CHAPTER 16

Mechanisms and Maps of Nucleotide Excision Repair

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16.1 Introduction

Nucleotide excision repair (NER) is the process by which helix-distorting DNA lesions and bulky adducts, such as those induced by UV (ultraviolet light), cisplatin, cigarette smoke, and aflatoxin, are removed from the genome.[1] The UV-induced DNA lesions cyclobutane dimers (CPDs) and 6-4 pyrimidine-pyrimidine dimer (6-4PP), and cisplatin-induced DNA lesions are most commonly formed between adjacent nucleotides on the same strand. Mechanisms for resolving these damages were first suggested in studies that showed bacterial factors could partially resolve the negative effects of UV irradiation if exposed to visible light.[2,3] These studies led to the discovery of the direct repair enzyme, photolyase, in *E. coli*. Photolyases are enzymes that when activated by blue light cleave UV-induced DNA damage into two adjacent, normal thymine bases.[4–6] Following UV irradiation, *E. coli* cells exposed to blue light eliminated thymine–thymine dimers, while *E. coli* cells kept in the dark maintained the same level of these adducts. Surprisingly, when glucose was added to the suspension buffer, both *E. coli* cells exposed to blue light and cells kept in the dark exhibited thymine–thymine dimer removal with increased levels of dimers present in small DNA fragments and decreased levels found in bulk chromosomal DNA.[7–11] Additionally, small gap synthesis was

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observed in both *E. coli* and mammalian cells following UV irradiation.[10,12] These studies illuminated another repair mechanism that involved the excision of these dimers from genomic DNA, later described as NER.

The general process of NER follows 5 steps: (1) bulky adduct damage is first recognized; (2) dual incisions are made on the 5′ follows end of the damage creating a small oligodeoxynucleotides containing the damage; (3) the excised oligodeoxynucleotide is released from the DNA; (4) the remaining gap in the DNA is filled in by repair synthesis; and (5) the newly synthesized DNA is ligated.[1,13–17] Of note, while this general process is conserved through evolution, proteins specific to NER are not conserved from prokaryotes to eukaryotes.[1] This chapter will cover the specific mechanisms of these steps in *E. coli* and mammalian cells, methods for mapping where this repair occurs, the consequences of inadequate repair, and the role of NER in disease.

16.2 Mechanisms of NER

The molecular mechanism of NER follows the general 5 steps described in the introduction. This damage recognition step can occur in two manners: global or transcription-coupled. For global repair, it occurs throughout the genome and damage recognition is independent of transcription. Transcription-coupled repair (TCR) only occurs for damage on the transcribed strand of actively transcribed genes and relies solely on the stalling of an elongating RNA polymerase at the damage site for damage recognition.[1,18–20] The original studies to uncover the molecular mechanism of NER were conducted in *E. coli* and then in humans.[1,13,15,21] Thus, this section will first focus on describing the molecular mechanism of NER in *E. coli* and will then cover the mechanism in humans.

16.2.1 NER in *E. coli*

16.2.1.1 Global Repair

NER is carried out by three key proteins in *E. coli* with additional accessory proteins to help with release of the excised oligodeoxynucleotides and to facilitate transcription-coupled repair (TCR) as shown by the path through the circle in Figure 16.1. The three key proteins, UvrA (100 kDa), UvrB (85 kDa), and UvrC (66 kDa), work in concert to create dual incisions at specific distances from the damage in order to remove the damage from DNA.[1,13,22–26] Specifically, damage is recognized by UvrA, an ATPase.[27,28] The UvrA homodimer then recruits a second ATPase, UvrB, to the site and a stable UvrB–DNA complex is formed by an ATP hydrolysis-dependent reaction. Once this stable complex is formed, UvrA is released and UvrC is recruited to the complex by UvrB. UvrC creates both the 5′ and 3′ incisions using its two nuclease active sites in an asynchronous, but concerted manner. These incisions are made seven nucleotides from the 5′ end and three nucleotides from the 3′ end of the adduct to create a 12 base long oligodeoxynucleotide containing the thymine dinucleotide adduct (12mer). UvrD,

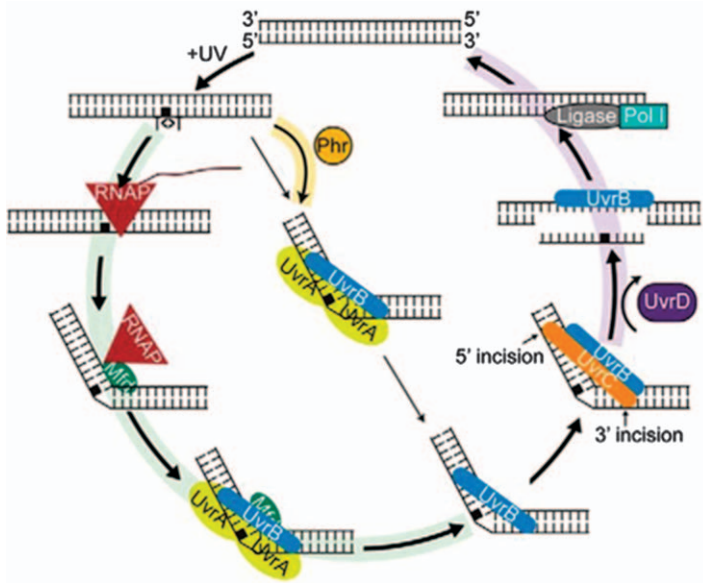

Figure 16.1 This figure shows the molecular mechanism of *E. coli* NER. DNA is first damaged. The outer pathway shows transcription coupled repair, while the pathway through the circle shows global repair. Damage is recognized and UvrA, B, and C are recruited and lead to excision of the damaged DNA. The gap is then filled in through repair synthesis and ligated to complete the repair process.
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an accessory NER protein, is a helicase and through this activity causes the release of both the excised oligodeoxynucleotides and UvrC.[27–45]

Following release from the repair complex, the excised oligodeoxynucleotide is degraded by exonucleases (Exo I, Exo VII, and RecJ).[22] In the absence of UvrD however, the excised oligodeoxynucleotides remains in complex with UvrC which blocks this degredation.[28,30,45] UvrC is the least abundant of the three proper repair proteins and thus, without this release to a new repair cycle, the number of excised oligodeoxynucleotides will saturate at the number of UvrC molecules.[13,46] DNA polymerase I conducts repair synthesis of the gap after it displaces UvrB.[30] If DNA polymerase I is not present, repair synthesis can be conducted by DNA polymerase II or DNA polymerase III.[47] This repair patch is then ligated by DNA ligase and NER of the bulky adduct is complete.[30,41] After completing the cycle, the repair proteins will begin repairing a new damage site.

16.2.1.2 Transcription-coupled Repair

The process of NER can be coupled with transcription, which stimulates the repair of the transcribed DNA strand.[1,22] DNA damage recognition is the

rate-limiting step of NER in both prokaryotes and eukaryotes. This is because most of the bulky adducts repaired through excision repair have only minor differences from normal, undamaged bases.[1,22,48] The effect of transcription on accelerating damage recognition is weaker for more easily recognized adducts (such as (6-4) photoproducts and benzo[α]pyrene diol epoxide- deoxyguanosine) and stronger for poorly recognized adducts (such as cyclobutane pyrimidine dimers (CPDs) and platinum di-guanine adducts).[49]

Damage recognition can be accelerated when RNA polymerase stalls after encountering a DNA lesion, as shown by the outer path of the circle in Figure 16.1.[22] The stalled RNA polymerase then forms a stable complex with the nascent RNA and the damaged DNA.[50,51] While this complex stimulates repair by removing inhibitory effects from histones in reconstituted transcription-coupled repair (TCR) reactions, the large complex blocks the access of repair factors to the damage *in vivo*, inhibiting repair in *E. coli* cells.[50] The stimulating effects of this complex on repair in cells are through another accessory repair protein, the 130 kDa Mfd translocase,[21,51–59] in which Evelyn Witkin reported that the *mfd* gene was responsible for protecting cells from a particular type of UV-induced mutagenesis.[59] When the transcription-repair coupling factor was purified, it was found to be a protein encoded by the *mfd* gene, providing an explanation for its protective effects on DNA. The Mfd protein both helps displace RNA polymerase to make the damaged DNA accessible to repair factors and helps recruit the NER factors, UvrA, B, and C, to the damaged site. These actions accelerate damage recognition and, in turn, repair.[21,51–59]

16.2.2 Mammalian NER

16.2.2.1 Global Repair

Creating the dual incision necessary for NER in *E. coli* is conducted by three main proteins, human NER requires sixteen proteins incorporated into six repair factors to accomplish this, as shown in Figure 16.2.[1,22,60] NER in humans is initiated by XPC, RPA, and XPA recognizing damage and recruiting the TFIIH repair complex. XPC acts as a molecular matchmaker, using energy from hydrolyzing ATP to bring the factors together into a complex. The TFIIH repair complex includes the helicases XPB and XPD. XPB and XPD help with damage recognition through kinetic proofreading using energy from ATP hydrolysis and unwinding DNA to create a repair bubble of about 25 base pairs surrounding the DNA lesion.[47,48,61,62] XPC then dissociates from the repair complex. This is followed by the recruitment of XPF and XPG which create 5′ and 3′ incisions using the SMX family structure-specific endonuclease active site. In humans, the 5′ incision is made nineteen to twenty-two bases away from the damage, while the 3′ wincision is made five to six bases away from the damage creating an excised oligodeoxynucleotide length of twenty-four to thirty bases.[15,47,61,63,64]

The excised oligodeoxynucleotide is then released in complex with TFIIH; this complex is stable for about ten minutes *in vivo*.[65] Once TFIIH dissociates

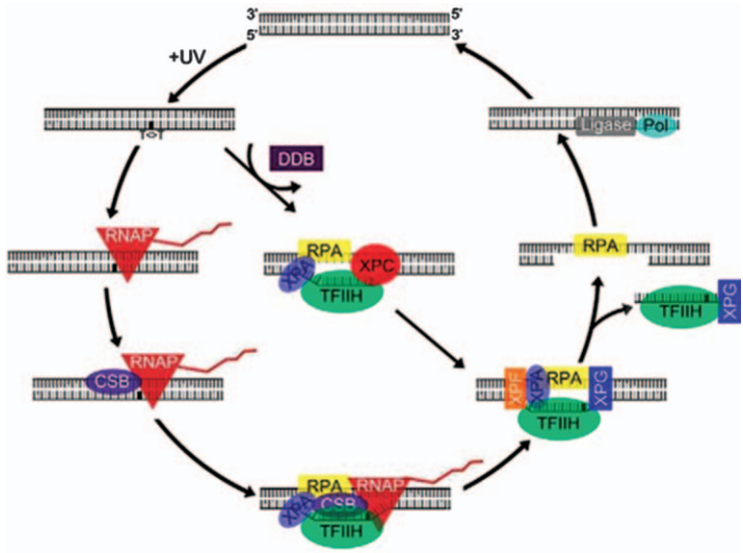

Figure 16.2 The molecular mechanism of mammalian NER. DNA is damaged and the damage is recognized either by stalled RNA polymerase and CSB (transcription-coupled repair, outer circle) or with the help of an accessory protein (global repair, inner pathway). The damaged DNA is excised in an approximately 26 base pair long oligodeoxynucleotide. The excised oligodeoxynucleotide is released in complex with repair proteins. The gap is filled in with repair synthesis and ligated.
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from the excised oligodeoxynucleotide, the oligodeoxynucleotide is then degraded by nucleases.[65–71] Following the release of the damaged DNA, DNA polymerases fill in the gap in the DNA with repair synthesis. The specific DNA polymerase used for repair synthesis in human cells depends on the cell cycle stage. In proliferating cells, DNA pol δ/ε fill in the gap, while in non-proliferating cells, other polymerases such as DNA pol κ/λ complete this task.[47,61,72] The size of the repair patch is the same size as the gap left following release of the excised oligodeoxynucleotide. This indicates that nick translation does not occur during this process.[73] Rarely, Exo I extends the excision gap by over twenty additional nucleotides. The single-stranded DNA at this gap is covered and protected by RPA and will serve as a signal for ATR checkpoint kinase.[74] After ligation of the repaired patch by either DNA ligase I or the XRCC1- ligase3 complex, the process of NER is complete.[47,61,75]

16.2.2.2 Transcription-coupled Repair

NER can also be transcription-coupled in humans in a process depicted by the outer circle in Figure 16.2.[22] RNA polymerase II forms a stable RNA pol II–RNA–DNA complex similar to the complex formed in *E. coli*. While in

E. coli Mfd is the essential factor to recruit repair factors to the stalled RNA polymerase, in humans transcription-coupling of repair is coordinated by one of two translocases, CSB or CSA.[76–78] When CSB recognizes a stalled RNA polymerase II, it prevents backtracking of the polymerase. It also promotes forward translocation on non-damaged templates such as pause-inducing repetitive A-tract sequences. Likewise, the binding of CSB to RNA polymerase II promotes transcriptional bypass of less bulky DNA lesions. The binding of CSB to RNA polymerase II alters the protein interaction landscape of the polymerase. This may facilitate the recruitment of NER factors and promote subsequent repair of the damaged DNA.[79] Of note, when TCR occurs in mammalian cells, XPC is not involved in the repair process.[76–78]

16.3 Mapping DNA Damage and NER

While methods such as slot blot enable researchers to study damage removal over time, these methods study NER efficiency at a global level. Pinpointing the specific, genomic location of damage formation and repair provides a more complete understanding of the factors that regulate these processes. To this end, the Sancar laboratory created two methods, Damage-sequencing and eXcision Repair-sequencing (XR-seq), to identify these locations at single-nucleotide resolution.[80,81] The methods and findings of these tools are described in this section.

16.3.1 Damage-sequencing

Damage-sequencing, a method for mapping specific sites of adduct formation, was created using the knowledge that DNA polymerases can cease DNA replication at sites of damage. Analysis of the data created with this method shows how different damaging agents induced DNA damage formation and repair are impacted by different genetic and genomic factors.[80,81]

16.3.1.1 Damage-seq Method

High-fidelity DNA polymerases will stop replicating DNA right before the DNA damage sites. This property provides the basis for mapping damage locations at single-nucleotide resolution. The Damage-seq protocol is previously published but follows the steps shown in the left panel of Figure 16.3. Cells are treated with a damaging agent such as UV or cisplatin. Genomic DNA is then isolated from the cells and sonicated to create fragments of about 600 base pairs, which then undergo end-repair. Following end-repair, the fragments are ligated to the first adaptor, denatured to single-stranded fragments, and immunoprecipitated with anti-damage antibodies to select for only the damaged strand. A biotinylated primer is then annealed to the adaptor and subsequently extended by a high-fidelity DNA polymerase. This extension will stop when the polymerase reaches a bulky adduct. Streptavidin-coated beads

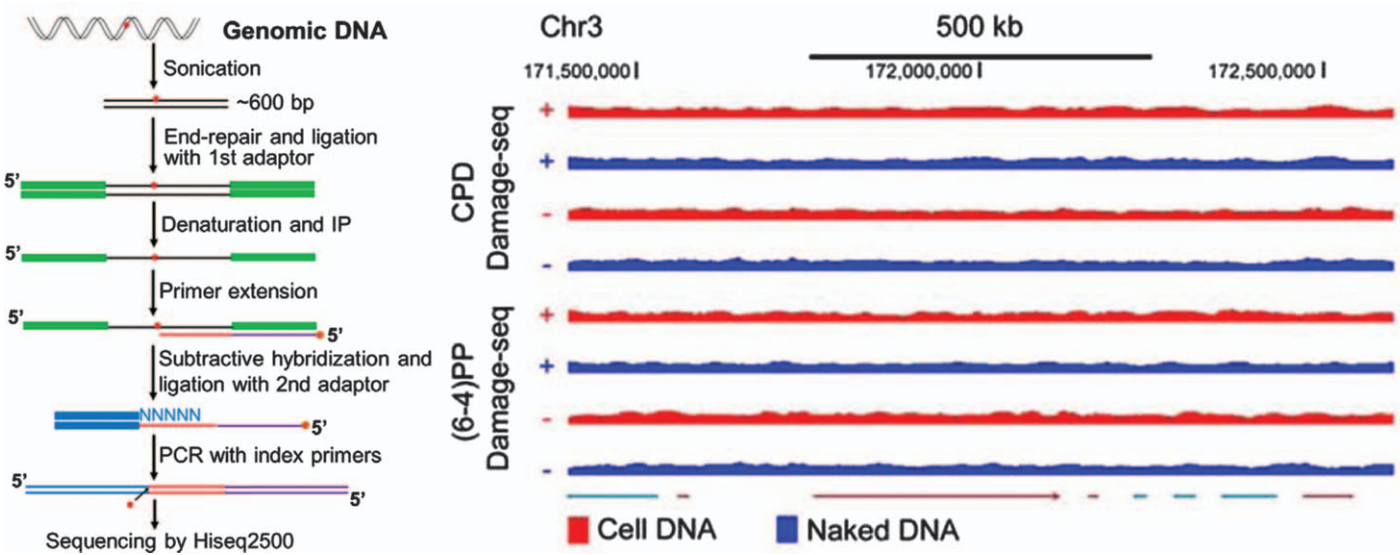

Figure 16.3 The protocol for Damage-sequencing (right) involves exposing cells to a damaging agent, fragmenting the DNA, purifying damaged segments, replicating the DNA with a polymerase that will stop at the damage, and amplifying the damaged segments. Damage-seq data (left) following UV exposure. Damage is uniform across the genome.
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are then used to purify the extended primers and extension products from undamaged strands are removed by subtractive hybridization, using an oligodeoxynucleotide with the 5u-sequence of the first adaptor, as undamaged strands contain the complementary sequence to this. Following these purification steps, the second adaptor is ligated and the ligation products are amplified with PCR and sequenced with Next Generation Sequencing (NGS). Any reads with the 5′ sequence of the first adaptor are discarded to ensure that only fragments from the damaged strand are analyzed, and the remaining reads are then aligned to the reference genome.[80,81]

16.3.1.2 Findings from Damage-seq

CPD and (6-4)PP damage-seq following UV exposure is shown in the right panel of Figure 16.3 for both naked DNA and cellular DNA. These low coverage patterns indicate that damage formation is similar between naked DNA and cellular DNA. Higher read coverage is needed to determine the role of chromatin state and genomic location damage formation.[22,81]

These findings were mostly reproduced when the same experiments were conducted using cisplatin and oxaliplatin as the damaging agent. The main difference is that while these drugs also form similar damage patterns between cellular DNA and naked DNA, transcription factor binding blocks platinum damage formation, most likely through physically blocking access to the DNA. Meanwhile, transcription factors can increase, decrease, or have no effect on UV damage formation. There is no rule to predict what the effect of a transcription factor binding DNA will be on UV damage formation, and any effect seen is more likely due to structural changes in the DNA following transcription factor binding.[22,80,81]

16.3.2 Excision Repair Sequencing

XR-seq uses our ability to capture all the small pieces of the excised oligodeoxynucleotides released during the NER through immunoprecipitations with highly specific antibodies to identify the precise location and relative amount of NER throughout the genome. This process can be used to determine what factors influence repair, how repair changes over time, and if repair patterns correlate with response to a damaging agent.[78,80,81]

16.3.2.1 XR-seq Method

The ability to isolate the excised oligodeoxynucleotides by immunoprecipitation (IP) of the TFIIH complex created the foundation for mapping repair events throughout the genome at single-nucleotide resolution. The detailed protocol has been published,[82] and the general procedure is depicted in Figure 16.4. The first step of the XR-seq protocol is to treat cells with a damaging agent (such as UV or cisplatin) and, following a designated incubation time, cells are lysed by homogenization. The lysis procedure and

IP antibodies vary depending on the organism being studied. For mammalian cells or tissue, it is important that the lysis buffer does not contain sodium dodecyl sulfide or any other protein-denaturing factors, as the first IP uses antibodies against repair proteins to purify the excised oligodeoxynucleotides. For *E. coli*, *S. cerevisiae*, and plants, the first IP uses antibodies against the DNA damage. The excised oligodeoxynucleotides are then eluted and adapters are ligated to the oligodeoxynucleotides. Following this step, the excised oligodeoxynucleotides are purified further through a damage IP to ensure that only DNA fragments containing damage are prepared for sequencing. The damage is then reversed to allow for PCR amplification of the excised oligodeoxynucleotides. These samples are then sequenced with NGS. These reads can then be aligned to the genome to show exactly where repair occurs. The NER maps show that there are regions of high repair and low repair and can lead to better understanding of what genetic, genomic, and epigenetic features influence repair. The maps also can show repair at specific loci, enabling more thorough studies of how driving mutations occur.[82] For DNA damage types that cannot be directly reversed, such as BPDE (benzo[*a*]pyrene diol epoxide), a general method named translesion XR-seq (tXR-seq) was developed to measure repair of all types of DNA lesions processed through NER. In place of damage reversal, this method uses a

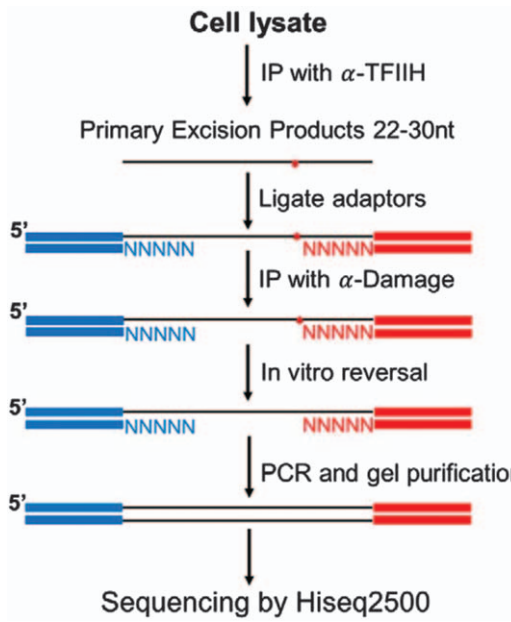

Figure 16.4 XR-seq is conducted by exposing cells to a damaging agent, lysing cells, conducting an IP for repair proteins to extract the excised oligodeoxynucleotides, and purifying and amplifying the oligodeoxynucleotides. Reproduced from ref. 22 with permission from The American Society for Biochemistry and Molecular Biology, Copyright 2017.

translesion DNA synthesis polymerase to bypass the damage and enable PCR amplification.[82]

16.3.2.2 Findings from XR-seq

Repair maps created by XR-seq show that there are preferentially repaired regions and regions of less repair. This indicates that many, often interconnected, factors including chromatin architecture, transcription, and DNA binding protein can direct where repair occurs. Studies using XR-seq can better define the mechanisms by which these factors direct the location of NER.[78,80,81,83–85]

XR-seq studies of *E. coli* have provided more insights into the mechanism of TCR. Repair patterns from wild type (WT), *mfd*$^{-}$, and *uvrD*$^{-}$ are shown on the left panel of Figure 16.5. These patterns confirm the model of TCR shown in the outer circle pathway of Figure 16.1. These studies also show the profound effect of TCR as the transcribed strand in WT cells is decidedly preferentially repaired. They also further confirm the role of Mfd as the transcription-repair coupling factor as repair maps from *mfd*$^{-}$ *E. coli* showed no preferential repair of the transcribed strand of genes with no confounding features. Furthermore, the repair maps from *mfd*$^{-}$ *E. coli* confirm that without an accessory transcription-coupling protein, the stalled RNA polymerase blocks repair. As evident by comparing the XR-seq signal on the transcribed strand to the non-transcribed strand in the *mfd*$^{-}$ lane of Figure 16.5, the non-transcribed strand is preferentially repaired. With only global repair, the expected outcome would be equal repair of both strands; however, the preferential repair of the non-transcribed strand indicates the transcribed strand is being blocked, likely by a stalled RNA polymerase.[22,32,45,50,52,53,59]

XR-seq studies, shown on the right panel of Figure 16.5, of XPC mutant human cell lines show only repair of the transcribed strand of actively transcribed genes. This indicates that there is no global repair, thus displaying the critical role of XPC in global repair and confirming its absence from TCR.[45] Repair patterns from CSB mutant human cell lines, shown in the right panel of Figure 16.5, show a loss of TCR confirming its role as a transcription-coupling accessory repair protein.[78] Additional analysis of XR-seq data showed that while RNA polymerase II shows strong TCR, RNA polymerase I does not exhibit TCR of ribosomal DNA.[86]

XR-seq studies have also shown that chromatin state can influence patterns of NER. Integrating XR-seq data with genome-wide data on chromatin states in specific cell types shows that open chromatin regions are repaired faster than heterochromatic regions, as seen in the top panel of Figure 16.6. In fact, CPD damage from a physiologic dose of UV in closed chromatin regions can take over two days to be resolved following UV exposure.[81,83,87] Likewise, TCR of cisplatin-induced damage is completed in approximately two days; however, global repair of cisplatin-induced damage in the non-transcribed strand and in intergenic regions can take weeks.[88]

Both *in vitro* and *in vivo* studies show that naked DNA is more efficiently repaired compared to nucleosomal DNA. In cells, this is seen by a periodicity

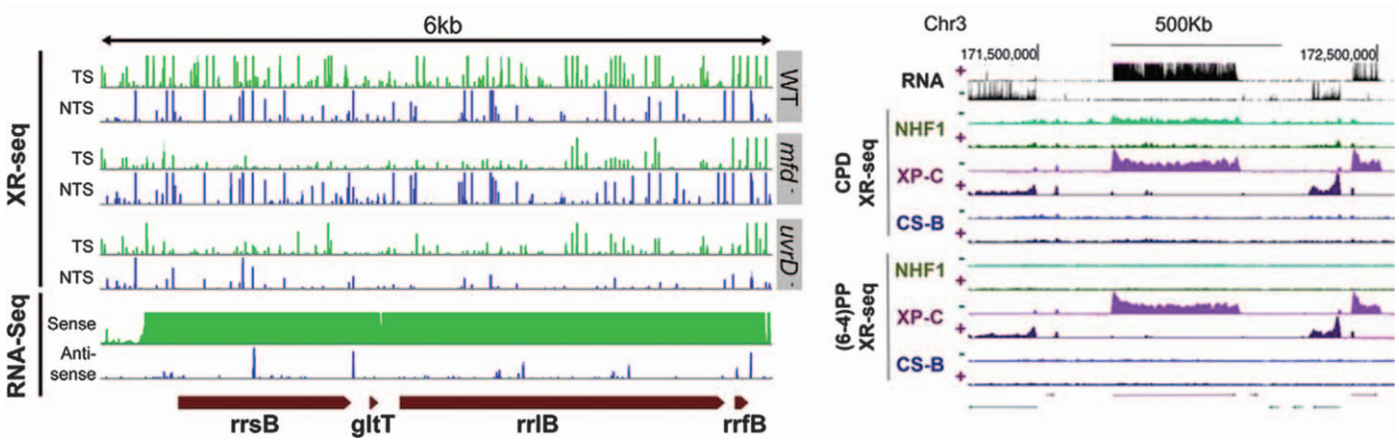

Figure 16.5 XR-seq data from *E. coli* (left) and human cell lines (right) showing repair patterns in wild type cells (WT, NHF1), transcription-coupled mutants (mfd-, CS-B), mammalian global repair mutants (XP-C), and *E. coli* cells that can't release repair proteins following an excision repair cycle (Uvrd^{-}).
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of higher amounts of NER that is antiphase with the nucleosome center as shown in the bottom left panel of Figure 16.6. The difference in repair efficiency between naked and nucleosomal DNA is stronger in *in vitro* studies than in *in vivo* studies, since cells contain nucleosome remodeling factors that can help facilitate damage access.[48,61,80,87,89,90]

Transcription factors can also influence repair efficiency as shown in the bottom right panel of Figure 16.6. When transcription factors bind to a target sequence with high affinity, they inhibit repair. In turn, this should translate to higher mutation rates in high-affinity transcription factor binding motif regions compared to control sequences. Mutation signature analysis using data from The Cancer Genome Atlas on both skin cancer and lung cancer, both of which are commonly attributed to bulky adduct damage, confirm this hypothesis. Transcription factors with weaker binding affinities show lower levels of repair inhibition and decreased frequency of mutations compared to transcription factors with higher binding affinity.[91–95]

In summary, the ability to map DNA damage and repair at single-nucleotide resolution has illuminated factors that influence damage formation and repair. Damage appears to be uniform across the genome at low coverage while repair is influenced by chromatin structure, DNA binding proteins, and transcription.

16.4 NER and Disease

NER is the sole mechanism for removing bulky DNA adducts caused by many known carcinogens, such as UV, and BPDE, and a commonly used chemotherapy, cisplatin, and its derivatives. This section will explore the role of NER in preventing tumor formation and in directing cellular response to chemotherapy.[1,22]

16.4.1 NER and Tumor Development

Carcinogenesis is attributed to mutations in key genes. In our day-to-day lives, we are constantly exposed to mutagens and proper repair of the damage caused by these exposures is essential for preventing tumor development. This section will explore the role of NER in tumorigenesis, specifically focusing on the outcomes of inefficient repair.

16.4.1.1 Xeroderma Pigmentosum

Human NER proteins are named for the human disease xeroderma pigmentosum (XP). XP is a hereditary disease that makes patients highly

Figure 16.6 Analysis of XR-seq data from human cells shows the impact of chromatin state (top), nucleosomes (bottom left), and transcription factor binding (bottom right) on damage and repair.
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sensitive to UV light with an approximately 5000-fold increase in sunlight-induced skin cancer compared to people without this disease. In 1968, James Cleaver discovered that these patients were defective in NER.[96] Seven of the key NER factors, XPA through XPG, were identified by studying the mutations present in XP patients. The increased risk for skin cancer in these patients is attributed to the inefficient NER capacity.[96–98]

16.4.1.2 Circadian Rhythm Control of NER

XPA, a rate-limiting protein in NER,[99] is under circadian control in mice. This means that the gene expression of XPA is controlled by the core circadian clock transcription translation feedback loop, oscillating with a 24-hour period.[99–101] The studies that defined this pattern show that XPA gene expression peaks at 5 pm and troughs at 5 am (see the top left panel of Figure 16.7). Follow-up studies show that total NER oscillates in the same manner.[100] Furthermore, TCR of a circadian controlled gene fluctuates following the oscillatory expression pattern of the gene.[102]

Given that NER is the sole repair mechanism for UV damage in mouse skin cells and that UV exposure can induce invasive skin tumors, it follows suit that exposure to UV at different circadian times would lead to different effects on tumorigenesis. If this connection is true, UV exposure at times of lower levels of NER would lead to increased numbers of tumors compared to mice treated at times of higher levels of NER. To test if the cyclical pattern of NER had a physiological impact, mice were exposed to UV irradiation at either 5 am (minimum repair) or 5 pm (maximum repair). Decreased NER efficiency does appear to increase risk of tumor development in response to UV. As shown in the top right and bottom panels of Figure 16.7, mice exposed to UV at the minimum NER time-point had a 4–5 fold increase in invasive skin carcinoma development compared to mice treated at the maximum NER time-point. It is still unclear if NER is under circadian control in humans. Thus, future studies are needed to determine if response to bulky-adduct formation varies depending on the time of day in humans.[103]

16.4.2 NER and Tumor Response to Cisplatin

Platinum-based chemotherapies, including cisplatin, oxaliplatin, and carboplatin, are a mainstay of solid tumor treatment and are used to treat a wide array of cancers including lung, liver, colorectal, testicular, ovarian, head and neck, and cervical.[104–106] Unfortunately, these therapies are very toxic and patients can experience numerous side effects including severe emesis, nephrotoxicity, ototoxicity, and neurotoxicity.[107] Platinum-based chemotherapies work by creating Pt-d(GpG) adducts with the platinum atom covalently bound to the N7 nitrogen in adjacent guanines.[104–106] This damage can either induce cell death, be ignored through translesion synthesis, or be repaired. These adducts are solely repaired by NER.[108–110] A study using XR-seq to profile NER in different mouse organs following

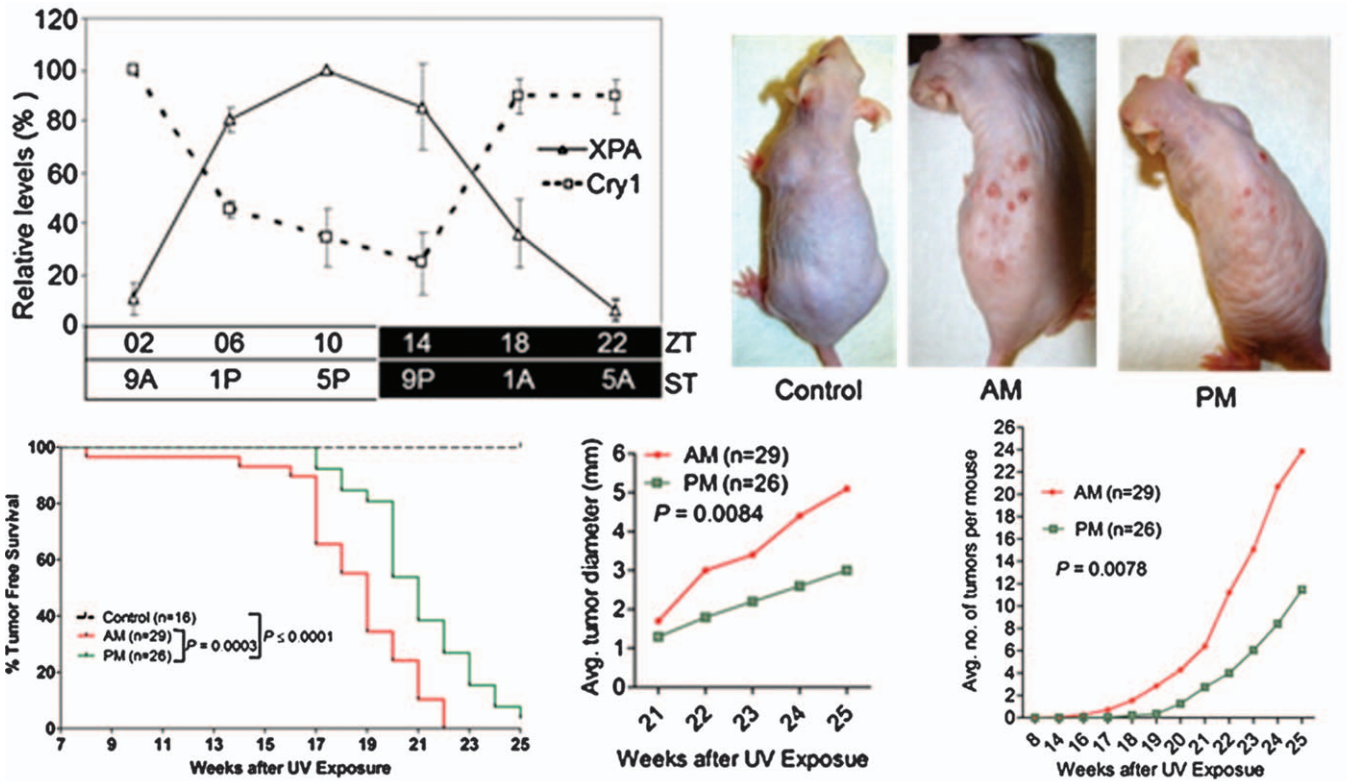

Figure 16.7 XPA is under circadian control (top left) and is antiphase with cryptochrome, a core circadian factor. Mice exposed to UV in the morning develop more and larger tumors than mice exposed to UV in the evening (top right and bottom). Reproduced from ref. 103, https://doi.org/10.1073/pnas.1115249108, with permission from the National Academy of Sciences, under the terms of a CC BY 4.0 license, https://creativecommons.org/licenses/by/4.0/.

treatment with cisplatin, including the organs most commonly associated with platinum toxicity, shows that repair profiles match the transcriptional profile of the organ.[111] Given the importance of NER in removing platinum damage, many studies have explored how genetic variants, alterations in gene expression, and protein function changes related to NER may correlate with patient outcomes and response to platinum-based chemotherapies. However, the link between alterations in NER and response to platinum-based chemotherapies remains unclear. This section will summarize the current literature on the connection between NER and platinum response.

16.4.2.1 Repair Gene Variants and Alterations in Gene Expression

Polymorphisms in genes coding for NER proteins are reported to be predictive of tumor response to platinum treatment in esophageal cancer cells,[112] non-small cell lung cancer,[113–116] and in multiple other cancers.[117] A number of other variants in genes encoding NER factors have been identified as indicators of cancer prognosis and/or treatment response; however, the genes that significantly correlate with outcomes vary between screens, even in similar patient populations. For example, in a screen of 25 single nucleotide polymorphisms (SNPs) in eight excision repair genes, variants in *ERCC1* (Excision Repair Cross-Complementation Group 1) and *XPC* or their regulatory regions correlated with survival in non-small cell lung cancer patients.[114] In a separate screen of 173 SNPs in 27 excision repair genes, Song *et al.*[115] claim that polymorphisms in *XPA* and *ERCC6* were associated with progression-free survival in non-small cell lung cancer patients, while polymorphisms in *ERCC6* correlated with overall survival. In a third screen of 17 SNPs in eight excision repair genes, only a SNP in *XRCC1* correlated with survival, while *ERCC1* and *ERCC3* correlated with platinum response.[116] Interestingly, despite their inclusion in all screens, variants in *ERCC1* and *XPC* were only shown to correlate with survival in one study.

Increased expression and activity of excision repair proteins is reported to correlate with platinum resistance in many cancers including ovarian,[118,119] testicular,[120] lung,[121–123] melanoma,[124] nasopharyngeal,[125–127] pancreatic,[128] and colorectal cancer.[117,129] However, similar to the studies on genetic variants, the correlations can be inconsistent between studies of similar patient populations. High *ERCC1* mRNA expression in non-small cell lung cancer tumor cells has been shown to correlate with poor disease-free survival in patients, independent of treatment with a platinum agent.[130] Likewise, non-small cell lung cancer patients with low *ERCC1* expression levels benefitted more from cisplatin treatment than patients with high *ERCC1* expression levels.[131] In patients with gastric cancer who were treated with surgery alone, low *ERCC1* mRNA correlated with longer overall survival. However, *ERCC1* mRNA levels could not serve as a prognostic factor for patients with breast cancer.[130] In a cohort of head and neck squamous cell carcinoma patients, it was found that there was no significant correlation

between expression levels of *ERCC1*, *XPA*, or *XPF* and overall survival. However, when the cohort was broken down based on the site of the tumor, it was found that *ERCC1* expression correlated with survival in patients with oral cavity tumors, while *XPA* expression correlated with survival in patients with oropharyngeal tumors.[127] In a comparison of platinum-sensitive and platinum-resistant gastric cancer cell lines, it was found that the platinum-sensitive cell line had lower levels of *XPC* leading to impaired repair and higher induction of cell death.[132] The mRNA levels of twelve DNA repair and multi-drug resistance genes were tested for correlation with cisplatin resistance and it was found that abundance of *ERCC2*, *XPA*, and *XRCC1* correlated with cisplatin resistance.[133] In an effort to better account for the complexity of the repair pathways, Kang *et al.*[134] determined a molecular score for DNA damage repair pathways based on expression levels of 23 platinum-repair related genes. Higher scores on this molecular panel correlated with improved survival and response rate.

16.4.2.2 Repair Capacity

A number of studies have explored the platinum sensitizing effect of knocking down expression levels of genes encoding NER factors. Knocking down *CSB* in either a prostate or colorectal cancer cell line, increased tumor cell sensitivity to cisplatin, independent of p53 or mismatch repair status.[135] Furthermore, a CRISPR/Cas9 screen identified transcription-coupled NER as a protective mechanism against cisplatin-induced cell death.[136] Likewise, knocking down both *ERCC1* and *XPF* lead to decreased repair of cisplatin-induced DNA damage and increased cisplatin-induced cell death in a non-small cell lung cancer cell line.[137] Small molecule inhibitors of ERCC1-XPF also decreased DNA repair and increased cytotoxicity following treatment with cisplatin in lung cancer cell lines.[138]

Despite the studies linking NER efficiency to the response to platinum-based chemotherapies, there are a number of confounding factors in this connection. Most excision repair proteins serve multiple functions and do not act alone. A study on neuroblastoma cells showed that resistance to platinum is likely related to mechanisms preventing platinum from binding to DNA and mechanisms that inhibit the downstream cell death signaling. The study also showed that resistance is not directly dependent on enhanced DNA repair capacity.[139] Furthermore, a comparison between a cisplatin-sensitive non-small cell lung cancer and its cisplatin-resistant derivative cell line showed that there was no difference in platinum-induced DNA damage removal between the two cell lines.[140] A recent review on the topic pointed out more inconsistencies in our current understanding of the role of repair as a mechanism of resistance. It called for new studies to examine repair with a more definite method than those used in previous experiments.[141]

Overall, while deficiencies in NER can increase susceptibility to cancer, the role of NER in the response of a tumor cell to cisplatin and its derivatives is still unclear.

16.5 Summary

NER protects the genome by removing bulky adduct damage. It does so through a process of damage recognition, dual incision, release of the damage-containing excised oligodeoxynucleotides, repair synthesis, and ligation of the gap. This process can occur in a transcription-coupled manner with the use of additional proteins. The novel Damage-seq and XR-seq allow us to identify where damage and repair occur at single-nucleotide resolution and provide a more complete understanding of the repair process. While mutations in NER factors can lead to a dramatic increase in developing melanoma following UV exposure, the role of NER efficiency in determining cellular response to other DNA damaging agents, such as cisplatin is still unclear.

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