that *EXO1* extends the size of the post-replicative gaps, which favors damage avoidance over TLS.

Results and Discussion

In order to investigate the timing of lesion bypass, we examined the replication of several DNA blocking lesions and the partition between TLS and DA bypass. For this purpose, we used our recently developed system that allows us to introduce a single lesion at a precise genomic locus in *S. cerevisiae* and monitor the bypass of this lesion by either TLS or DA (Masłowska et al., 2019). In short, a plasmid containing the single lesion of interest is inserted at a specific locus within the yeast genome using the Cre recombinase and modified lox sites. Lesion bypass is monitored by counting blue and white colonies as the lesion is located within the *lacZ* reporter gene (Supp. Figure 1). It is important to note that the tolerance events (DA or TLS) are plotted as a percentage compared to the integration of a non-damaged vector. Hence, the total percentage of tolerance might be lower than 100%, reflecting a lower survival of the cells with the lesion compared to the non-damaged control.

TLS and DA are in competition

Using this system, we have previously monitored the bypass of a common UV lesion, the (6-4)TT photoproduct and had shown that Translesion Synthesis (TLS) is in competition with Damage Avoidance (DA). Indeed, the inactivation of *ubc13* that is responsible for PCNA poly-ubiquitination (the signal activating the DA pathway) led to a decrease in the use of DA (from ~100% to 58%), and a concomitant 10-fold increase in the level of TLS (from 4% to 42%), without significantly affecting cells survival. Similarly, inactivation of *rad51* (the recombinase in charge of the DA process) produced a comparable decrease in DA and increase in TLS (Masłowska et al., 2019).

In the *ubc13* and *rad51* strains, some DA still persisted as we still observed a significant number of white colonies in our assay. These colonies could have arisen from *RAD51*-independent template switching mechanisms that rely on *RAD52* (Gangavarapu et al., 2007). The (6-4)TT photoproduct is primarily bypassed by the combination of TLS polymerases Pol ζ and Rev1.

Here, we have repeated the experiment using the G-AAF lesion, which is also predominantly bypassed by the same two DNA polymerases (although a minor fraction of the bypass also involves Pol η) (Masłowska et al., 2022; Pagès et al., 2008). As shown in Figure 1A, inactivation of either *ubc13* or *rad51* led to a decrease in DA and an increase in TLS, indicating a

competition between these two pathways. These results with the G-AAF lesion are similar to the ones previously obtained with the (6-4)TT lesion.

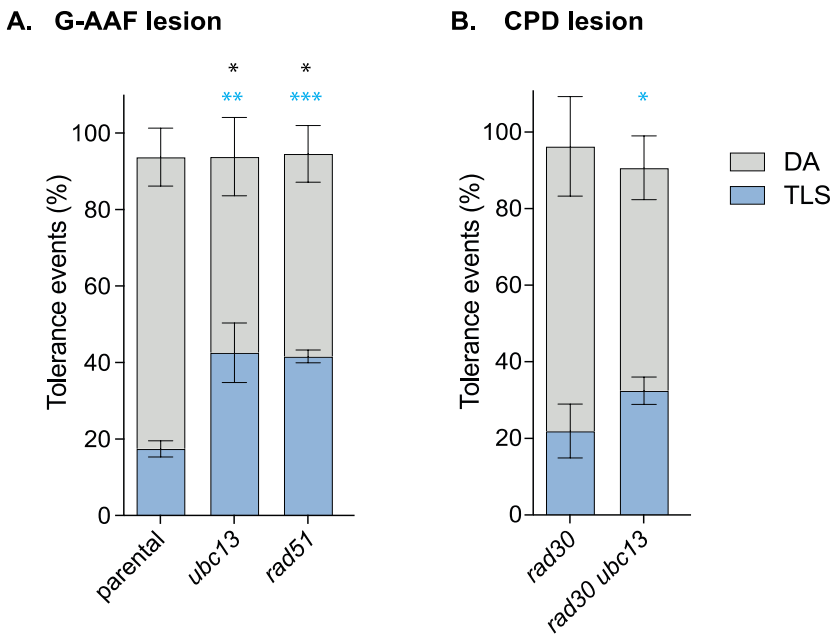

Figure 1: Partitioning of DNA damage tolerance pathways **A:** at a G-AAF lesion. **B:** at a CPD lesion. Tolerance events are shown as the percentage of bypass of the lesion compared to the non-damaged control. A total tolerance events below 100% reflects a lower survival of the damaged vector compared to the control.
Unpaired t-test was performed to compare TLS and DA values from the different mutants to the parental strain (A) or to the *rad30* strain (B). (*$p < 0.05$; **$p < 0.005$; ***$p < 0.0005$)

Surprisingly, when we had used another common UV lesion, the TT-CPD lesion that is bypassed primarily by DNA Polymerase η (encoded by *RAD30*), and partially by the combination of Pol ζ-Rev1, we had not observed any increase in TLS when *ubc13* or *rad51* were inactivated (Masłowska et al., 2019). It appears that the two pathways were not in competition for this lesion.

Here, we monitored the bypass of this same lesion in the absence of Pol η, when its bypass relies only on Pol ζ-Rev1 (Figure 1B). We did this time observe an increase in TLS when *ubc13* was inactivated.

Model: competition between TLS and DA occurs behind the fork at post-replicative gaps.

As we did not observe a competition between DA and TLS for the CPD lesion when it is bypassed by Pol η, we formulated the following model:

We hypothesized that the observed competition between DA and TLS (an increase of TLS in the *ubc13* strain) takes place behind the fork during a gap filling reaction. Following the encounter with a blocking lesion, a repriming event generates a single-strand DNA gap that will be filled post-replicatively by TLS or DA, thereby placing the two pathways in competition. As the expression level of Rev1 is significantly higher in G2/M compared to the S phase (Waters and Walker, 2006), it implies that Rev1 primarily acts post-replicatively rather than at the fork. On the other hand, Pol η is expressed constantly throughout the cell cycle (Waters and Walker, 2006), it should therefore be able to bypass lesions both at the fork and post-replicatively.

In our model, the majority of CPD lesions would be efficiently bypassed by Pol η at the fork without the need for repriming, alleviating the competition with DA.

If this model is valid, forcing Pol η to bypass the CPD lesion post-replicatively (rather than at the fork) should induce competition between TLS with DA for this lesion, as observed for the other two lesions tested.

To test this model, and in order to force Pol η action to occur only post-replicatively, we restricted its expression to the G2/M phase of the cell cycle. If Pol η is absent during the S phase, it cannot act at the replication fork. This, by allowing its expression only in G2/M, we ensured its activity occurs post-replicatively.

We used two different strategies for this purpose: i) we expressed *RAD30* from the CLB2 promoter (including the cyclin degron) system (Karras and Jentsch, 2010), limiting its expression to the G2/M phase of the cell cycle; ii) we used the auxin-inducible degron (AID) system (Morawska and Ulrich, 2013), to induce Pol η degradation in G1 and S phase, therefore allowing its expression only in G2/M (Figure 2).

We synchronized our cells by arresting them in G1 using alpha-factor, and while monitoring the cell cycle by FACS upon release from the arrest, we verified the level of expression of Pol η by Western blot.

As shown in Figure 2, the Clb2-controlled *RAD30* construction allows peak expression of Pol η in G2/M. However, while the level of expression is very low in G1 and at the beginning of S phase, it already starts increasing in mid to late S phase. The auxin-controlled *RAD30* construction shows a more effective restriction of expression during G1/S, and a strong level

of expression in G2/M. Another advantage of this latter construction is that it allows a physiological level of expression of Pol η as it is controlled by its native promoter.

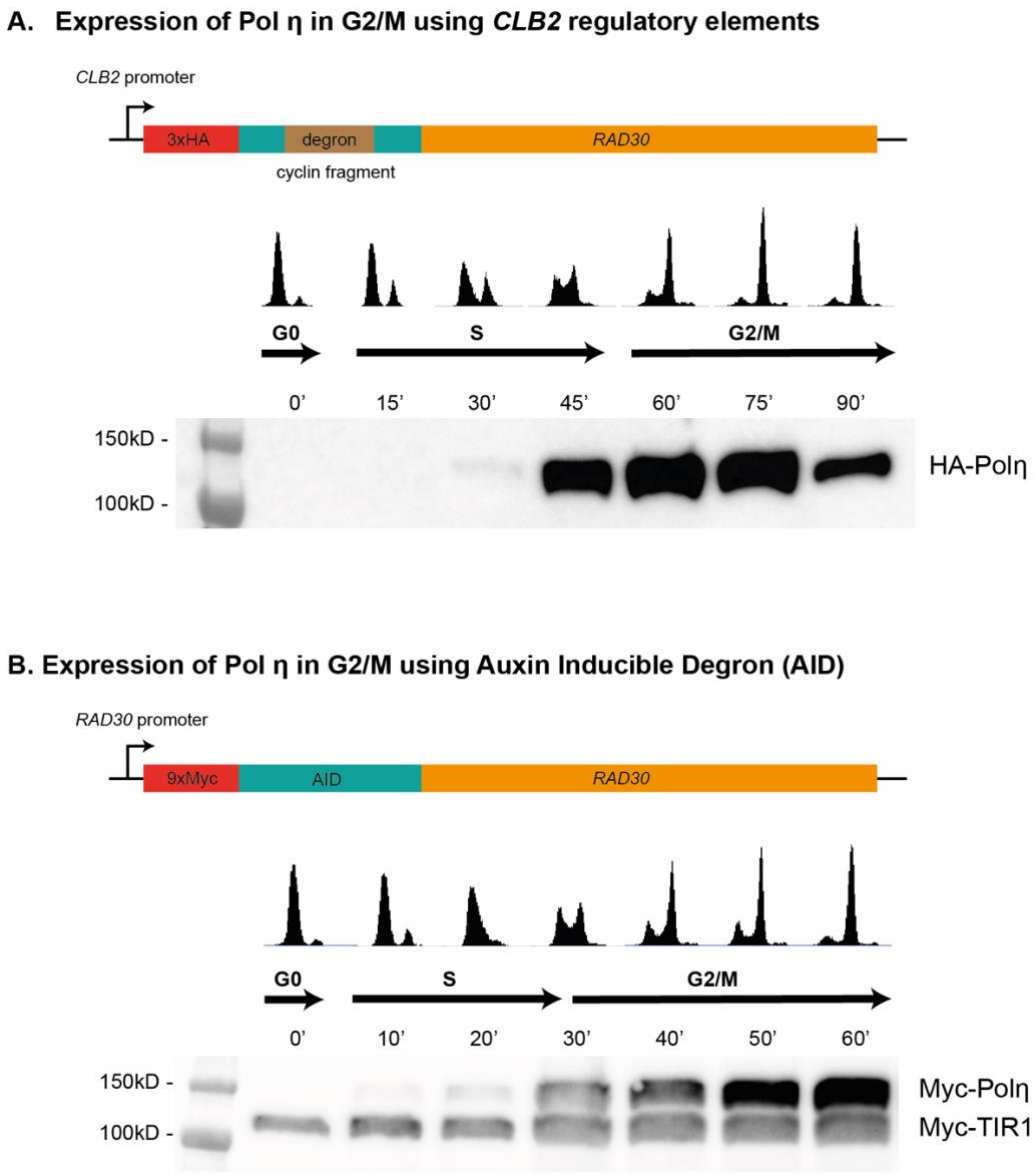

Figure 2: Constructions allowing Pol η expression in the G2/M cell cycle phase **A.** Using the CLB2 promoter and cyclin degron element. **B.** Using the auxin-inducible degron. Auxin was present at 1 mM concentration during cell synchronization with alpha factor and removed upon cells release. Western blots show the level of expression of HA-Pol η or Myc-Pol η at different phases of the cell cycle.

Using these two strains, we measured the level of TLS and DA upon introduction of a single CPD lesion into the yeast genome during the S phase of the cell cycle (by electroporating the damaged vector right after washing away the alpha-factor and auxin). As shown in Figure 3A,

compared to the parental strain, the expression of Pol η exclusively in G2/M leads to a significant reduction in the TLS level at the CPD lesion, accompanied by a simultaneous increase in the level of DA. It is important to note that the TLS level, although reduced, does not reach the same low levels observed in the absence of Pol η (Figure 1B). This could be due to the fact that either some Pol η is still present in S phase and contributes to TLS, or that it continues to participate in TLS in G2/M, resulting in a higher level than in the complete absence of the polymerase. According to our hypothesis, the observed decrease in TLS can be attributed to the fact that when Pol η is acting post-replicatively (in G2/M), TLS is in competition with DA.

To validate this hypothesis, we repeated the experiment in a strain where *ubc13* was inactivated in order to prevent DA (Figure 3B). The inactivation of *ubc13* did not lead to an increase in TLS in the parental strain where Pol η is expressed all along the cell cycle as previously shown (Masłowska et al., 2019). However, when Pol η expression was restricted to G2/M, a situation where TLS is potentially in competition with DA, the inactivation of *ubc13* did lead to an increase in TLS. Remarkably, inactivation of *ubc13* in the two strains expressing Pol η in G2/M effectively restored TLS to levels comparable to the parental strain where Pol η is expressed constantly throughout the cell cycle.

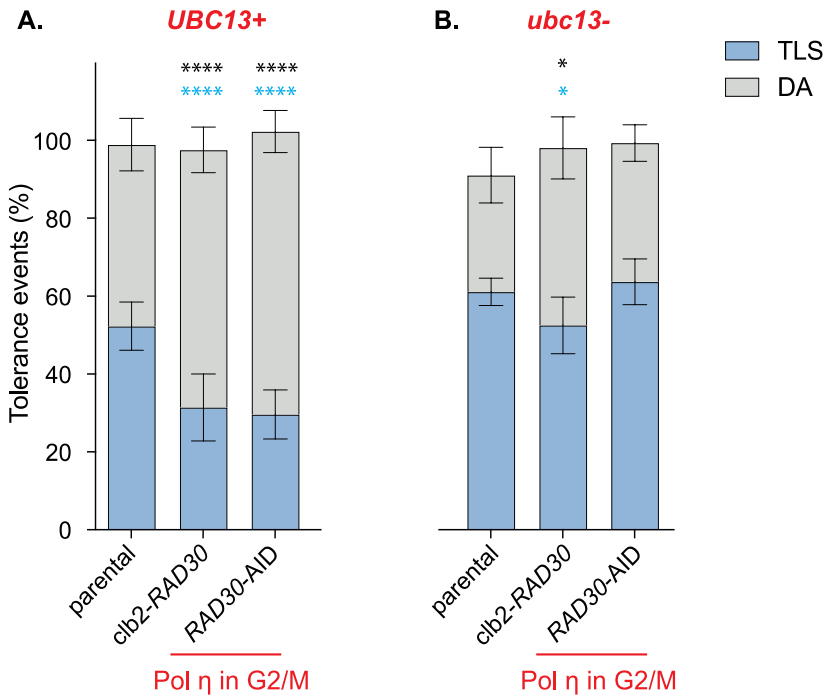

Figure 3: Partitioning of DDT pathways at a single CPD lesion in strains expressing Pol η only in G2/M. **A**. in cells procificent for DA (*UBC13+*), **B.** in cells deficient for DA (*ubc13*Δ). Unpaired t-test was performed to compare TLS and DA values from the different mutants to the parental strain. (*p < 0.05; **p < 0.005; ***p < 0.0005)

These experiments validate our model. It appears therefore that when a lesion is bypassed at the replication fork (such as the TT-CPD bypassed by Pol η), TLS is not in competition with DA, resulting in a high TLS level. However, when the same lesion is bypassed post-replicatively, TLS is then in competition with DA, and the level of TLS is reduced.

It is interesting to note that we obtained similar results with both constructions that express Pol η in G2/M. As stated earlier, the CLB2 construction allows some level of expression of Pol η already in mid to late S-phase. Given that our lesion is located close to an early replication origin (see description of the system in (Masłowska et al., 2019)), we can expect it to be encountered by the replication fork early in the S-phase, when Pol η is still absent or at least very weakly expressed in our strain. Whether Pol η reappears in mid to late S-phase (CLB2 construction) or in G2/M (AID construction), the replication fork has already bypassed the lesion and the gap has formed, implying competition with DA. This suggests that DA is not restricted to G2/M phase, but can occur in S-phase as soon as post-replicative gaps are formed.

This further implies that TLS has priority over DA. Indeed, when Pol η is absent, we showed that DA can occur in early S-phase. So in the normal conditions, when Pol η is present, the level of TLS is high, implying that TLS won the competition over DA. This priority given to TLS seems to have been conserved through evolution as we have previously shown that TLS had also priority over DA in the bacteria *E. coli* (Naiman et al., 2014).

Gap extension by Exo1 promotes DA and reduces TLS

Our results indicate that post-replicative gaps play a crucial role in the regulation of DNA damage tolerance. To further investigate these findings, we explored the involvement of *EXO1* in DNA damage tolerance. *EXO1* encodes a 5'->3' exonuclease that has been mostly described for its role in recombination at double strand breaks (during meiosis initially, but also in mitotic cells) and telomere maintenance (for a comprehensive review, see (Tran et al., 2004)). Additionally, studies have demonstrated that Exo1 can extend ssDNA gaps during Nucleotide Excision Repair (NER) (Giannattasio et al., 2010). We have recently shown in bacteria that the

extension of ssDNA gaps was crucial for DA to occur efficiently. Specifically, in the absence of the 5'->3' exonuclease RecJ, we have observed a decrease in DA and a concomitant increase in TLS (Chrabaszcz et al., 2018; Laureti et al., 2022). The involvement of yeast Exo1 in postreplication repair has been previously proposed (Tran et al., 2007) (Vanoli et al., 2010) (Karras et al., 2013), suggesting that gap extension is also required for DA in yeast. It has also been shown that ExoI acts at postreplicative gaps to initiate damage signaling in response to MMS treatment (García-Rodríguez et al., 2018).

Therefore, we set out to correlate the impact of Exo1 on the size of the gaps with the choice of DNA damage tolerance pathway.

-Exo1 promotes ssDNA gap extension

We evaluated how Exo1 modulates the size of ssDNA gaps. For this purpose, we exposed yeast cultures synchronized in the G1 phase to a single dose of UV (4 J.m-2) (Figure 4A) and monitored ssDNA in fixed cells by fluorescence microscopy using GFP-tagged Rfa1, the large subunit of the RPA complex (Figure 4C-D). We had previously shown that RPA foci serve as a proxy for postreplicative gaps (Wong et al., 2020). Cell-cycle stage was also monitored by flow cytometry (FACS) (Figure 4B).

During early S phase, the number of cells with RPA foci and the number of foci arising per cell should roughly correspond to those cells that received replication-blocking damage and thus the numbers of postreplicative gaps. As expected, inactivation of *EXO1* did not significantly affect these parameters (Figure 4C). However, the overall intensity of RPA foci per nucleus, reflecting the total amount of ssDNA, was significantly lower in the *exo1* mutant (Figure 4D), indicating a reduction in the size of the postreplicative gaps. We therefore conclude that Exo1 extends the length of the ssDNA gaps generated at UV-induced lesions.

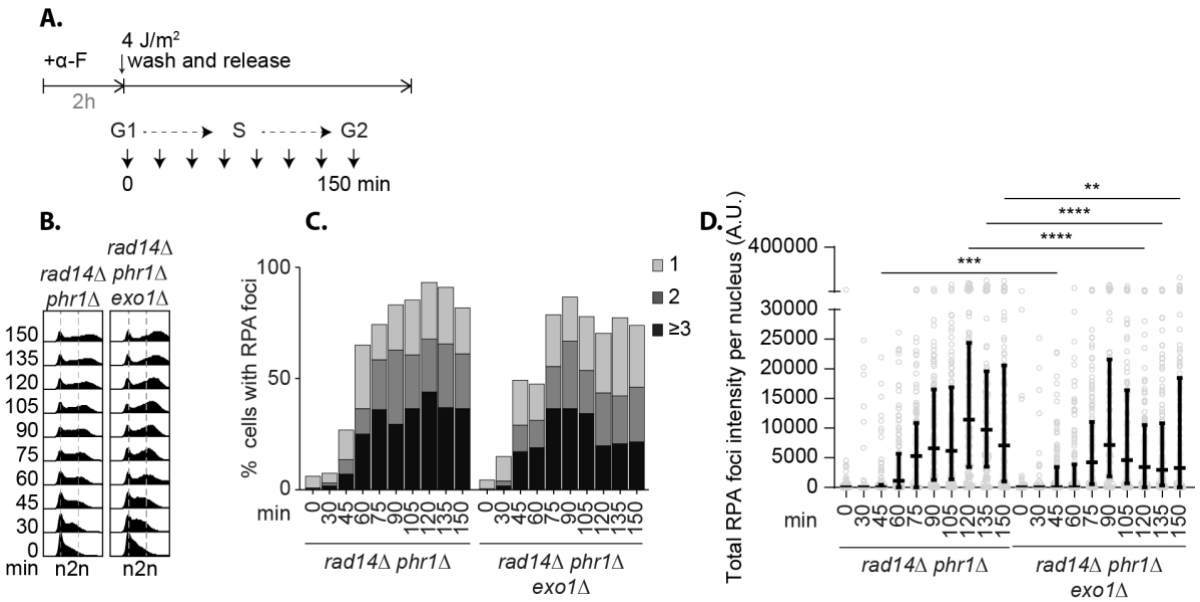

Figure 4: A. Experimental scheme: cells arrested in G1 phase with alpha-factor (αF) were exposed to 4J.m^{-2} UV before release into the cell cycle without. **B.** FACS monitoring of the progression through the cell cycle. **C.** Percentage of cells with Rfa1GFP foci, detected by fluorescence microscopy. **D.** Total Rfa1GFP foci intensity per nucleus.
Mann-Whithney test ** p<0.01; ***p<0.001; ****p<0.0001

-Exo1 favors DA over TLS

We then explored the effect of *EXO1* inactivation on the balance between TLS and DA. We inactivated *EXO1* and monitored the bypass of 3 different DNA lesions. As depicted in Figure 5, we observed a strong increase in TLS for the TT(6-4) (>9 fold) and for the G-AAF (>3 fold) lesions in the absence of Exo1. In the absence of gap extension by Exo1, homologous recombination with the sister chromatid (DA) becomes less efficient, allowing TLS to occur at a higher rate. This confirms that TLS at these two lesions predominantly occurs at the post-replicative gaps.

In contrast, we did not observe any significant increase in TLS at the CPD lesion in the absence of *exo1*, confirming our model that gap extension has little effect on this lesion since it is bypassed at the fork and not post-replicatively.

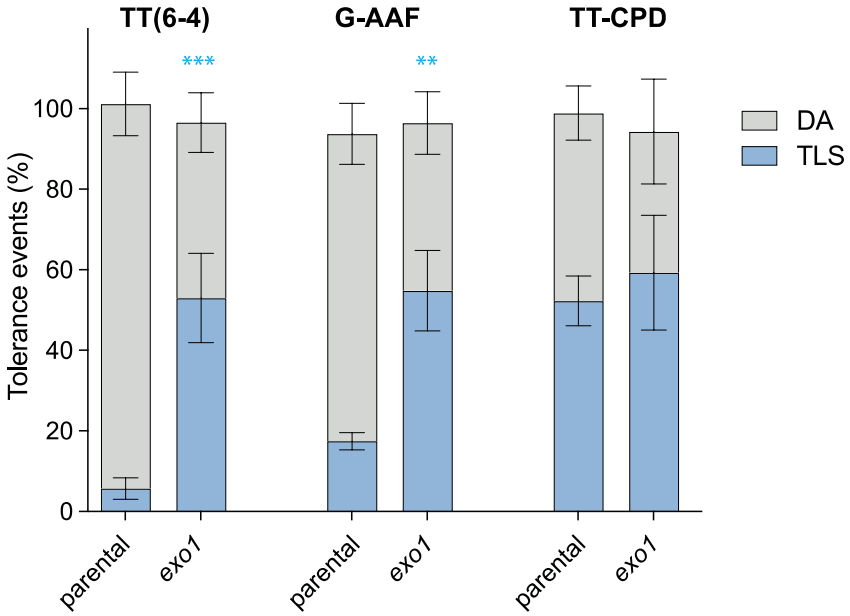

Figure 5: Partitioning of DDT pathways at 3 different DNA lesion in the presence (parental) or absence of Exo1. Unpaired t-test was performed to compare TLS and DA values from the different mutants to the parental strain. (*p < 0.05; **p < 0.005; ***p < 0.0005)

Conclusions

In conclusion, we have shown that TLS could occur both at the replication fork and behind the fork. When it occurs at the fork, it has priority over DA. On the other hand, while when occurring post-replicatively, TLS is reduced because it is then in competition with DA (Figure 6).

By promoting the extension of post-replicative gaps, Exo1 facilitates homologous recombination, favoring DA and in turn reducing TLS when the two pathways are in competition.

The timing of expression of the TLS polymerases determines when TLS occurs. Rev1 is primarily expressed in G2/M. Therefore, its activity, and hence the activity of Pol ζ with which it interacts (Acharya et al., 2006), is limited to post-replicative gaps. This results in a reduced level of TLS by these two polymerases when DA in functional. On the other hand, Pol η is expressed continuously throughout the cell cycle, allowing it to efficiently perform TLS at the fork during S phase without competing with DA.

It will be interesting to test whether constant expression of Rev1 throughout the cell cycle would lead to an elevated level of TLS by enabling its action at the replication fork, thereby alleviating the competition with DA. However, the control of Rev1 appears not to be transcriptional (Waters and Walker, 2006) making its modulation challenging and such investigation difficult.

Considering that TLS by Rev1-Pol ζ at various lesions is much more mutagenic than TLS by Pol η at CPD lesions, over evolution, cells might have restricted the expression of Rev1 to G2/M, providing the advantage to tune down this mutagenic pathway by allowing competition with DA.

On the other hand, direct bypass of the CPD lesion at the replication fork could be advantageous for the cell. CPDs are the most abundant DNA damage directly induced by UV light, and their removal by NER is delayed compared to TT(6-4) (Cadet and Douki, 2018).

In conclusion, cells potentially limit mutagenesis by permitting error-free TLS bypass directly at the fork, while restricting error-prone bypass to post-replicative gaps where it competes with error-free DA. This dual approach allows for efficient damage tolerance while minimizing the risk of mutagenic events.

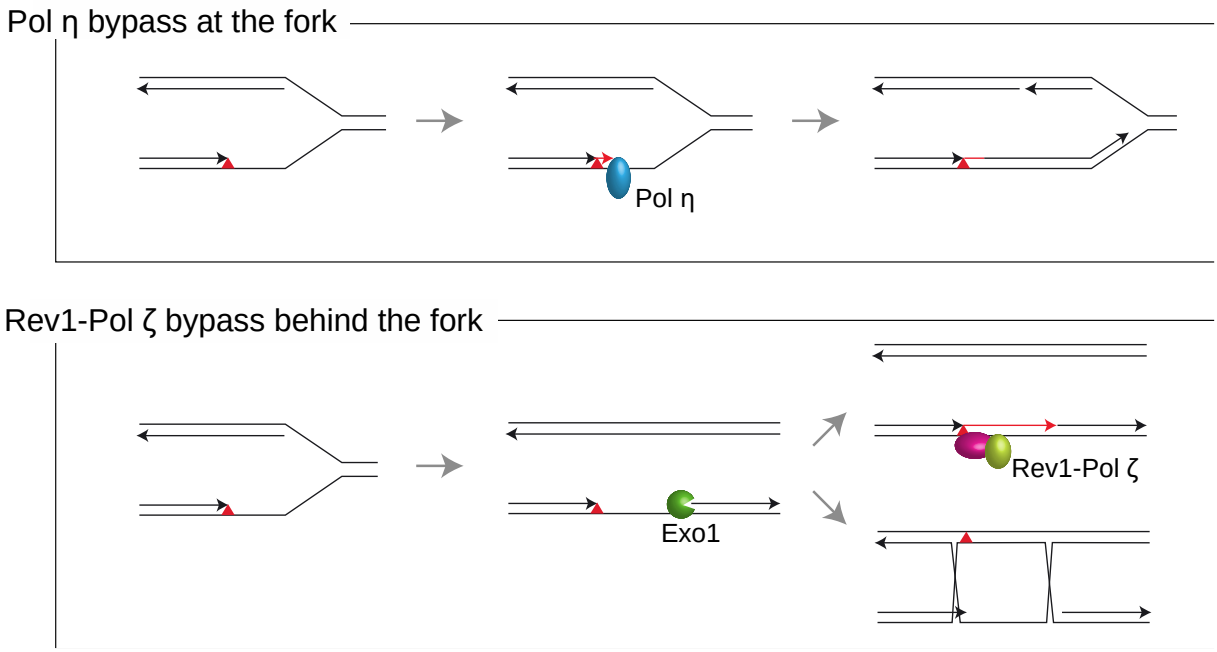

Figure 6: Model for lesion bypass at the fork or behind the fork. The bypass of CPD lesion by Pol η occurs at the fork and is not in competition with DA. Lesion bypass by Rev1-Pol ζ occurs behind the fork at a single-stranded DNA gap. This gap is extended by Exo1 which favors DA. DA is in competition with Rev1-Pol ζ TLS.

Materials and methods

Yeast strains

All strains used in the present study are derivative of strain EMY74.7 (Johnson et al., 1998) (MATa his3-Δ1 leu2-3,112 trp1Δ ura3-Δ met25-Δ phr1-Δ rad14-Δ msh2Δ:hisG). In order to study tolerance events, all strains are deficient in repair mechanisms: nucleotide excision repair (*rad14*), photolyase (*phr1*), and mismatch repair system (*msh2*). Gene disruptions were achieved using PCR-mediated seamless gene deletion (Akada et al., 2006) or URAblaster (Alani et al., 1987) techniques.
Strains carrying polymerase η (*RAD30*) under control of auxin-inducible degron (AID) were created by inserting the pKAN-PRAD30-9myc-AID*(N) cassette into the Rad30 native locus.
Strains carrying polymerase η (*RAD30*) under control of regulatory elements of cyclins Clb2 (from pGIK43) or Clb5 (from pKM101) were created by inserting cyclin cassettes into native Rad30 locus.
TIR1 strains were created by integration of osTIR1 cassette from pNHK53 (encoding OsTIR1 under control of the ADH1 promoter) into yeast chromosome VII.
Plasmid pNHK53 was obtained from the National BioResource Project–Yeast (Nishimura et al., 2009), plasmid pKAN-PCUP1-9myc-AID*(N) from Addgene (Morawska and Ulrich, 2013), and plasmid pGIK43 from Georgios Karras.

Integration

Integration of plasmids carrying (6-4)TT/N2dG-AAF lesions (or control plasmids without lesion) and result analysis was performed as previously described (Masłowska et al., 2019).
For experiments involving cell-cycle restricted polymerase η cells were synchronized in G0 phase using alpha-factor. After synchronization, the wash, conditioning and electroporation steps were carried out directly. For strains with AID degrons auxin was present at 1mM during incubation with alpha-factor.
Lesion tolerance rates were calculated as the relative integration efficiencies of damaged vs. non-damaged vectors normalized by the transformation efficiency of a control plasmid (pRS413) in the same experiment. DA events are calculated by subtracting TLS events from the total lesion tolerance events. All experiments were performed at least in triplicate. Graphs and statistical analysis were done using GraphPad Prism applying unpaired t-test. Bars represent the mean value ± s.d.
For a more comprehensive method description, see (Masłowska et al., 2019).

Synchronisation

Yeast cultures were grown till OD600=0.8. Alpha-factor (GeneScript) was then added at the concentration 10 µg/ml, and cells were further incubated for 90 min. Synchronization was verified by microscopy, and further by flow cytometry.

Detection of proteins

Total lysates of synchronized yeast cultures were prepared by quick trichloroacetic acid (TCA) extraction: cells (pelleted 10 ml of culture) were resuspended in 250 µl of 20% TCA and vortexed with glass beads for 30s. After centrifugation at 3000 × g for 10 min, the supernatant was removed and the pellet resuspended in LDS loading buffer and incubated at 75 °C for 10

min. Proteins were analysed by SDS–PAGE/Western blotting using monoclonal antibodies anti c-Myc 9E10 or anti HA 12CA5 (Thermo).

Flow cytometry

Cells were fixed in 70% ethanol overnight and washed with 50 mM TE, pH 7.5. After incubation with 0.1 mg/ml DNase-free RNAse A for 4 h at 42°C, and 0.5 mg/ml Proteinase K for 30 min at 50°C, DNA was stained with SYTOX green. Cells were analyzed by flow cytometry, using a Accuri C6 Plus flow cytometer (BD Biosciences).

Live cell imaging and image analysis

Imaging and image analysis was performed twice, essentially as previously described (Wong et al., 2020). Cells synchronized in G1 with alpha-factor were irradiated with UV, washed, and released into the cell cycle. At indicated time points after release, cells were plated on concanavalin A-coated chambered coverslips with glass bottom (Ibidi) and imaged with a DeltaVision Elite system (GE Healthcare) equipped with a 60× oil immersion objective (NA = 1.42), scientific CMOS camera, InsightSSI solid state illumination, and SoftWoRx software with built-in deconvolution algorithms in an environmentally controlled chamber at 30°C. GFP signals were imaged with a FITC filter and DIC was used for brightfield images. Z stacks with 21 planes (step size = 0.2 µm) were acquired for each image.

Images were analyzed using a customized scripts written in ImageJ macro language with ImageJ FIJI software (https://fiji.sc/). Scripts are available at https://github.com/helle-ulrich-lab/image-analysis-PORTs.

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References

Acharya, N, RE Johnson, S Prakash, and L Prakash (2006), 'Complex formation with Rev1 enhances the proficiency of Saccharomyces cerevisiae DNA polymerase zeta for mismatch extension and for extension opposite from DNA lesions.', *Mol Cell Biol*, 26 (24), 9555-63.

Akada, Rinji, Takao Kitagawa, Shohei Kaneko, Daiso Toyonaga, Sachiko Ito, Yoshito Kakihara, Hisashi Hoshida, Shigeru Morimura, Akihiko Kondo, and Kenji Kida (2006), 'PCR-mediated seamless gene deletion and marker recycling in Saccharomyces cerevisiae.', *Yeast (Chichester, England)*, 23 (5), 399-405.

Alani, E, L Cao, and N Kleckner (1987), 'A method for gene disruption that allows repeated use of URA3 selection in the construction of multiply disrupted yeast strains.', *Genetics*, 116 (4), 541-45.

Bianchi, Julie, et al. (2013), 'PrimPol Bypasses UV Photoproducts during Eukaryotic Chromosomal DNA Replication.', *Mol Cell*, 52 (4), 566-73.

Cadet, Jean and Thierry Douki (2018), 'Formation of UV-induced DNA damage contributing to skin cancer development', *Photochemical & Photobiological Sciences*, 17 (12), 1816-41.

Chrabaszcz, Élodie, Luisa Laureti, and Vincent Pagès (2018), 'DNA lesions proximity modulates damage tolerance pathways in Escherichia coli.', *Nucleic acids research*, 8 437.

Daigaku, Yasukazu, Adelina A Davies, and Helle D Ulrich (2010), 'Ubiquitin-dependent DNA damage bypass is separable from genome replication.', *Nature*, 465 (7300), 951-55.

Fumasoni, Marco, Katharina Zwicky, Fabio Vanoli, Massimo Lopes, and Dana Branzei (2015), 'Error-Free DNA Damage Tolerance and Sister Chromatid Proximity during DNA Replication Rely on the Polα/Primase/Ctf4 Complex.', *Mol Cell*,

Gangavarapu, Venkateswarlu, Satya Prakash, and Louise Prakash (2007), 'Requirement of RAD52 group genes for postreplication repair of UV-damaged DNA in Saccharomyces cerevisiae.', *Molecular and cellular biology*, 27 (21), 7758-64.

García-Gómez, Sara, et al. (2013), 'PrimPol, an archaic primase/polymerase operating in human cells.', *Mol Cell*, 52 (4), 541-53.

García-Rodríguez, Néstor, Magdalena Morawska, Ronald P Wong, Yasukazu Daigaku, and Helle D Ulrich (2018), 'Spatial separation between replisome- and template-induced replication stress signaling.', *Embo J*, e98369.

Giannattasio, M, C Follonier, H Tourrière, F Puddu, F Lazzaro, P Pasero, M Lopes, P Plevani, and M Muzi-Falconi (2010), 'Exo1 competes with repair synthesis, converts NER intermediates to long ssDNA gaps, and promotes checkpoint activation.', *Mol Cell*, 40 (1), 50-62.

Heller, Ryan C and Kenneth J Marians (2006), 'Replication fork reactivation downstream of a blocked nascent leading strand.', *Nature*, 439 (7076), 557-62.

Johnson, R E, C A Torres-Ramos, T Izumi, S Mitra, Satya Prakash, and Louise Prakash (1998), 'Identification of APN2, the Saccharomyces cerevisiae homolog of the major human AP endonuclease HAP1, and its role in the repair of abasic sites.', *Genes Dev*, 12 (19), 3137-43.

Karras, Georgios I and Stefan Jentsch (2010), 'The RAD6 DNA damage tolerance pathway operates uncoupled from the replication fork and is functional beyond S phase.', *Cell*, 141 (2), 255-67.

Karras, Georgios Ioannis, Marco Fumasoni, Grzegorz Sienski, Fabio Vanoli, Dana Branzei, and Stefan Jentsch (2013), 'Noncanonical role of the 9-1-1 clamp in the error-free DNA damage tolerance pathway.', *Mol Cell*, 49 (3), 536-46.

Laureti, L, L Lee, G Philippin, M Kahi, and V Pagès (2022), 'Single strand gap repair: The presynaptic phase plays a pivotal role in modulating lesion tolerance pathways.', *PLoS Genet*, 18 (6), e1010238.

Lopes, Massimo, Marco Foiani, and José M Sogo (2006), 'Multiple mechanisms control chromosome integrity after replication fork uncoupling and restart at irreparable UV lesions.', *Mol Cell*, 21 (1), 15-27.

Masłowska, Katarzyna H, Luisa Laureti, and Vincent Pagès (2019), 'iDamage: a method to integrate modified DNA into the yeast genome.', *Nucleic acids research*, 18 (20), 563-e124.

Masłowska, KH, F Villafañez, L Laureti, S Iwai, and V Pagès (2022), 'Eukaryotic stress-induced mutagenesis is limited by a local control of translesion synthesis.', *Nucleic Acids Res*, gkac044.

Morawska, Magdalena and Helle D Ulrich (2013), 'An expanded tool kit for the auxin-inducible degron system in budding yeast.', *Yeast (Chichester, England)*, 30 (9), 341-51.

Naiman, Karel, Gaëlle Philippin, Robert P Fuchs, and Vincent Pagès (2014), 'Chronology in lesion tolerance gives priority to genetic variability.', *Proceedings of the National Academy of Sciences of the United States of America*, 111 (15), 5526-31.

Nishimura, K, T Fukagawa, H Takisawa, T Kakimoto, and M Kanemaki (2009), 'An auxin-based degron system for the rapid depletion of proteins in nonplant cells.', *Nat Methods*, 6 (12), 917-22.

Pagès, Vincent and Robert P P Fuchs (2002), 'How DNA lesions are turned into mutations within cells?', *Oncogene*, 21 (58), 8957-66.

Pagès, Vincent, Anne Bresson, Narottam Acharya, Satya Prakash, Robert P Fuchs, and Louise Prakash (2008), 'Requirement of Rad5 for DNA polymerase zeta-dependent translesion synthesis in Saccharomyces cerevisiae.', *Genetics*, 180 (1), 73-82.

Rupp, W D and P Howard-Flanders (1968), 'Discontinuities in the DNA synthesized in an excision-defective strain of Escherichia coli following ultraviolet irradiation.', *Journal of molecular biology*, 31 (2), 291-304.

Sogo, José M, Massimo Lopes, and Marco Foiani (2002), 'Fork reversal and ssDNA accumulation at stalled replication forks owing to checkpoint defects.', *Science (New York, NY)*, 297 (5581), 599-602.

Tran, Phuoc T, Julien P Fey, Naz Erdeniz, Lionel Gellon, Serge Boiteux, and R Michael Liskay (2007), 'A mutation in EXO1 defines separable roles in DNA mismatch repair and post-replication repair.', *DNA repair*, 6 (11), 1572-83.

Tran, PT, N Erdeniz, LS Symington, and RM Liskay (2004), 'EXO1-A multi-tasking eukaryotic nuclease.', *DNA Repair (Amst)*, 3 (12), 1549-59.

Vanoli, Fabio, Marco Fumasoni, Barnabas Szakal, Laurent Maloisel, and Dana Branzei (2010), 'Replication and recombination factors contributing to recombination-dependent bypass of DNA lesions by template switch.', *PLoS genetics*, 6 (11), e1001205.

Waters, Lauren S and Graham C Walker (2006), 'The critical mutagenic translesion DNA polymerase Rev1 is highly expressed during G(2)/M phase rather than S phase', *Proceedings of the National Academy of Sciences of the United States of America*, 103 (24), 8971-76.

Wong, RP, N García-Rodríguez, N Zilio, M Hanulová, and HD Ulrich (2020), 'Processing of DNA Polymerase-Blocking Lesions during Genome Replication Is Spatially and Temporally Segregated from Replication Forks.', *Mol Cell*, 77 (1), 3-16.e4.