



# HHS Public Access

Author manuscript

*DNA Repair (Amst)*. Author manuscript; available in PMC 2021 July 17.

Published in final edited form as:

*DNA Repair (Amst)*. 2021 May ; 101: 103075. doi:10.1016/j.dnarep.2021.103075.

## Tracking *Escherichia coli* DNA polymerase V to the entire genome during the SOS response

Mahmood Faraz<sup>a</sup>, Roger Woodgate<sup>b</sup>, Anders R. Clausen<sup>a,\*</sup>

<sup>a</sup>Department of Medical Biochemistry and Cell Biology, University of Gothenburg, 40530 Gothenburg, Sweden

<sup>b</sup>Laboratory of Genomic Integrity, National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, MD, 20892-3371, USA

### Abstract

Ribonucleotides are frequently incorporated into DNA and can be used as a marker of DNA replication enzymology. To investigate on a genome-wide scale, how *E. coli* pol V accesses undamaged chromosomal DNA during the SOS response, we mapped the location of ribonucleotides incorporated by steric gate variants of pol V across the entire *E. coli* genome. To do so, we used strains that are deficient in ribonucleotide excision repair (*rnhB*), deficient in pol IV DNA polymerase, constitutively express all SOS-regulated genes [*lexA(Def)*] and constitutively “activated” RecA\* (*recA730*). The strains also harbor two steric gate variants of *E. coli* pol V (Y11A, or F10L), or a homolog of pol V, (pol V<sub>R391-Y13A</sub>). Ribonucleotides are frequently incorporated by the pol V-Y11A and pol V<sub>R391-Y13A</sub> variants, with a preference to the lagging strand. In contrast, the pol V-F10L variant incorporates less ribonucleotides and no strand preference is observed. Sharp transitions in strand specificity are observed at the replication origin (*oriC*), while a gradient is observed at the termination region. To activate RecA\* in a *recA*<sup>+</sup> strain, we treated the strains with ciprofloxacin and genome-wide mapped the location of the incorporated ribonucleotides. Again, the pol V-Y11A steric gate variant exhibited a lagging strand preference. Our data are consistent with a specific role for pol V in lagging strand DNA synthesis across the entire *E. coli* genome during the SOS response.

### Keywords

SOS response; DNA polymerase V; Steric gate mutant; R391; Ribonucleotide incorporation; Ribonucleotide Excision Repair

---

This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

\*Corresponding author. anders.clausen@gu.se (A.R. Clausen).

CRedit authorship contribution statement

**Mahmood Faraz:** Writing - original draft, Data curation, Investigation, Methodology, Formal analysis, Software, Visualization.

**Roger Woodgate:** Conceptualization, Data curation, Funding acquisition, Methodology, Project administration, Resources, Supervision, Validation, Visualization, Writing - review & editing. **Anders R. Clausen:** Conceptualization, Methodology, Resources, Validation, Funding acquisition, Writing - review & editing.

Declaration of Competing Interest

The authors declare no competing interest.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:<https://doi.org/10.1016/j.dnarep.2021.103075>.

## 1. Introduction

Most damage-induced mutagenesis in *E. coli* is dependent upon DNA polymerase V (pol V) [1,2]. Pol V is encoded by the *umuDC* genes and is induced as part of the stress-inducible SOS response [3]. As part of the SOS response, the RecA recombinase is induced and forms nucleoprotein filaments [4]. This is often referred to as “activated” RecA, or RecA\*. In this so-called activated form, RecA\* mediates the self-cleavage of the transcriptional repressor of the SOS response, LexA, leading to the expression of all LexA-regulated genes, including UmuD and UmuC. Dimeric UmuD is not active for mutagenesis until it undergoes a similar RecA\* cleavage reaction to generate UmuD’<sub>2</sub> [5–7]. UmuD’<sub>2</sub> interacts with UmuC to form heterotrimeric UmuD’<sub>2</sub>C = pol V [8]. However, pol V exhibits weak activity *in vitro* [9]. Its activity is, however, significantly increased *in vitro* in the presence of RecA\* [10]. It is believed that activation occurs when a single RecA monomer is transferred from the 3’ end of a RecA nucleoprotein filament along with ATP to UmuD’<sub>2</sub>C to generate a UmuD’<sub>2</sub>C-RecA-ATP complex, that is the biologically active form of pol V (pol V Mut) [11].

In addition to promoting DNA damage-induced translesion synthesis (TLS), in certain genetic backgrounds, pol V Mut confers a considerable spontaneous mutator activity to *E. coli* [12,13]. Studies using *lacZ* reporter assays suggest that much of this mutator activity is targeted to the lagging DNA strand, at least in the context of the *lacZ* allele [14]. We were interested in determining whether this strand bias would extend to the entire *E. coli* genome. To do so, we generated *E. coli* strains in which pol V would be expected to be highly active [*recA730 lexA51(Def)*] and also deficient in the major ribonucleotide excision repair pathway, due to a deletion of RNase HII ( *rnhB*) [15]. We then utilized so-called “steric gate” mutant variants of pol V that have altered abilities to incorporate ribonucleotides during replication of undamaged DNA [16] and have used the HydEn-seq method [17] to track pol V-dependent misincorporated ribonucleotides across the *E. coli* genome. In the presence of RecA\* (genetically activated *recA730*, or *recA*<sup>+</sup> treated with ciprofloxacin), pol V variants with an increased ability to incorporate ribonucleotides were tracked to the lagging strand across the entire genome. In particular, there was a sharp strand transition at the single *E. coli* origin of replication (*oriC*). The lagging strand bias is clearly pol V-dependent, since the bias disappeared in the pol V-F10L variant that exhibits decreased ribonucleotide incorporation [16]. Our study, therefore, provides the first evidence that under appropriate activating conditions, pol V has access to the entire undamaged *E. coli* genome and particularly, the lagging strand.

## 2. Materials and methods

### 2.1. Bacterial strains and plasmids

All bacterial strains and plasmids used in this study are listed in Table 1.

### 2.2. Bacterial growth conditions

Bacterial cells were streaked out from frozen stocks on LB agar plates containing kanamycin (50 µg/mL), spectinomycin (25 µg/mL), and zeocin (25 µg/mL). 4–6 colonies were diluted in 2–3 mL LB media containing antibiotics and incubated at 37 °C overnight. The overnight

culture was then diluted into 25 mL LB media containing antibiotics to have  $OD_{600} = 0.05$ . Where noted, ciprofloxacin was added to the media to a final concentration of 30 ng/mL. Cells were grown 2–3 hours ( $OD_{600} = 0.4$ – $0.6$ ) and then centrifuged to harvest the cell pellet. The saved pellet was used for DNA extraction.

### 2.3. HydEn-seq method

The HydEn-seq method and sequence data analyses have been previously described [17]. We used *E. coli* K12, DH10B reference genome build 2008-03-17 from the NCBI for sequence alignment. The strand bias plots were analyzed as previously described [18].

### 2.4. Data availability

The sequencing data of this study have been deposited in the Gene Expression Omnibus (GEO) database, <https://www.ncbi.nlm.nih.gov/geo> with the accession no. GSE158570. Custom scripts are available upon request to the corresponding author.

## 3. Results

### 3.1. Genome-wide tracking of ribonucleotides in the *E. coli* genome by HydEn-seq method

To track the replication enzymology of pol V, we used the HydEn-seq method that has been previously used to track *S. cerevisiae* DNA polymerases  $\alpha$ ,  $\epsilon$ ,  $\delta$ , and  $\eta$  [17,19]. In our current study, we have tracked incorporated ribonucleotides by pol V steric gate mutants Y11A, and F10L, and pol V<sub>R391</sub>-Y13A. These alleles were chosen based upon previous observations regarding their ability to misincorporate ribonucleotides into the *E. coli* genome. F10 is adjacent to the steric gate and the F10L substitution results in an increase in ribonucleotide discrimination, compared to wild-type pol V [16]; Y11A effectively removes any ribonucleotide selectivity by pol V [16]; and pol V<sub>R391</sub>-Y13A is a steric gate mutant of pol V<sub>R391</sub>, which is a potent homolog of *E. coli* pol V [20]. We expected that ribonucleotide misincorporation would be highest in pol V<sub>R391</sub>-Y13A and lowest in pol V-F10L. DNA libraries were prepared from the various strains and subject to HydEn-seq analysis. We used Illumina NextSeq 500 system to run paired-end sequencing. After alignment with the *E. coli* reference genome, we mapped the location of ribonucleotides incorporated into the genome (5' DNA end after hydrolysis). We used the wild-type strain for background subtraction. To visualize the data, we have provided plots obtained from averaged values of three different biological replicates, or libraries (Fig. 1). The plots of pol V-Y11A (Fig. 1A, top) and pol V<sub>R391</sub>-Y13A (Fig. 1A, middle) support the hypothesis that both pols misincorporate ribonucleotides into the *E. coli* genome. On the other hand, the plot of the F10L variant was almost flat (Fig. 1A, bottom). This observation is, therefore, consistent with the previous *in vitro* study [16] indicating this variant does not incorporate ribonucleotides into the *E. coli* genome.

### 3.2. Identification of DNA replication origin and termination region in the *E. coli* genome

Previously, it was shown that the HydEn-seq method could determine the location of DNA replication origins and termination regions in the yeast [17]. In *E. coli*, there is only one replication origin, *oriC* at chromosomal position 3,925,744–3,925,975 [21]. However, there

are several termination sites in the termination region. Three main termination sites are named *TerA*, *TerB*, and *TerC* at chromosomal positions ~1430000, ~1770000, ~1690000 [22]. DNA replication starts at *oriC* in two opposite directions and ends in the termination region [23]. We used UCSC genome browser to display the data and track the chromosomal positions of *oriC*, *TerA*, *TerB*, and *TerC* sites (Fig. 1). This analysis revealed clear signal transitions that occur at *oriC* in pol V-Y11A (Fig. 1A, top and B), and especially in pol V<sub>R391</sub>-Y13A plot (Fig. 1A, middle). Transitions at the termination region were much less obvious with either mutant compared to the *oriC*.

### 3.3. pol V-Y11A and pol V<sub>R391</sub>-Y13A participate in genome replication with a lagging strand preference during the SOS response

For the obtained data shown in Fig. 1, we used wild-type strain for background subtraction and calculated the fraction of reads that are mapped to the leading and lagging strands. Interestingly, Y11A and Y13A steric gate variants show higher strand specificity for the lagging strand (Fig. 1A, top and middle). Since the F10L mutant does not incorporate ribonucleotides at a very high level, we were unable to observe a strand preference for F10L (Fig. 1A, bottom). The lagging strand preference allows us to investigate where in the genome pol V might have access. As the signals (*y*-axis) are distributed all over the genome from the replication origin to the termination region, we conclude pol V has access to the entire *E. coli* genome when it is highly activated in a *recA730 lexA51(Def)* background. This is a novel and prominent finding of this study.

### 3.4. Ciprofloxacin induces pol V-Y11A to misincorporate ribonucleotides with a lagging strand preference

In the previous experiments, pol V was highly activated due to the presence of RecA\* encoded by *recA730*. We wanted to determine if a similar pol V strand specificity might be observed in *recA*<sup>+</sup> cells that were “stressed” by exposure to the antibiotic ciprofloxacin. Therefore, we treated *recA*<sup>+</sup> *lexA*<sup>+</sup> and *recA*<sup>+</sup> *lexA51(Def)* strains harboring pol V-Y11A and pol V wild-type plasmids with 30 ng/mL ciprofloxacin (Fig. 1B). Ciprofloxacin will eventually kill the cells and it is not possible for cells to form colonies overnight in 30 ng/mL Ciprofoxacin. However, as shown by Henrikus et al. [24], exposure of cells to 30 ng/mL ciprofloxacin for up to 3 h is a strong inducer of the SOS response (as measured by many end-points), which is why we chose the 30 ng/mL ciprofloxacin for 3 h regime for our studies.

We used treated pol V wild-type for background subtraction. Similar to the previous data shown in Fig. 1A, pol V-Y11A plots, the strains treated with ciprofloxacin showed a lagging strand preference (Fig. 1B). The strand bias was much less evident in the *recA*<sup>+</sup> *lexA*<sup>+</sup> strain treated with ciprofloxacin (Fig. 1B, bottom) and this is likely due to the overall lower steady-state levels of pol V induced after treatment compared to fully derepressed levels in the *lexA51(Def)* allele. These observations, therefore, confirm a pol V lagging strand bias in both *recA*<sup>+</sup> *lexA*<sup>+</sup> and *recA*<sup>+</sup> *lexA51(Def)* backgrounds.

### 3.5. Template switch is sharper at *oriC* compared to the termination region

Previously, the HydEn-seq method revealed the template switch sharpness during *S. cerevisiae* DNA replication [17]. To investigate this aspect in our current study, we focused on the single *oriC* and termination region in *E. coli*. We retrieved the data for pol V-Y11A, pol V<sub>R391-Y13A</sub>, and pol V-F10L. We prepared new plots by using an origin-specific window of ~300 kbp versus a terminus-specific window of ~500 kbp and calculated the slope of transitions (black lines in Fig. 2A–F). Both pol V Y11A and pol V<sub>R391-Y13A</sub> showed much stronger strand transitions at *oriC* compared to the termination region. This observation is consistent with the fact that DNA replication initiates only from one single site (*oriC*) [21] but terminates at a number of termination sites [22]. Next, we calculated the transition slope of *oriC* and termination region for pol V-Y11A in the ciprofloxacin experiments (Fig. 2, G–J). In these graphs, pol V-WT was used for background subtraction. Strains with the *lexA51(Def)* allele showed sharper lagging strand transitions at *oriC* (Fig. 2G) compared to *lexA*<sup>+</sup> (Fig. 2H). The calculations from Fig. 2A–J are shown in Fig. 2K.

## 4. Discussion

The present study provides a framework to investigate the chromosomal distribution of DNA synthesis reaction in *E. coli* by a translesion DNA polymerase, pol V. By using a series of *lacZ* alleles that have been inserted into the *E. coli* chromosome in opposite directions, the Fijalkowska and Schaaper laboratories have elegantly shown that the majority of pol V-dependent spontaneous mutagenesis occurs on the lagging strand during genome duplication [14]. However, a limitation of the *lacZ* reversion assay is that it monitors the reversion of a single nucleotide in a fixed sequence context. To examine whether pol V replication occurs on the lagging strand genome-wide, we have taken advantage of pol V variants with mutations at the steric gate residue, and which are known to readily incorporate ribonucleotides into the *E. coli* genome [16,20], and have used HydEn-seq methodology [17] to map the location of pol V-dependent ribonucleotide misincorporation across the entire 4.6 Mbp genome.

While HydEn-seq detects a lagging strand preference for pol V, it is unable to display a similar leading-over-lagging preference as seen for pol  $\eta$  or other replicative DNA polymerases [17,19]. We speculate that even though the steric gate variant incorporates ribonucleotides with a preference for the lagging strand, the relative contribution of pol V to replication of the entire genome is minor.

Previously, Walsh et al., showed pol V<sub>R391-Y13A</sub> generates higher levels of SOS-induced spontaneous mutagenesis than pol V-Y11A, as it binds more repeatedly to the undamaged genome and for a longer time [20]. Accordingly, we saw a clear genome-wide lagging strand bias for pol V<sub>R391-Y13A</sub>.

The strand preference in our study might be a structural result of the pol V gating defects. However, our conclusion is consistent with previous observations with wild-type pol V using the *lacZ* reporter assay [14].

Based on the data from HydEn- seq method (Figs. 1 and 2), we propose a model of how pol V can gain access to the *E. coli* genome *in vivo* (Fig. 2L). In our model, SOS-induced pol V participates in the genome replication with a lagging strand preference (red arrows). However, as we subtracted the lagging and leading strands signals of the wild-type enzyme from the steric gate mutant, we cannot exclude the possibility that pol V also participates to some extent in leading strand synthesis. The lagging strand preference seen in our model is consistent with previous studies in which the *lacZ* reporter system was used to investigate the role of pol V during SOS mutagenesis [14], but now extends to the *entire E. coli* genome and multiple sequence contexts. As previously discussed [14], pol V may have better access to the lagging strand because the strand is replicated discontinuously and therefore provides more 3' termini. The lagging strand also generates more single-stranded DNA and is a better target for RecA\*, which is required for maximal activation of pol V *in vivo*.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

## Acknowledgments

We would like to thank the Genomics Core Facility at the University of Gothenburg for sequencing.

## Funding source

This study was supported by the Swedish Research Council ([www.vr.se](http://www.vr.se)) [2018-05121 to A.R.C.] and NICHD/NIH Intramural Research Program. The study sponsors had no involvement in the study design; collection, analysis and interpretation of data; the writing of the manuscript; or the decision to submit the manuscript for publication

## Abbreviations:

<b>pol V</b>	DNA polymerase V
<b>TLS</b>	Translesion DNA synthesis
<b>RER</b>	Ribonucleotide Excision Repair

## References

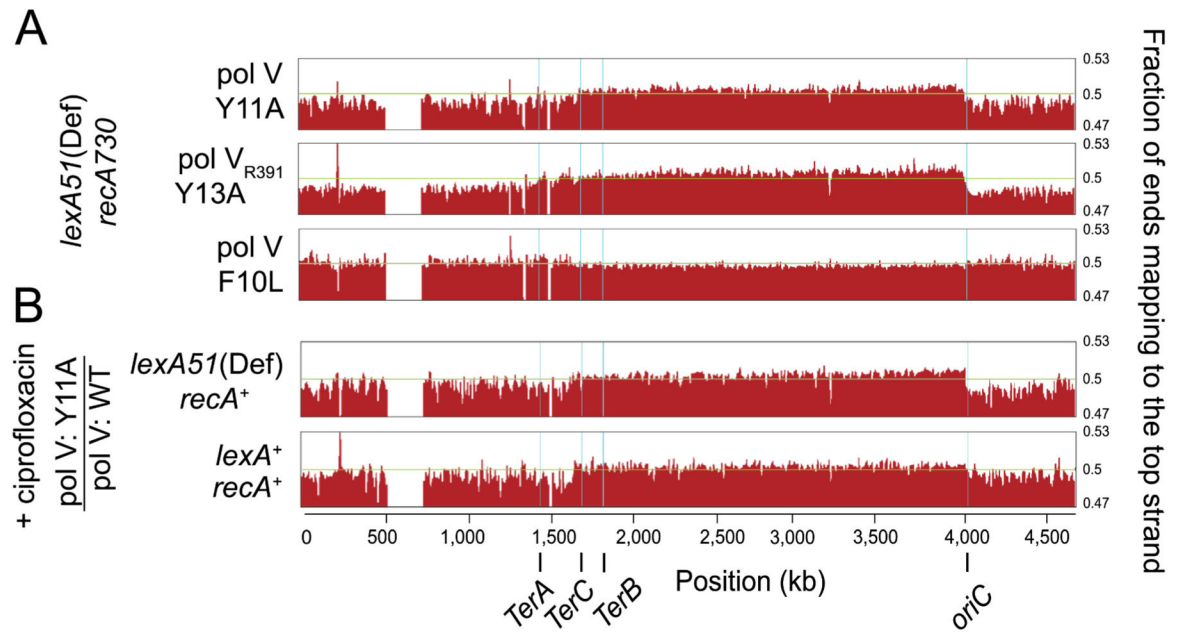
- [1]. Kato T, Shinoura Y, Isolation and characterization of mutants of *Escherichia coli* deficient in induction of mutations by ultraviolet light, *Mol. Gen. Genet* 156 (1977) 121–131. [PubMed: 340898]
- [2]. Steinborn G, *Uvm* mutants of *Escherichia coli* K12 deficient in UV mutagenesis. I. Isolation of *uvm* mutants and their phenotypical characterization in DNA repair and mutagenesis, *Mol. Gen. Genet* 165 (1978) 87–93. [PubMed: 362169]
- [3]. Bagg A, Kenyon CJ, Walker GC, Inducibility of a gene product required for UV and chemical mutagenesis in *Escherichia coli*, *Proc. Natl. Acad. Sci. U. S. A* 78 (1981) 5749–5753. [PubMed: 7029544]
- [4]. Patel M, Jiang Q, Woodgate R, Cox MM, Goodman MF, A new model for SOS-induced mutagenesis: how RecA protein activates DNA polymerase V, *Crit. Rev. Biochem. Mol. Biol* 45 (2010) 171–184. [PubMed: 20441441]
- [5]. Shinagawa H, Iwasaki H, Kato T, Nakata A, RecA protein-dependent cleavage of UmuD protein and SOS mutagenesis, *Proc. Natl. Acad. Sci. U. S. A* 85 (1988) 1806–1810. [PubMed: 3126496]

- [6]. Burckhardt SE, Woodgate R, Scheuermann RH, Echols H, UmuD mutagenesis protein of *Escherichia coli*: overproduction, purification, and cleavage by RecA, Proc. Natl. Acad. Sci. U. S. A 85 (1988) 1811–1815. [PubMed: 3279417]
- [7]. Nohmi T, Battista JR, Dodson LA, Walker GC, RecA-mediated cleavage activates UmuD for mutagenesis: mechanistic relationship between transcriptional derepression and posttranslational activation, Proc. Natl. Acad. Sci. U. S. A 85 (1988) 1816–1820. [PubMed: 3279418]
- [8]. Woodgate R, Rajagopalan M, Lu C, Echols H, UmuC mutagenesis protein of *Escherichia coli*: purification and interaction with UmuD and UmuD', Proc. Natl. Acad. Sci. U. S. A 86 (1989) 7301–7305. [PubMed: 2552436]
- [9]. Tang M, Shen X, Frank EG, O'Donnell M, Woodgate R, Goodman MF, UmuD' (2)C is an error-prone DNA polymerase, *Escherichia coli* pol V, Proc. Natl. Acad. Sci. U. S. A 96 (1999) 8919–8924. [PubMed: 10430871]
- [10]. Schlacher K, Cox MM, Woodgate R, Goodman MF, RecA acts *in trans* to allow replication of damaged DNA by DNA polymerase V, Nature 442 (2006) 883–887. [PubMed: 16929290]
- [11]. Jiang Q, Karata K, Woodgate R, Cox MM, Goodman MF, The active form of DNA polymerase V is UmuD' (2)C-RecA-ATP, Nature 460 (2009) 359–363. [PubMed: 19606142]
- [12]. Sweasy JB, Witkin EM, Sinha N, Roegner-Maniscalco V, RecA protein of *Escherichia coli* has a third essential role in SOS mutator activity, J. Bacteriol 172 (1990) 3030–3036. [PubMed: 2188949]
- [13]. Fijalkowska IJ, Dunn RL, Schaaper RM, Genetic requirements and mutational specificity of the *Escherichia coli* SOS mutator activity, J. Bacteriol 179 (1997) 7435–7445. [PubMed: 9393709]
- [14]. Maliszewska-Tkaczyk M, Jonczyk P, Bialoskorska M, Schaaper RM, Fijalkowska IJ, SOS mutator activity: unequal mutagenesis on leading and lagging strands, Proc. Natl. Acad. Sci. U. S. A 97 (2000) 12678–12683. [PubMed: 11050167]
- [15]. McDonald JP, Vaisman A, Kuban W, Goodman MF, Woodgate R, Mechanisms employed by *Escherichia coli* to prevent ribonucleotide incorporation into genomic DNA by Pol V, PLoS Genet. 8 (2012), e1003030. [PubMed: 23144626]
- [16]. Vaisman A, Kuban W, McDonald JP, Karata K, Yang W, Goodman MF, Woodgate R, Critical amino acids in *Escherichia coli* UmuC responsible for sugar discrimination and base-substitution fidelity, Nucleic Acids Res. 40 (2012) 6144–6157. [PubMed: 22422840]
- [17]. Clausen AR, Lujan SA, Burkholder AB, Orebaugh CD, Williams JS, Clausen MF, Malc EP, Mieczkowski PA, Fargo DC, Smith DJ, Kunkel TA, Tracking replication enzymology *in vivo* by genome-wide mapping of ribonucleotide incorporation, Nat. Struct. Mol. Biol 22 (2015) 185–191. [PubMed: 25622295]
- [18]. Orebaugh CD, Lujan SA, Burkholder AB, Clausen AR, Kunkel TA, Mapping ribonucleotides incorporated into DNA by hydrolytic end-sequencing, Methods Mol. Biol 1672 (2018) 329–345. [PubMed: 29043634]
- [19]. Kreisel K, Engqvist MKM, Kalm J, Thompson LJ, Bostrom M, Navarrete C, McDonald JP, Larsson E, Woodgate R, Clausen AR, DNA polymerase  $\eta$  contributes to genome-wide lagging strand synthesis, Nucleic Acids Res. 47 (2019) 2425–2435. [PubMed: 30597049]
- [20]. Walsh E, Henrikus SS, Vaisman A, Makiela-Dzbenska K, Armstrong TJ, Lazowski K, McDonald JP, Goodman MF, van Oijen AM, Jonczyk P, Fijalkowska IJ, Robinson A, Woodgate R, Role of RNase H enzymes in maintaining genome stability in *Escherichia coli* expressing a steric-gate mutant of pol VR391, DNA Repair (Amst.) 84 (2019), 102685. [PubMed: 31543434]
- [21]. Oka A, Sugimoto K, Takanami M, Hirota Y, Replication origin of the *Escherichia coli* K-12 chromosome: the size and structure of the minimum DNA segment carrying the information for autonomous replication, Mol. Gen. Genet 178 (1980) 9–20. [PubMed: 6991883]
- [22]. Hill TM, Arrest of bacterial DNA replication, Ann. Rev. Microbiol 46 (1992) 603–633. [PubMed: 1444268]
- [23]. Bramhill D, Kornberg A, A model for initiation at origins of DNA replication, Cell 54 (1988) 915–918. [PubMed: 2843291]
- [24]. Henrikus SS, Henry C, McGrath AE, Jergic S, McDonald JP, Hellmich Y, Bruckbauer ST, Ritger ML, Cherry ME, Wood EA, Pham PT, Goodman MF, Woodgate R, Cox MM, van Oijen AM, Ghodke H, Robinson A, Single-molecule live-cell imaging reveals RecB-dependent function of

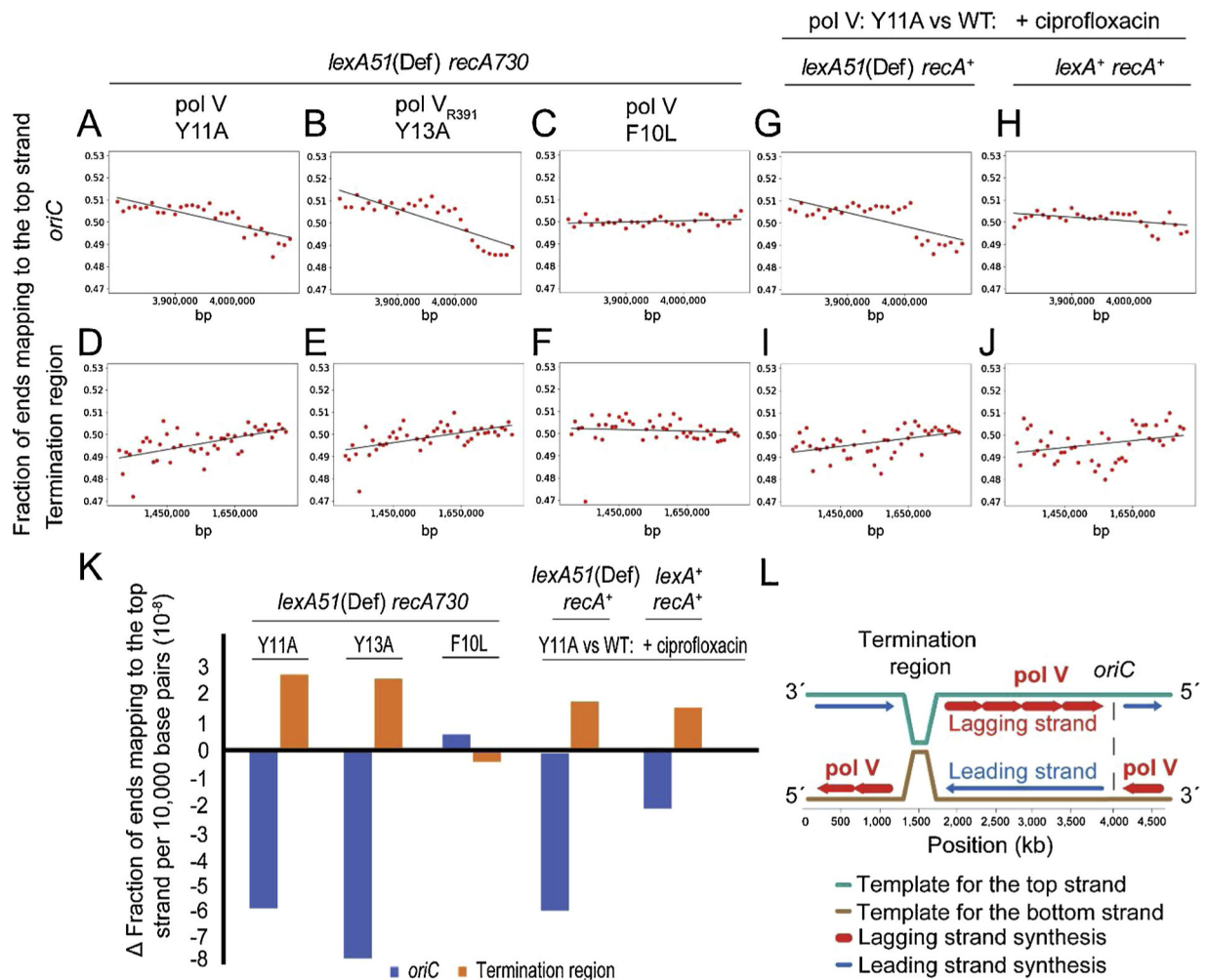
DNA polymerase IV in double strand break repair, *Nucleic Acids Res.* 48 (2020) 8490–8508.  
[PubMed: 32687193]

- [25]. Szekeres ES Jr., Woodgate R, Lawrence CW, Substitution of *mucAB* or *rumAB* for *umuDC* alters the relative frequencies of the two classes of mutations induced by a site-specific T-T cyclobutane dimer and the efficiency of translesion DNA synthesis, *J. Bacteriol* 178 (1996) 2559–2563.  
[PubMed: 8626322]



**Fig. 1.**

Genome-wide tracking of pol V strand-specific ribonucleotide incorporation. Pol V-Y11A (A, top), pol V<sub>R391</sub>-Y13A (A, middle), pol V-F10L (A, bottom), and pol V-Y11A treated with ciprofloxacin (B) are shown with the fraction of end reads mapped to the top strand in bins of 10,000 bp after background subtraction using the wild-type strain. The 0.47 and 0.53 numbers shown on the right side of the graphs are vertical viewing range in UCSC genome browser. For better illustration, three individual data points at positions 1,270,000, 1,360,000, and 1,370,000 were removed when they were out of the specified range. The numbers marked with the ticks below the graphs show the chromosomal position in the *E. coli* genome. Replication origin, *oriC* and replication terminators, *TerA*, *TerB*, and *TerC* are marked with a light blue vertical line in the graph. The light green horizontal line represents a top strand specificity of 0.5. The plots are the average values of three independent biological replicates or libraries. In ciprofloxacin experiments (B), *E. coli* cells with wild-type *recA* genetic background were grown in the presence of ciprofloxacin with a final concentration of 30 ng/mL for approximately 3 h. The blank area in the graph represents the locations in the genome with repetitive sequences that are not possible to be aligned at a single location to the reference genome.



**Fig. 2.** DNA strand specificity switch at *oriC* and termination region. HydEn-seq data of pol V-Y11A (A and D), pol V<sub>R391</sub>-Y13A (B and E), pol V-F10L (C and F), and ciprofloxacin experiments (G-J) were plotted at the DNA replication origin, *oriC* (A-C, G and H) and termination region (D-F, I and J) with the window size of ~300 kbp for origin and ~500 kbp for termination region in *x*-axis (A-J). Red dots in the scatter plots are the averaged data of three independent libraries similar to Fig. 1. The black line represents the data slope within the window. (K) The calculated slopes were used to generate a bar chart with the slopes value. The blue and brown colors represent *oriC* and termination region slopes, respectively. The *x*-axis shows different steric gates and the *y*-axis represents the slopes. (L) A schematic diagram of pol V replication enzymology in *E. coli*. The figure shows *E. coli* genome with one replication origin (*oriC*) (marked with dashed black vertical lines) and a termination region (buckets). Pol V participates in the whole genome replication with lagging strand preference (red arrows vs. blue arrows). The *x*-axis shows the chromosomal position in *E. coli* genome.

**Table 1**

Strains and plasmids used in this study.

Strains/plasmids	Relevant genotype/ characteristic	Reference
RW838 <sup>a</sup>	<i>E. coli</i> : <i>lexA51(Def) recA730 (umuDC)596::ermGT dinB61::ble rnhB782::kan</i>	[15]
RW916 <sup>a</sup>	<i>E. coli</i> : <i>lexA51(Def) (umuDC)596::ermGT dinB61::ble rnhB782::kan</i>	LGI <sup>c</sup> stocks
RW988 <sup>a</sup>	<i>E. coli</i> : <i>(umuDC)596::ermGT dinB61::ble rnhB782::kan polB::ΩSpc</i>	LGI stocks
JW0178 <sup>b</sup>	<i>E. coli</i> : <i>rnhB782::kan</i>	<i>E. coli</i> Genetic Stock Center
pRW134	pGB2, <i>umuD'C</i> (pol V wild-type)	[25]
pJM963	pGB2, <i>umuD'C</i> -Y11A (pol V steric gate)	[16]
pJM964	pGB2, <i>umuD'C</i> -F10L (pol V variant)	[16]
pJM1282	pGB2, <i>rumA'B</i> -Y13A (pol V <sub>R391</sub> steric gate)	[20]

<sup>a</sup>Full genotype: *thr-1 araD139 (gpt-proA)62 lacY1 tsx-33 glnV44 galK2 hisG4 rpsL31 xyl-5 mtl-1 argE3 thi-1 sulA211*.

<sup>b</sup>Full genotype: *(araD-araB)567 lacZ4787 rph-1 (rhaD-rhaB)568 hsdR514*.

<sup>c</sup>LGI: Laboratory of Genomic Integrity.