

**GENOME-WIDE EFFECTS OF DNA REPLICATION ON  
NUCLEOTIDE EXCISION REPAIR OF UV-INDUCED DNA  
LESIONS.**

by  
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## ABSTRACT

### GENOME-WIDE EFFECTS OF DNA REPLICATION ON NUCLEOTIDE EXCISION REPAIR OF UV-INDUCED DNA LESIONS.

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Damage-seq, replication, strand asymmetry

Replication can cause unrepaired DNA damages to turn into mutations that might lead to cancer. Nucleotide excision repair is the leading repair mechanism that prevents melanoma cancers by removing UV-induced bulky adducts. However, the role of replication on nucleotide excision repair in general is yet to be clarified. Recently developed methods Damage-seq and XR-seq map damage formation and nucleotide excision repair events respectively, in various conditions. Here, we applied Damage-seq and XR-seq methods to UV-irradiated HeLa cells synchronized at two stages of the cell cycle: early S phase, and late S phase. We analyzed the damage and repair events along with replication origins and replication domains of HeLa cells. We found out that in both early and late S phase cells, early replication domains are more efficiently repaired relative to late replication domains. The results also revealed that repair efficiency favors leading strand around replication origins, which is correlated with melanoma mutations.

## ÖZET

### TEZ BAŞLIĞI

### TEZ YAZARI

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*To my fiancée...*

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CHR: Commission on Human Rights .....	2
DAW: Division for the Advancement of Women .....	5
DESA: Department of Economic and Social Affairs .....	9

## 1. INTRODUCTION

### 1.1 UV-Induced Damages in Humans

Ultraviolet (UV) light is the major cause of skin cancers in humans (Kiefer, 2007). It is a portion of the electromagnetic (EM) spectrum which is emitted from the sun together with visible light and heat. Based on its wavelength, UV light divides into three subgroups: UVA (wavelength 315-400 nm), UVB (wavelength 280-315 nm), and UVC (wavelength 100-280 nm). While the less energetic UVA makes up the majority of UV light passing the atmosphere, all UVC and approximately 90% of UVB is either blocked or absorbed by the ozone layer. Even in these conditions, we are not fully protected from the damaging effects of UV light. So that, UV irradiation accounts for approximately 30.000 DNA lesions' formation per cell per hour.

The most abundant UV lesions in cellular DNA are pyrimidine dimers (Kielbassa, Roza & Epe, 1997), which are formed by the covalent bonds between the adjacent pyrimidines (Whitmore, Potten, Chadwick, Strickland & Morison, 2001). Different in their chemical structure, two types of pyrimidine dimers exist; one is called cyclobutene pyrimidine dimers (CPDs), and the other is called pyrimidine (6-4) pyrimidone photoproducts [(6-4)PPs]. While both UVC and UVB can induce these dimer formations, UVA is only capable of induce CPDs. Nonetheless, UVA induction can convert already formed (6-4)PPs into their Dewar valence isomers. Moreover, UVA can induce oxidative DNA damages through photosensitized reactions (Hu & Adar, 2017). Thanks to the development of time resolved spectroscopy techniques in recent years, dynamics of pyrimidine dimer formation is well known. The formation and biological properties of UV lesions will be briefly discussed in the subsections below.

### 1.1.1 Cyclobutene Pyrimidine Dimers (CPDs)

CPDs are the most frequent pyrimidine dimers that are arising from the covalent linkages between the consecutive pyrimidines, and it is characterized by the four-member ring structure that are double bonded from the pyrimidine 5 and 6 (Whitmore et al., 2001). In vivo, CPDs can be observed in four different configurations: cis-syn, cis-anti, trans-syn, or trans-anti. (Khattak & Wang, 1972) While it is generally observed in cis-syn form when the DNA is double-stranded (Wacker, Dellweg, Träger, Kornhauser, Lodemann, Türck, Selzer, Chandra & Ishimoto, 1964), in denatured DNA and single-stranded regions, trans-syn configuration exists (Taylor & Brockie, 1988). Although it is rare, nonadjacent pyrimidines can also form CPDs in single-stranded regions (Nguyen & Minton, 1988). Moreover, different configurations can affect the ability of repair enzymes to recognize these lesions and correct them, which cause mutability differences between the configurations (Friedberg, Walker, Siede & Wood, 2005).

Apart from the configuration of the lesions, their dipyrimidine doublets (TT, TC, CT, and CC) can contribute to CPD formation at different rates depending on the type of UV exposure or the nucleotide content of the DNA. According to the study of Douki and Cadet, under UVC and UVB exposure, double-stranded mammalian DNA produces TT, TC, CT, and CC CPDs in 100:50:25:10 ratios, respectively (Douki & Cadet, 2001). While TT CPDs accounts for more than half of the total CPDs after the exposure of UVC and UVB, for UVA exposure, this ratio rises to 90% (Mouret, Philippe, Gracia-Chantegrel, Banyasz, Karpati, Markovitsi & Douki, 2010). On the other hand, TT CPDs are the abundant products of UV exposure for mammalian DNA, but the abundance might be greatly influenced by the GC percentage of the DNA. For example, in the bacterial DNA that possess a rich GC percentage, TT CPDs are the minor products of UV exposure (Patrick, 1977).

### 1.1.2 Pyrimidine (6-4) Pyrimidone Photoproducts [(6-4)PPs] and their

#### Dewar Valence Isomers

(6-4)PPs form with occurrence of a pyrimidone ring by the bonding between C6 position of the 5'-end base and C4 position of the 3'-end base. In fact, this structure forms indirectly following the UV exposure, after a cyclic reaction intermediate, which can be either an oxetane if thymine is the 3'-end base, or azetidine if cytosine is the 3'-end base. Because of its indirect formation, (6-4)PPs appear thou-

sand times slower than CPDs (Schreier, Schrader, Koller, Gilch, Crespo-Hernández, Swaminathan, Carell, Zinth & Kohler, 2007).

Under UVC and UVB exposure, formation of (6-4)PPs is approximately five times less than that of CPDs (Douki & Cadet, 2001). Moreover, TT dipyrimidines that are the most abundant sites for CPDs are less frequent for (6-4)PPs. Instead, TC and CCs are the frequent sites for (6-4)PPs, while CT (6-4)PPs are uncommon. Another unique property of (6-4)PPs is its conversion into Dewar valence isomers with the photoisomerization process (Taylor & Cohrs, 1987). Although UVB irradiation can trigger the process, with the combination of UVB and UVA exposure, the yield increases significantly.

## 1.2 Nucleotide Excision Repair in Humans

Throughout the generations, cells manage to evolve highly specialized repair mechanisms to cope with a variety of lesions that threaten the genome integrity and survival. Considering the diversity of these lesions, it would be unexpected to have only a single mechanism that can preserve the integrity of the genome. Hence, there are several repair mechanisms that cells utilize which are eminently conserved between species. Due to the removal of both strands, repair of a double strand break is demanding. There are two mechanisms that can be triggered by double strand breaks: homologous recombination, and non-homologous end-joining. Homologous recombination uses the sister-chromatid as a template to repair double strand breaks in an error-free manner. In addition, if sister-chromatid is not available for use, non-homologous end-joining directs the fusion of broken ends in an error-prone manner. Although being error-prone, non-homologous end-joining is the dominant mechanism for double strand break repair in mammals. Reasons of this dominance are the distant proximity of chromatids to each other, and the DNA folding that makes the homologous sequence less reachable. In addition, imperfect matches by homologous recombination can lead to tragic outcomes such as creating repeated sequences (Li, Wehrenberg, Waldman & Waldman, 2018).

On the other hand, when a damage occurs at a single strand, the opposite strand can be used as a template. In such cases, DNA excision repair mechanisms remove the lesion site and re-synthesize the gap using the template strand. While oxidation, deamination and alkylation damages are repaired by base excision repair (Klungland,

Höss, Gunz, Constantinou, Clarkson, Doetsch, Bolton, Wood & Lindahl, 1999), mismatches that escape proofreading are identified and corrected by mismatch repair (Modrich, 1997). And lastly, bulky adducts caused by UV irradiation, environmental mutagens, and chemotherapeutic agents are removed by nucleotide excision repair (Reardon & Sancar, 2005). Nucleotide excision repair contains two sub pathways that differ from each other at the damage recognition step: Global Repair (GR) and Transcription-Coupled Repair (TCR). TCR is specialized in recognizing adducts in transcribed regions, while GR can recognize bulky adducts at any site. Subsections below will address the assembly and main properties of nucleotide excision repair in more detail.

### **1.2.1 Repair of UV-induced damages by Nucleotide Excision Repair**

Identified firstly at *E. coli* by two independent studies published in 1964 (Boyce & Howard-Flanders, 1964; Setlow & Carrier, 1964), nucleotide excision repair can repair variety of bulky adducts from UV-induced pyrimidine dimers to chemotherapeutic agents such as cisplatin (Yimit, Adebali, Sancar & Jiang, 2019). Although repair mechanisms are highly conserved among the species, nucleotide excision repair in humans appeared to be surprisingly different from that of *E. coli*. While *E. coli* contains three proteins (UvrA, B, C) for the incision of damaged fragments, human nucleotide excision repair has sixteen proteins for the task. More interestingly, there is not an evolutionary relevance between these human and *E. coli* proteins. In addition, the excised fragments are usually around 12 nucleotides long in *E. coli*. For humans, the length of these fragments are around 30 nucleotides (Sancar, 2016). Human nucleotide excision repair can be generally discussed in three steps: 1) damage recognition, 2) dual incision and excision of damaged fragments, and 3) re-synthesis and ligation.

#### **1.2.1.1 Damage Recognition**

As it is mentioned earlier, GR and TCR have distinct damage recognition steps. GR scans the whole genome to detect helix distortions caused by bulky adducts, whereas TCR responds only to a stalled RNA polymerase II during transcription.

In GR, three proteins (XPC, RAD23B, CETN2) work in coordination to recog-



nize the lesion site (Sugasawa, Ng, Masutani, Iwai, van der Spek, Eker, Hanaoka, Bootsma & Hoeijmakers, 1998). XPC is the first protein to interact with the lesion by binding to the small single-stranded DNA (ssDNA) that is left unpaired due to the pyrimidine dimer formation at the opposite strand. The ability of XPC to bind unpaired ssDNA enables GR to detect a variety of lesions, since the unpaired ssDNA is a common characteristic of bulky adducts. After XPC binding, RAD23B and CETN2 interact with and stabilize XPC. However, helix distortions must be apparent to XPC for an efficient detection. (6-4)PPs are recognized relatively in ease because of having a prominent distortion (Mizukoshi, Kodama, Fujiwara, Furuno, Nakanishi & Iwai, 2001), whereas the distortion of CPDs cause only a 9° unwinding with a 30° bent (Park, Zhang, Ren, Nadji, Sinha, Taylor & Kang, 2002), which can be considered mild. For the detection of CPDs, proteins DDB1 and DDB2 form a complex called ultraviolet radiation–DNA damage-binding protein (UV-DDB). The complex directly interacts with the lesion, and DDB2 kinks the lesion to increase unwinding (Scrima, Koníčková, Czyzewski, Kawasaki, Jeffrey, Groisman, Nakatani, Iwai, Pavletich & Thomä, 2008), as a result the ssDNA becomes detectable for XPC.

The recognition mechanism of TCR is triggered by the blockage of RNA polymerase II (RNAPII), which transcribes the active gene during transcription elongation. When RNAPII stalls following an encounter with a lesion, it subsequently recruits the nucleotide excision repair proteins (Svejstrup, 2002). Afterwards, RNAPII dynamically interacts with UV-stimulated scaffold protein A (UVSSA), ubiquitin-specific-processing protease 7 (USP7), Cockayne syndrome protein CSB. CSB is an ATP-dependent chromatin remodeling factor that contains a helicase motif, surprisingly without a helicase activity (Selby & Sancar, 1997b). Moreover, studies in early 2000s revealed that point mutations in the ATPase domain of CSB protein significantly cripples the cell’s ability to escape the inhibited RNA synthesis (Citterio, Rademakers, van der Horst, van Gool, Hoeijmakers & Vermeulen, 1998; Muftuoglu, Selzer, Tuo, Brosh Jr & Bohr, 2002), which suggests that CSB plays a key role for the TCR assembly. Furthermore, recruitment of repair factors that work on incision of the damaged fragment also mediated by CSB (Fousteri, Vermeulen, van Zeeland & Mullenders, 2006). More identified functions of CSB include transcription elongation, chromatin maintenance and remodeling, histone tail binding, and strand annealing (Selby & Sancar, 1997a). Another important Cockayne syndrome protein is CSA, which is also recruited by CSB. CSA mediates the recruitment of PCNA, RFC and pol  $\delta$ . Therefore, it is a key protein for the later events of the repair.

The recruited core nucleotide excision repair factors and some TCR specific factors such as UV-stimulated scaffold protein A (UVSSA), ubiquitin-specific-processing protease 7 (USP7), XPA-binding protein 2 (XAB2) and high mobility group

nucleosome-binding domain-containing protein 1 (HMGN1), gather on the lesion site where RNAPII stalls. However, because RNAPII stalls on the lesion, it covers the lesion so that the TCR complex cannot reach it (Tornaletti, Reines & Hanawalt, 1999). To proceed, RNAPII should somehow move from the 35 nucleotides length of strand where it is positioned. There are three proposed mechanisms for that purpose which are degradation, dissociation and backtracking. Because backtracking is already known to be occurring at transcription proofreading and at natural transcription pause sites, it is the most accepted mechanism among these three (Marteijn, Lans, Vermeulen & Hoeijmakers, 2014).

#### **1.2.1.2 Dual Incision and Excision of Damaged Fragment**

After RNAPII backtracks, transcription initiation factor IIH (TFIIH) initiates to unwind DNA with its helicase subunits. The TFIIH complex is formed of 10 proteins. While XPB and XPD have helicase activity, CDK-activating kinase (CAK) subcomplex is responsible for the initiation of TFIIH complex. The initiation is also known as DNA damage verification step which is the last reversible step of nucleotide excision repair (Marteijn et al., 2014). With the initiation of TFIIH complex, the lesion becomes ready to be removed. Then XPF-ERCC1 and XPC endonucleases interact with the lesion site to catalyze the lesion from two sides together with the TFIIH complex. Meanwhile, replication protein A (RPA) not only protects the non-damaged single strand, but also interacts with and coordinates most subunits of TFIIH complex. The cleavage of the lesion site that yields 22-30 nucleotide long single stranded gap, is termed dual incision (Marteijn et al., 2014).

#### **1.2.1.3 Re-synthesis and Ligation**

After the dual incision, the occurred gap must be filled with the ligation process. During replication, the proteins proliferating cell nuclear antigen (PCNA), replication factor C (RFC), DNA pol  $\delta$ , DNA pol  $\epsilon$  and DNA ligase 1 mediates re-synthesis and ligation. However, if the cell is non-replicating, then DNA pol  $\kappa$  and XRCC1–DNA ligase 3 fill the gap (Marteijn et al., 2014).

### 1.2.2 NER associated diseases

There are three human diseases that are known to be directly associated with nucleotide excision repair. These diseases are xeroderma pigmentosum (XP), cockayne syndrome (CS) and trichothiodystrophy (TTD) (De Boer & Hoeijmakers, 2000; Lehmann, 2003). XP discovered in 1968 as a hereditary disease that causes a defective nucleotide excision repair (Cleaver, 1968). XP patients are extremely photosensitive, so that they have approximately 5000-fold increased risk of UV-induced skin cancer. Dry parchment skin and pigmentation related anomalies are some of the hallmarks of this disorder (De Boer & Hoeijmakers, 2000). Seven genes that are associated with the disease, known as XP complementation groups (XP-A, B, C, D, E, F, G) (Cleaver & Bootsma, 1975), and proteins that are produced by all these genes have a role in GR. Except XPC and XPE, they are also involved in TCR (Van Hoffen, Venema, Meschini, Van Zeeland & Mullenders, 1995).

CS first reported in 1936 as a disease related to deafness and dwarfism (Cockayne, 1936). In the upcoming years, problems at joints, vision, and calcifications in the brain are further reported (Cockayne, 1946; Neill & Dingwall, 1950). Moreover, these patients have aging related issues, and like XP patients, they are photosensitive, though not as severe as XP patients, therefore, their risk of having UV-induced skin cancer is not increased. As a consequence of all these abnormalities, most severe types of CS patients have a lifespan of as short as 7 years. Two genes, CSA and CSB are known to be related to the disease, which are both TCR proteins. Thereby, it was thought that CS patients are TCR defective. However, since TCR deficiency is not enough to explain all these severe symptoms alone, a deficiency in transcription is also argued (Drapkin, Reardon, Ansari, Huang, Zawel, Ahn, Sancar & Reinberg, 1994).

TTD patients can display a broad range of symptoms from having brittle hair to low fertility and impaired intelligence. If the disorder is caused by one of the XPB, XPD or TTDA genes, which are all code for a component of TFIIH complex, TTD patients can become nucleotide excision repair deficient, hence photosensitive. Even though TFIIH complex can be functional, the levels of TFIIH complex decreases significantly (Giglia-Mari, Miquel, Theil, Mari, Hoogstraten, Ng, Dinant, Hoeijmakers & Vermeulen, 2006).

## 1.3 Replication and its contribution to Mutagenesis

Owing to many potential origins of replication (ORIs), a mammalian cell replicates in approximately 10 hours (Takebayashi, Ogata & Okumura, 2017). During the cell division, only a portion of these ORIs fires, and they fire in an asynchronized manner except the ORIs that are in proximity to each other. By firing simultaneously, these close packed ORIs coordinates the replication of regions longer than mega bases, termed as “replication domains” (Jackson & Pombo, 1998). Replication domains are divided into 4: early replication domains, late replication domains and the zones between these domains are up transition zones and down transition zones (Farkash-Amar, Lipson, Polten, Goren, Helmstetter, Yakhini & Simon, 2008; Hansen, Thomas, Sandstrom, Canfield, Thurman, Weaver, Dorschner, Gartler & Stamatoyannopoulos, 2010; Hiratani, Ryba, Itoh, Yokochi, Schwaiger, Chang, Lyou, Townes, Schübeler & Gilbert, 2008; Koren, Handsaker, Kamitaki, Karlić, Ghosh, Polak, Egan & McCarroll, 2014; Nakayasu & Berezney, 1989; O’keefe, Henderson & Spector, 1992). Generally, the interior regions of the nucleus are replicated earlier than nuclear periphery regions, thus located at early replication domains (Dimitrova & Berezney, 2002). Multiple studies indicated that these domains are different each other in the mutation frequencies. Suggested by the genome-wide analysis of mutation rates, early replication domains have reduced levels of mutation comparing to late replication domains (Lawrence, Stojanov, Polak, Kryukov, Cibulskis, Sivachenko, Carter, Stewart, Mermel, Roberts & others, 2013; Stamatoyannopoulos, Adzhubei, Thurman, Kryukov, Mirkin & Sunyaev, 2009). Also, in most cancers, base substitution mutation elevates in late replication domains (Schuster-Böckler & Lehner, 2012).

Replication is driven by replication forks which are formed when a predefined ORI fires (Langston, Indiani & O’Donnell, 2009). Replication fork usually proceeds bidirectionally, with the coordinated work of polymerases  $\epsilon$  and  $\delta$ . During the movement of the fork, polymerases  $\epsilon$  continuously synthesizes leading strands, whereas polymerase  $\delta$  discontinuously synthesizes lagging strands. Moreover, bidirectionality creates an asymmetric work labor, so that two polymerases work on opposite strands towards different directions. In other words, in the left replicating fork, polymerases  $\epsilon$  proceeds on the plus strand, while in the right replicating fork, it progresses on the minus strand. Studies suggests that this asymmetric progress of polymerases around the associated ORI are reflected to the mutation profiles, where lagging strand reported to harbor more mutations than leading strand (Haradhvala, Polak, Stojanov, Covington, Shinbrot, Hess, Rheinbay, Kim, Maruvka, Braunstein & others, 2016; Lujan, Williams, Pursell, Abdulovic-Cui, Clark, McElhinny & Kunkel, 2012; Reijns, Kemp, Ding, de Procé, Jackson & Taylor, 2015; Shinbrot, Henninger, Weinhold, Covington, Göksenin, Schultz, Chao, Doddapaneni, Muzny, Gibbs & others, 2014).

The occurred asymmetry on mutation profiles is reasoned by the error-prone bypass mechanism on the leading strand that makes it vulnerable to mutations (Seplyarskiy, Akkuratov, Akkuratova, Andrianova, Nikolaev, Bazykin, Adameyko & Sunyaev, 2019). Other studies argued that the attachment of helicase to leading strand increases the damage response, thus leading to effective repair of the strand (Hedglin & Benkovic, 2017; Yeeles, Poli, Mariani & Pasero, 2013). Furthermore, many mutational signatures are reported to have a significant replication strand asymmetry (Tomkova, Tomek, Kriaucionis & Schuster-Böckler, 2018).

## 1.4 Mapping Damage Formation and Nucleotide Excision Repair Events

### using Damage-seq and Excision-seq (XR-seq) Methods, Respectively

Mapping of UV-induced damages and their repair is essential to understand the role of nucleotide excision repair on mutagenesis. Since the birth of the field of DNA repair, which began with the discovery of photolyase in 1958 (Rupert, Goodgal & Herriott, 1958; Sancar, 2016), many methods are introduced to map DNA damage and repair (Li & Sancar, 2020). However, not until the emergence of next-generation sequencing techniques, genome-wide mapping of DNA damage and repair at single-nucleotide resolution could be performed. Today, there are several methods that can accomplish the task. Among these methods, Damage-seq and Excision-seq (XR-seq) can map UV-induced DNA damages and repair of these damages by nucleotide excision repair, respectively, which will be explained in the subsections below.

#### 1.4.1 Damage-seq

Damage-seq mechanism can sensitively detect a variety of DNA lesions such as CPDs, (6-4)PPs, and cisplatins, mainly using the DNA polymerase II stalling to its advantage (Hu, Lieb, Sancar & Adar, 2016). In fact, the method can be adapted to any DNA damage that stalls DNA polymerase II, where the damage-specific antibody is present (Sancar, 2016). After the induction of the damage, the genomic DNA is sonicated, ligated to first primers, and denatured. Then, damage sites are immunoprecipitated by damage-specific antibodies and enriched. Following the enrichment, a biotinylated primer is annealed and extended by a polymerase called

Q5 DNA polymerase, which extends the primer until it reaches the damage without synthesizing the site of the damage. Next, a second adaptor is ligated to the extended primer for amplification by PCR. Lastly, the amplified oligomers can be sequenced and analyzed.

#### **1.4.2 Excision-seq (XR-seq)**

XR-seq method can measure the repair of DNA damages that is coordinated by nucleotide excision repair, using the 22-30 nt long excised oligomers that are produced after the dual incision of lesion site (Hu, Li, Adebali, Yang, Oztas, Selby & Sancar, 2019; Hu et al., 2016). Excised oligomers are immunoprecipitated by TFIIH and ligated by adaptors from both sides. Next, the oligomers are filtered according to the damage of interest by immunoprecipitating with damage-specific antibodies. Then, using photolyases, lesions of the left oligomers are reversed for a proper PCR amplification process and the oligomers are sequenced.

## 2. RESULTS

### 2.1 Genome-wide mapping of UV-induced damages and their repair

#### synchronized at two stages of the cell cycle: early S phase, and late S phase

This paper presents an experimental setup followed by bioinformatic analysis of genomic data, where we purified and sequenced fragments of UV-induced damages and their repair in HeLa cells that are synchronized either at early S phase or at late S phase. After synchronizing cells using double-thymidine treatment, we further treated cells with 20J/m<sup>2</sup> UV-B exposure. Immediately after the exposure, we adopted Damage-seq to quantify occurred damages by the exposure, before nucleotide excision repair initiates. To quantify repair, we adopted XR-seq and quantified CPD repair at 12 minutes and 2 hours; while (6-4)PP repair were quantified only at 12 minutes (Figure 1A). Finally, we performed each experiment twice to obtain biological replicates.

Quality control analyses were performed on early S phased (6-4)PPs at 12 minutes (Figure 1B-D) and other samples (Figure S2-8) indicates high data qualities and consistent results between replicates. In agreement with the dual incision mechanism of nucleotide excision repair (J. C. Huang, Svoboda, Reardon, & Sancar, 1992; Li et al., 2017; Reardon & Sancar, 2005), XR-seq oligomers are in the size range of 20-30 nt, with a median of 26 nt. Moreover, dipyrimidine content of 26 nt oligomers enriches at position 19-20 (Figure 1B) where the DNA lesion occurs (J. C. Huang et al., 1992). Also, (6-4)PP samples exhibit high levels of TC dipyrimidine repair (Figure 1B, S2-4 ) whereas CPD samples exhibit elevated TT dipyrimidine repair (Figure S5-9), which are the most abundant sites for formation of these photoproducts (Mouret et al., 2010). Because this study focuses on the global repair, contribution of transcription-coupled repair can create a bias. Importantly, the re-

pair levels at transcribed and non-transcribed strand are equivalent (Figure 1C, S2-9), suggesting little or no contribution of transcription-coupled repair. Correlation plots between the biological replicates indicates reasonable reproducibility, having correlation coefficients 0.86 and above (Figure 1D, S2-9).

## 2.2 Early replication domains are repaired more efficiently than late

**replication domains, however, the repair rate of late replication domains elevates while replication proceeds.**

To determine how excision repair rates are influenced by replication domains during replication, we compared repair efficiency of early replication domains (ERDs) and late replication domains (LRDs). We obtained replication domains of HeLa cells from a study where a supervised method called Deep Neural Network-Hidden Markov Model was developed to define replication domains from Repli-seq data (Liu et al., 2016). We mapped damage and repair events to corresponding replication domains. To eliminate the effect of a potential bias in damage formation, we normalized repair quantities (XR-seq) by the captured damage events (Damage-seq) in each genomic window (Figure 2A). This approach enabled us to assess the efficiency of repair per damage at a given region, which we refer to as repair rate. Based on an analysis with a Hi-C dataset, the human genome was classified into A/B compartments, which are associated with open and closed chromatin regions, respectively (Lieberman-Aiden et al., 2009). Recently, it was also shown that ERDs and LRDs strongly correlate with A/B compartments respectively (Pope et al., 2014; Ryba et al., 2010). Because ERDs are correlated to open chromatins, these regions are more reachable for excision repair machinery than LRDs. Expectedly, repair rates of ERDs are elevated at the center and gradually reduced towards flanking sites, while LRDs exhibit an opposite pattern (Figure 2A, S18-23). These results suggest that ERDs and their flanking regions are efficiently repaired, whereas less reachable LRDs are poorly repaired. Moreover, LRDs are known to contain higher mutation frequency than other regions (Lawrence et al., 2013; Stamatoyannopoulos et al., 2009), hence; low repair rate of UV damages located at LRDs might be a key factor of mutagenesis in melanoma cancers. On the other hand, the difference between early and late S phases indicates that repair rate is elevated in favor of LRDs when replication timing moves from early to late (Figure 2A-B, S24-29). This time dependent increase in the repair rate of LRDs can be caused by the unfold-



ing of heterochromatin during replication. With the unfolding of the chromatin, more LRD regions will be accessible where the DNA lesions can be recognized and removed by nucleotide excision repair. Also we observe a reduction of repair rate at ERDs, however this reduction might be caused by the relativity of the XR-seq method; increased repair rate at LRDs results in a relative decrease in the repair rate at ERDs, even if repair rate does not quantitatively change at ERDs. In addition, (6-4)PP repair at 12 minutes exhibits minor differences between early and late S phases (Figure 2), potentially because of its fast repair after the damage occurrence (Hu, Adebali, Adar, & Sancar, 2017). Conversely, CPD repair rate at 12 minutes and 2 hours demonstrate significant increase for LRDs and decrease for ERDs (Figure 2B, p-values  $< 2.2e-16$ ). Comparison of 12 minutes to 2 hours shows that the repair rate bias is less prominent for later repair. It takes longer time to repair CPDs relative to (6-4)PPs.

### **2.3 Variety of chromatin states are associated with dominant repair.**

Active chromatin states are repaired effectively, basically because those regions are more accessible to nucleotide excision repair (Adar, Hu, Lieb, & Sancar, 2016). We addressed how repair machinery in ERDs, and LRDs is differentially influenced by the chromatin states during the replication. We retrieved chromatin states of HeLa cells segmented by ChromHMM from UCSC website (Ernst & Kellis, 2017). We intersected the chromatin states with replication domains and mapped damage and repair reads to those regions, for each chromosome. After calculating the repair rates (Figure 3A, S13A-17A), we further assessed early S phase repair relative to late S phase (early/late repair/damage) to observe the replication timing differences in efficiency in the function of chromatin states (Figure 3B, S13B-17B). Generally, repair efficiency is higher in the active chromatin states such as promoters and strong enhancers, which is in agreement with the previous studies (Adar et al., 2016; Hu, Lieb, Sancar, & Adar, 2016). Those regions sustain high repair rates, even at LRDs during the early S phase, that should be condensed and harder to reach (Figure 3A). On the other hand, all the transcription associated chromatin states together with “FaireW” and “Low” chromatin states are highly affected by the replication timing (Figure 3B). “FaireW” represents the regions that are associated to the regulatory activities (Giresi, Kim, McDaniell, Iyer, & Lieb, 2007), whereas “Low” stands for low activity regions that neighboring active sites. On ERDs, although both chromatin

states have relatively low repair at early and late S phases, they demonstrate a drastic increase when replication proceeds from early to late. However, on LRD regions, it is hard to make any assumptions considering the wide interquartile range of boxplots of many chromatin states (Figure 3B).

## 2.4 ORIs display distinct melanoma mutation counts and strand

### asymmetry based on their replication domains.

Replication domains are 1 to 2 Mb-sized DNA chunks that involves many small ORIs. Even though analyzing replication domains can exhibit the association of nucleotide excision repair and replication timing on a greater scale, the association of these small ORIs and nucleotide excision repair cannot be explained by only using replication domains. Therefore, we retrieved two independent datasets that are derived from two different methods: okazaki fragment sequencing (OK-seq) and short nascent strand sequencing (SNS-seq). OK-seq quantifies the replication initiation zones that are the sets of closely positioned ORIs using highly purified Okazaki fragments (Petryk et al., 2016), whereas SNS-seq can precisely identifies individual ORIs (Besnard et al., 2012; Langley, Graf, Smith, & Krude, 2016). Using these datasets together with a melanoma mutation dataset that we retrieved from the International Cancer Genome Consortium (ICGC) data portal (see Methods), we examined the mutation profiles at the sites of ORIs where the replication initiates. Because nucleotide excision repair is highly associated to melanoma cancers, we argued that this relation must be reflected to the mutation counts of melanoma. For both OK-seq and SNS-seq data, we assorted the regions based on their corresponding replication domains to detect how mutation profiles of ORIs affected by the domains they are located. Then, we counted the mutations on these regions that are centered at individual ORIs (SNS-seq data, Figure 4A) or initiation zones (OK-seq data, Figure 4B-C) and normalized the mutations with cytosine counts in each bin, to eliminate any bias in potential mutation sites. Also, we gradually extended the region length of initiation zones from 20 kb to 200 kb (Figure 4B-C) for observing the replication effect on a range of scales. Mutation counts of initiation zones differ depending on the replication domains they are located. In agreement with previous studies (Lawrence et al., 2013; Schuster-Bockler & Lehner, 2012; Stamatoyannopoulos et al., 2009), the mutation counts of initiation zones at LRDs elevate, while ERDs contain the initiation zones with the lowest mutation counts (Figure 4B-C). These differences

that are related to the replication domains are also persistent for the individual ORIs (Figure 4A). Furthermore, initiation zones at up (UTZs) and down transition zones (DTZs), which are the domains that connect ERDs to LRDs, have mutation counts in between of ERDs and LRDs. Moreover, the flanking sites of initiation zones at transition zones that are close to ERDs have lower counts, whereas the sites that are close to LRDs have higher (Figure 4C, left). Mutation counts not only exhibit a replication domain related difference, but also reveal a strand asymmetry around the initiation zones (Figure 4B-C). The asymmetry suggests that lagging strand (minus strand at left direction; plus strand at right direction) have more mutations than leading, independent of the replication domains. While the initiation zones at LRDs show an explicit strand asymmetry compared to the initiation zones at ERDs, the initiation zones at ERDs have a wider strand asymmetry than that of LRDs. One possible reason can be the amount of ORIs they harbor; earlier studies suggest that ERDs contain significantly higher number of replication origins (Besnard et al., 2012), and the cumulative effect of these ORIs can create a strand asymmetry that is visible on a wider region. Additionally, replication fork movement at LRDs (1.5–2.3 kb/min) is faster than it is at ERDs (1.1–1.2 kb/min) (S. Takebayashi et al., 2005), which can cause more mutation and increased asymmetry between strands. Conversely, individual ORIs obtained from SNS-seq data do not show an explicit strand asymmetry (Figure 4A).

## **2.5 Asymmetric damage around initiation zones causes asymmetric**

### **repair profiles.**

To find whether there is a strand asymmetry at repair and damage profiles similar to melanoma mutations, we mapped repair and damage events to initiation zones independently. Interestingly, A strand asymmetry around the initiation zones is visible for both repair and damage profiles (Figure 5). The asymmetry suggests that lagging template strand harbors more damages and attracts more repair accordingly. Reasoning that nucleotide composition of initiation zones might contribute to the strand asymmetry, we decided to simulate damage and repair signals. We simulated signals via Art simulator (W. Huang, Li, Myers, & Marth, 2012), filtered the signals that resembled the real signals in nucleotide composition, and mapped the filtered ones to the human genome. The simulated signals indeed display a similar strand asymmetry, indicating the contribution of nucleotide composition of the genomic

regions surrounding the initiation zones. Nonetheless, simulated signals have lower RPKM values in general. Although nucleotide composition of these signals and real ones are similar, the real repair and damage events occurred at other regions. Therefore, it is expected to observe lower RPKM values for simulated signals.

## 2.6 Strand asymmetry of excision repair rate

After observing strand asymmetry at mutation counts of melanoma, and damage and repair events independently, we examined the repair rates of (6-4)PP and CPD samples around initiation zones for an asymmetry. Interestingly, a strand asymmetry that is inversely correlated with mutation counts of melanoma is prominent among the CPD damages (Figure 6). The asymmetry indicates an efficient repair of leading strand, which is in agreement with the mutation counts that displayed low mutation on leading strand. This pattern becomes more explicit at a wider scale (Figure S36,37). In addition, CPD samples at 2 hours have elevated asymmetry at a wider scale compared to the CPD samples at 12 minutes (Figure 6, S36, 37, 42-45), because CPDs can be effectively repaired 1 hour after the damage formation. On the contrary, samples with (6-4)PP damages are not showing any strand difference, because of the fast repair ability of nucleotide excision repair for these photo-products. Although we do not observe a distinct mutational strand asymmetry at the individual ORIs, we analyzed the damage and repair events individually, and repair rates around ORIs. Surprisingly, we observed a strand asymmetry at individual ORIs (Figure S46-53, 58-61). While the samples at 2 hours demonstrates an efficient repair at leading template, samples at 12 minutes display an asymmetry that favors lagging template repair. Even though replication forks often move bidirectionally, at some regions, forks tend to move continuously in one direction, either by the moving long distances as a single fork, or multiple forks that are fired simultaneously (S. I. Takebayashi et al., 2017). To observe the effect of replication fork movement, we decided to use the regions that replication forks move in one direction. We retrieved a data which is produced by OK-seq and contains regions that are dominantly replicated in a single direction, termed high replication fork directionality (RFDs) (Petryk et al., 2016). Repair rates at high RFDs display a decrease at the direction of replication fork on wider regions (Figure S62-67). This decrease can be caused by the replication fork itself, considering that the regions replication fork had passed opens and becomes reachable to nucleotide excision repair, while the

downstream will be relatively condensed. Also, CPDs at 2 hours demonstrate a visible strand asymmetry at both directions in favor of leading template strand (Figure S62-67). These results suggest that the unidirectional movement of replication fork creates a strand difference by synthesizing one strand as lagging and the opposite as leading for long regions because leading strand is repaired more efficiently.

### **3. DISCUSSION**

#### **3.1**

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