Published in final edited form as:

Photochem Photobiol. 2017 January ; 93(1): 178–191. doi:10.1111/php.12641.

Post-excision Events in Human Nucleotide Excision Repair†

Michael G. Kemp[1,*] and **Jinchuan Hu**[2]

[1]Department of Pharmacology and Toxicology, Wright State University Boonshoft School of Medicine, Dayton, OH 45435

[2]Department of Biochemistry and Biophysics, University of North Carolina School of Medicine, Chapel Hill, North Carolina 27599

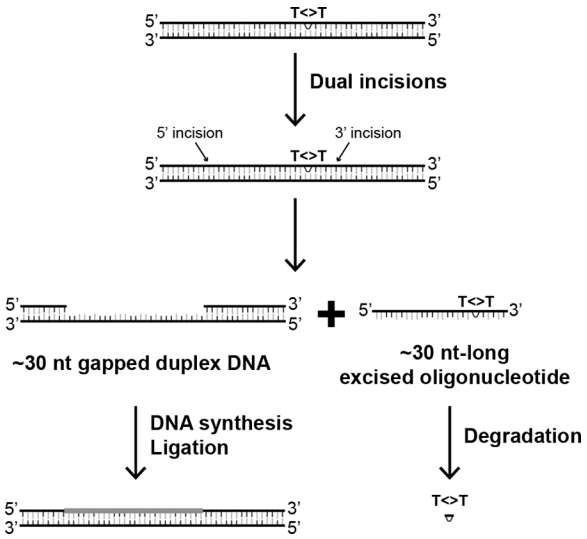
Abstract

The nucleotide excision repair system removes a wide variety of DNA lesions from the human genome, including photoproducts induced by ultraviolet (UV) wavelengths of sunlight. A defining feature of nucleotide excision repair is its dual incision mechanism, in which two nucleolytic incision events on the damaged strand of DNA at sites bracketing the lesion generate a damage-containing DNA oligonucleotide and a single-stranded DNA gap approximately 30 nucleotides in length. Although the early events of nucleotide excision repair, which include lesion recognition and the dual incisions, have been explored in detail and are reasonably well understood, the fate of the single-stranded gaps and excised oligonucleotide products of repair have not been as extensively examined. In this review, recent findings that address these less-explored aspects of nucleotide excision repair are discussed and support the concept that post-incision gap and excised oligonucleotide processing are critical steps in the cellular response to DNA damage induced by UV light and other environmental carcinogens. Defects in these latter stages of repair lead to cell death and other DNA damage signaling responses and may therefore contribute to a number of human disease states associated with exposure to UV wavelengths of sunlight, including skin cancer, aging, and autoimmunity.

Graphical Abstract

†This article is part of the Special Issue highlighting Dr. Aziz Sancar's outstanding contributions to various aspects of the repair of DNA photodamage in honor of his recent Nobel Prize in Chemistry.

*Corresponding author: mike.kemp@wright.edu (M. Kemp).



Schematic of human nucleotide excision repair. UV induces the formation of UV photoproducts in DNA, including a representative thymine dimer indicated in the figure. Two nucleolytic incision events take place ~20 ± 5 phosphodiester bonds 5' and 6 ± 3 nt phosphodiester bonds 3' of the UV photoproduct to generate an ~30-nt-long gapped DNA duplex and an 30-nt-long damage-containing DNA oligonucleotide. Completion of the DNA repair reaction requires DNA repair synthesis and ligation to fill in the gap and degradation of the excised, damage-containing DNA oligonucleotide.

INTRODUCTION

Ultraviolet (UV) wavelengths of sunlight induce the formation of photoproducts in genomic DNA that interfere with DNA metabolism and normal cellular physiology. Because these UV lesions block or slow the progression of DNA and RNA polymerases, the resulting replication and transcription stress have the potential to lead to mutagenesis, genomic instability, and cell death. These processes may therefore ultimately contribute to sunlight-induced skin carcinogenesis, aging, and other pathologies in susceptible individuals.

In humans and other placental mammals, the sole mechanism for removing UV photoproducts from DNA is nucleotide excision repair (1–4). A central feature of this repair system is its dual incision mechanism, which involves two nucleolytic events ~20 ± 5 phosphodiester bonds 5' and 6 ± 3 nt phosphodiester bonds 3' to the UV photoproduct (5). The reaction products of the nucleotide excision repair reaction therefore include a damage-containing oligonucleotide approximately 24- to 32-nt in length and a corresponding single-stranded DNA (ssDNA) gap approximately 30 nt long (6). A simple schematic of this repair process is outlined in Figure 1. Biochemical studies in vitro and in vivo have demonstrated that the length of the excised, UV photoproduct-containing oligonucleotides are identical in humans and many other eukaryotic organisms (5,7–11). Although additional nucleolytic degradation of the excised oligonucleotide is expected to take place to break down these byproducts of DNA repair, the ultimate fate of the adducted nucleotides is not known (12). Coincident with the dual incision event, a ssDNA gap is generated and must be filled in by a

DNA polymerase and then the remaining nick ligated to restore the DNA duplex to its initial, undamaged state. Failure to efficiently fill and seal excision gaps is associated with the activation of DNA damage response signaling (13), a reduced rate of repair, and cell death.

The discovery of excision repair in the 1960s was made possible by the use of methods that are now known to directly measure the two reaction products of the nucleotide excision repair reaction. Howard-Flanders and Setlow found that radiolabeled thymidine incorporated into the genomic DNA of bacterial and human cells was released ("excised") from DNA following UV irradiation in the form of small oligonucleotides (14–16). Similarly, Painter and Hanawalt observed the incorporation of radionucleotides into the DNA of non-S phase cells following UV (17,18), which represents the gap filling step of repair. The identification of UV-sensitive and cancer-prone human patients with deficiencies in nucleotide excision repair (19,20) demonstrated that DNA repair was important to human health and prompted the identification, cloning, and characterization of the genes and corresponding proteins that are responsible for UV photoproduct removal from genomic DNA in human cells. This work ultimately enabled the purification of the six core nucleotide excision repair factors (XPA, RPA, XPC, TFIIH, XPF-ERCC1, and XPG) and the development of a detailed, mechanistic model for the nucleotide excision repair reaction (21–26). Indeed, the ability to study the individual steps and components of nucleotide excision repair in isolation has been critical to the current understanding of the damage recognition and dual incision steps of repair. However, important questions in the field remain to be answered, particularly regarding the fate of the excision gap and excised oligonucleotide products of repair. In addition, the extent to which human diseases associated with exposure to UV wavelengths of sunlight are influenced by defects in these latter steps of repair remains to be determined. Studies over the past decade have begun to more fully examine these latter steps of excision repair. Thus, in this review, the pre-incision steps of nucleotide excision repair are briefly summarized before undertaking a more extensive discussion of recently published findings that address the processing of the excision gap and excised oligonucleotide products of the dual incision reaction. Important unanswered questions and links between defects in these post-excision steps of nucleotide excision repair and human disease are also highlighted.

PRE-INCISION STEPS OF NUCLEOTIDE EXCISION REPAIR

The first step in removing UV photoproducts and other bulky DNA adducts from DNA is the initial recognition of the damage, which takes place through one of two pathways outlined in Figure 2. In the so-called general or global genome repair pathway, UV lesions are recognized by an XPC (xeroderma pigmensotum group C)-dependent process that leads to the assembly of XPC, TFIIH (transcription factor II-H), RPA (replication protein A), and XPA (xeroderma pigmentosum group A) at the site of damage. The precise mechanism of damage recognition and the order of assembly of repair proteins at UV lesions have been the subject of a number of excellent studies (27–31) and have been discussed in greater detail elsewhere (1,4,32,33). Nonetheless, once the damage is identified, TFIIH unwinds the DNA around the lesion to generate a repair bubble of approximately 20 nt (23,25,34,35). These factors are not sufficient for the dual incision reaction to take place, however, and the subsequent recruitment of the endonucleases XPG (xeroderma pigmentosum group G) and

then XPF-ERCC1 (xeroderma pigmentosum group F) is therefore required for the dual incision events to take place 6 ± 3 nt phosphodiester bonds 3' and ~20 ± 5 phosphodiester bonds 5' of the lesion, respectively. It should be noted that the purification of the individual repair factors and the utilization of in vitro biochemical assays were essential for characterizing these damage recognition and dual incision steps of repair (22–26,36). Moreover, the ability to reconstitute the full dual incision reaction in vitro with only these six core repair factors (RPA, XPA, XPC, TFIIH, XPF-ERCC1, and XPG) (22,23) demonstrated that these factors alone are sufficient for damage recognition and excision. Thus, though there are many additional proteins and regulatory systems that can influence the efficiency of repair in certain physiological contexts and within specific chromosomal regions in vivo, these six core factors are the only essential components of the general excision repair pathway.

A second pathway for damage recognition and removal involves gene transcription and RNA polymerase. It was recognized more than 30 years ago that UV photoproducts on the transcribed strand of active genes are repaired at a faster rate than on non-transcribed strands and within inactive genes (37–39). UV photoproducts are barriers to RNA polymerase movement (40–43), and thus the stalling of an RNA polymerase at a lesion provides a mechanism for the initial recognition of the DNA damage. Because this sub-pathway of excision repair, termed transcription-coupled repair (44), has not been reconstituted in vitro, it is not understood at the level of detail as the general repair pathway. Nonetheless, genetic approaches have demonstrated that two additional factors, termed CSA and CSB (Cockayne Syndromes group A and B), are necessary for the removal of UV photoproducts from the transcribed strand of active genes and for the resumption of transcription (45–49). These factors are thought to facilitate the eventual assembly of a pre-incision complex containing RPA, XPA, and TFIIH (but not XPC). Similar to the global genome repair mechanism, the recruitment of the XPF and XPG nucleases then allows for the dual incisions and the removal of the damage.

As described earlier, an important aspect of nucleotide excision repair is the generation of a lesion-containing oligonucleotide and a corresponding single-stranded DNA gap approximately 30-nt in length. Though the excised 30-mer products of the general excision repair pathway were first observed nearly 25 years ago using cell-free extracts and defined DNA substrates in vitro (5,50), only recently have methods been developed to isolate, visualize, quantify, and map the excised oligonucleotides in UV-irradiated cells in vivo (51–54). This work, described in greater detail below, demonstrated that the lengths of the excised oligonucleotide products of excision repair are identical in cells deficient in either XPC or CSB (10,51), in which UV photoproducts can only be removed through the transcription-coupled or global genome repair pathway, respectively. Similarly, earlier studies had shown the excision gap size to be approximately 30 nt in length for both excision repair pathways (6,55). These results therefore demonstrate that the final products of nucleotide excision repair (a ssDNA gap and an excised, damage-containing oligonucleotide) are identical irrespective of how the damage was initially recognized.

Following nucleolytic cutting by XPF and XPG, the damage-containing oligonucleotide dissociates from the gapped, duplex DNA (24,56). As will be described in detail below, this

excised oligonucleotide is initially released in a tight complex with the repair factor TFIIH before associating with the ssDNA-binding protein RPA and undergoing further degradation (Figure 2) (10,56). The excision of the UV photoproduct-containing oligonucleotide leaves a ssDNA gap in the DNA that must be filled in and ligated to complete the repair reaction. These steps, also described below, require a heterotrimeric “clamp” protein known as PCNA (proliferating cell nuclear antigen), a DNA polymerase, and a DNA ligase. The diversity in polymerase and ligase usage that has been described for this step over the past decade was unanticipated and suggests additional levels of regulation of the latter steps of nucleotide excision repair.

POST-EXCISION STEPS OF NUCLEOTIDE EXCISION REPAIR

Fate of the Gap

In additional to physically removing lesions from DNA, the completion of nucleotide excision repair necessitates that the ssDNA gap is filled in by the action of a DNA polymerase and then the remaining nick ligated. Though these processes are expected to take place rapidly during normal repair, defects in gap filling and ligation likely take place under specific biological conditions or at specific chromosomal loci and may therefore contribute to genomic instability.

Gap filling DNA synthesis—DNA repair synthesis requires the action of a DNA polymerase to add nucleotides to the 3’-hydroxyl that is generated by the action of the XPF endonuclease. Because of its similarity to replicative DNA synthesis, gap filling DNA replication was assumed to involve the canonical replicative DNA synthesis enzymes. Indeed, in vitro reconstitution experiments with purified factors demonstrated that either of the high-fidelity replicative B-family DNA polymerases (Pols δ and ε) could support the filling in of the excision gap in conjunction with the replicative clamp protein PCNA and clamp loader RFC (replication factor C), (21,26,57,58). However, validation that these polymerases alone are responsible for gap filling in vivo was complicated by the fact that the polymerases play essential roles in chromosomal DNA replication.

Interestingly, more recent data has implicated a role for the error-prone Y-family polymerase, Pol κ, in nucleotide excision repair. Cells deficient in this polymerase were found to be partially defective in the removal of UV photoproducts from genomic DNA (59). Though not required for the dual incision reaction per se, experimental approaches in human cells have indicated that DNA polymerase activity contributes to pre-incision factor turnover at damage sites in UV-irradiated cells in vivo and thus impacts the overall rate of nucleotide excision repair (60,61). Nonetheless, to further address the roles for the different DNA polymerases in the repair of UV photoproducts, a subsequent analysis showed that all three DNA polymerases (δ, ε, and κ) contribute to the gap filling step of excision repair in vivo (62), with RNA interference-based approaches in asynchronously growing cultured cells indicating that Pol ε is responsible for approximately half of all repair synthesis and that Pols δ and κ carry out the remaining repair synthesis as part of a common pathway. Similarly, immunostaining approaches showed that all three polymerases were recruited to large, UV-damaged, sub-nuclear foci but that each polymerase showed unique requirements

for recruitment. Whereas the recruitment of Pols δ and κ required the canonical clamp loader RFC (62,63), Pol ε recruitment to damage sites instead required the action of an alternative clamp loader comprising the CTF18 protein in conjunction with the canonical small RFC subunits. A schematic summarizing these findings is provided in Figure 3. Furthermore, siRNA-mediated knockdown experiments showed that Pol κ recruitment to foci also required the DNA repair scaffold protein XRCC1 and the ubiquitination of the PCNA clamp by the Rad18 ubiquitin ligase complex. Evidence showing that Pol ε is involved in gap filling solely in dividing cells and not in serum-starved quiescent cells suggests that the utilization of specific DNA polymerases during gap filling may involve distinct cellular conditions, such as proliferation, dNTP levels, specific DNA structures, or other factors. It will be interesting in the future to apply whole genome mapping strategies to determine whether there are particular chromosomal regions or chromatin states that influence the preferential recruitment of specific DNA polymerases.

Ligation—Similar to the prevalent roles for DNA polymerases δ and ε in general DNA metabolism, DNA ligase I (Lig I) was largely assumed to be the enzyme that seals the nick in the DNA that remains following gap filling DNA synthesis. Indeed, experiments with purified proteins in vitro showed that Lig I was able to seal the nick to restore the DNA duplex to its native state (21,26,58). However, genetic studies yielded conflicting findings regarding the sensitivity of Lig I-deficient cells to UV and thus the role of Lig I in excision repair (64,65). Recent work using Lig I-deficient cells maintained in a confluent, non-dividing state to limit genomic stress from chromosomal DNA replication showed that Lig I-deficient cells exhibited no major defects in UV photoproduct removal (60). Instead, the loss of DNA ligase III (Lig III) was shown to impair the ligation of nucleotide excision repair-generated nicks and the removal of UV damage from genomic DNA. Like DNA synthesis, DNA ligation is not required for the dual incision reaction to take place but may affect repair protein turnover at localized sites of UV irradiation in cells in vivo to influence overall nucleotide excision repair rate (60). Furthermore, both Lig III and its binding partner XRCC1 were found to co-localize and associate with excision repair factors at UV-damaged sites in quiescent, non-cycling cells (60). Nonetheless, Lig I was found to be present within UV-damage foci in replicating cells, which indicates that Lig I may play a role in sealing at least a portion of the nicks that remain after gap filling DNA synthesis in dividing cells. Thus, Lig III and to a lesser extent Lig I are responsible for completing the final step of human nucleotide excision repair (Figure 3).

Gap enlargement, double-strand break formation, and activation of DNA damage response kinases—Though both the excised oligonucleotide and excision gap show a mean length of approximately 30 nt in human cells (5,6,10,55), longer ssDNA gaps have been detected in UV-irradiated *Saccharomyces cerevisiae* cells and were shown to be dependent on a 5'→3' exonuclease known as Exonuclease 1 (Exo1) (66). Moreover, this nucleolytic enlargement of the excision gap appeared to be required for maximal activation of a cell cycle checkpoint that delays the entry of UV-irradiated G1 cells into S phase. Consistent with this data in yeast, fluorescence microscopy studies have shown that human Exo1 accumulates at UV-damage sites in human cells and forms a complex with the core excision repair factor XPA (67). The recruitment of Exo1 to UV damage foci in vivo

required the 5’ incision by XPF but not the 3’ incision by XPG and was enhanced when gap filling was inhibited with chemicals that lower nucleotide levels and block chain elongation. Moreover, studies in non-cycling human cells in vivo (67,68) and with a reconstituted system composed of purified excision repair and DNA damage checkpoint proteins in vitro (68) demonstrated that maximal activation of the ATR (ataxia telangiectasia and rad3-related) DNA damage checkpoint kinase (69,70) required Exo1-nucleolytic processing of the excision repair gaps. These studies showed that the phosphorylation of multiple ATR kinase substrates, including RPA and the tumor suppressor protein p53, was defective in Exo1-deficient non-cycling cells following UV. A schematic of Exo1-dependent excision gap enlargement is provided in Figure 4.

Whether a cell cycle delay is the sole or even major functional pathway controlled by ATR in response to Exo1-enlarged excision gaps is unknown and requires further study. Replicative DNA polymerase stalling at DNA lesions and uncoupling from DNA helicase activity (71,72), which generates regions of single stranded DNA for recruitment of the ATR/ATRIP complex to DNA (73,74), is the most widely recognized trigger for the recruitment and activation of ATR (69,70,75). Thus, although ATR has well-described roles in controlling replication fork stability, origin firing, and cell cycle phase transitions in response to DNA damage encountered during chromosomal DNA replication (69,75), the functions of ATR in response to excision repair intermediates and other stimuli in non-replicating cells are less clear. Indeed, it should be noted that ATR can be activated by multiple mechanisms in UV-irradiated, non-replicating cells, including by direct association with bulky DNA adducts induced by UV and related chemical carcinogens (76–78), by nucleotide excision repair-dependent processing of DNA lesions (67,79–83), and by transcription stress (84,85). Thus, additional work is needed to determine the downstream functions of these alternative ATR signaling processes, including in response to excision gaps enlarged by Exo1. Nonetheless, the factors that affect whether excision gaps are enlarged by Exo1 or are efficiently filled in and ligated are not known. These issues have important implications for genomic instability in UV-irradiated cells because ssDNA gaps are likely prone to breakage, which could give rise to double-strand breaks that are potentially lethal to the cell or which could lead to gene deletions or to chromosomal rearrangements.

Indeed, a recent study found that double-strand breaks are formed in UV-irradiated, non-replicating, quiescent cells in a manner that requires the canonical excision repair factors (86). This phenomenon was associated with rapid and robust signaling by the ATM (ataxia telangiectasia-mutated) DNA damage response kinase (Figure 4), which has well-recognized roles in the cellular response to DNA double-strand breaks (87,88). Moreover, inhibition of ATM and the related kinase DNA-PK (89), which promotes the repair of double-strand breaks by non-homologous end joining pathway, was shown to sensitize non-replicating, quiescent cells to the lethal effects of UV and UV mimetic chemical carcinogens (84,86). Though dependent on the dual incisions by the core nucleotide excision repair machinery (86), the mechanism of double-strand break formation in UV-irradiated non-replicating cells remains unclear. Double-strand break formation and recognition by ATM and DNA-PK may require nucleolytic processing of nearby excision gaps on opposing strands of DNA by Exo1, nucleolytic targeting of ssDNA at excision gaps by other cellular nucleases, or RNA

polymerase-dependent transcription through nicks or unfilled excision gaps. Nonetheless, double-strand break formation following UV may to lead to loss of genetic information, gross chromosomal rearrangements, or aberrant cell death. Thus, it will be important to determine the mechanism of excision repair-dependent double-strand break formation in non-replicating UV-irradiated cells and its impact on human diseases associated with genomic instability, such as cancer and aging.

Fate of the Excised Oligonucleotide

The second product of the nucleotide excision repair reaction is a short, UV photoproduct-containing DNA oligonucleotide approximately 24- to 32-nt in length. The ultimate fate of these excised oligonucleotides, and in particular the UV-damaged nucleotide bases, has not been thoroughly studied (12). In this section we highlight several recent reports that have focused on understanding this unexplored area of nucleotide excision repair.

Association of excised oligonucleotides with TFIIH and RPA—Reconstituted excision repair reactions using purified proteins and defined DNA substrates demonstrated that the excised oligonucleotide is protein-bound in vitro (22,24), and a recent gel filtration chromatography analysis of in vitro repair reactions with a defined DNA damage substrate and cell-free extract showed that the excised oligomers are largely found in two distinct complexes (56). Targeted immunoprecipitation of specific excision repair factors subsequently established that the excised oligonucleotides are nearly entirely bound to the core repair factors TFIIH and RPA following the dual incision event in vitro (56). This finding was further confirmed in vivo through the use of a new in vivo excision assay that allows for the isolation and detection of excised oligonucleotides in UV-irradiated cells (10,90). Whether there are additional proteins that associate with the TFIIH- and RPA-excised oligonucleotide complexes has not been fully explored, though a least a fraction of the TFIIH-excised oligonucleotide complexes contain either XPF or XPG (10).

Interestingly, in vitro psoralen crosslinking experiments had previously shown that the XPD subunit of TFIIH and the 70-kDa and 32-kDa subunits of RPA are in close proximity to DNA lesions during excision repair (91). XPD is a DNA helicase, and its 5→3 helicase activity is known to be essential for nucleotide excision repair by unwinding the DNA duplex around the lesion (92,93). Moreover, biochemical analyses of XPD homologues have indicated that XPD helicase activity is inhibited by the presence of DNA lesions on the translocated strand of DNA (94,95) and that XPD is indeed capable of forming a stable nucleoprotein complex with damage-containing DNA in vitro (96). Structural studies and mutational analyses of XPD and its archaeal homologue have further verified that the protein is important in validating the presence of damage (97–99). Thus, during the process of damage recognition and verification, the stalling of XPD and TFIIH at the damage site likely results in the lesion and surrounding DNA being essentially buried within the XPD subunit of TFIIH. Upon the dual incisions by XPF and XPG, the damage-containing oligonucleotide would be expected to remain within the TFIIH holoenzyme and specifically bound to XPD. Based on in vitro biochemical experiments with immobilized DNA substrates that have shown that the excised oligomers do not remain stably associated with gapped duplex DNA following the dual incisions (22,24,56), the TFIIH-excised oligonucleotide complexes are

thought to readily dissociate from the excision gaps following XPF and XPG cutting (Figure 5).

Our understanding of how the excised oligonucleotides are processed following the dual incision event is limited. Experiments in which TFIIH-excised oligonucleotide complexes were isolated from in vitro excision repair reactions and then studied under defined reaction conditions revealed that the TFIIH-excised DNA complex is remarkably stable. In the absence of ATP, the excised oligonucleotides remain stably associated with TFIIH for at least 8 hours (56). Interestingly, in the presence of ATP or a non-hydrolyzable ATP analog, excised oligonucleotides slowly dissociated from TFIIH. These results suggest that ATP binding, but not hydrolysis, by a component of TFIIH may alter its conformation to allow for release of the excised, damage-containing oligonucleotide. It is expected that there may be additional factors that promote the release of excised oligonucleotides from TFIIH to allow for the high rate of excision repair observed in vivo. The identification of a purported release factor may therefore help shed new light on the regulation of this novel step of repair, which is likely important for recycling TFIIH for new rounds of repair or for TFIIH to function in gene transcription (35). Nonetheless, the fact that excised oligonucleotides associate tightly with TFIIH following the dual incision event has been a useful experimental tool that has allowed for the generation of high-resolution, genome-wide maps of UV photoproduct repair in human cells (51,52).

As mentioned above, excised oligonucleotides also associate with RPA both in vitro and in vivo (10,56,90). RPA binding to the excised oligonucleotide likely takes place after the release from TFIIH and may be coordinated with oligonucleotide degradation. This hypothesis is based in part on the fact that the lengths of the excised oligonucleotides that are bound to TFIIH and RPA are distinct. Oligonucleotides that are bound to TFIIH are in the range of 24- to 32-nt, and the majority of oligonucleotides that are bound to RPA are closer to 18- to 20-nt in mean length (10,56,90). The TFIIH- and RPA-bound excised oligonucleotides are thus frequently referred to as the full-length, primary excision products and the partially degraded excision products, respectively. Though full-length oligonucleotides can be found to be associated with RPA, particularly at early time points during repair in vitro (56), the smaller products overwhelmingly predominate at later time points of excision repair both in vitro (56) and in vivo (10,90). Whether the binding of RPA to the excised oligonucleotides is non-specific or a defined, intermediate step in nucleotide excision repair has yet to be resolved. However, as will be described in greater detail below, the use of chemical inhibitors of DNA repair synthesis and ligation in vivo has been shown to lead to a preferential enrichment of excised oligonucleotides that are bound to RPA (90). Thus, the association of excised oligonucleotides with RPA following their release from TFIIH may be a regulated process during the post-incision steps of nucleotide excision repair. However, additional work aimed at understanding the molecular details of oligonucleotide release from TFIIH is needed to test this hypothesis. Nonetheless, the binding of excised oligonucleotides to RPA could prevent RPA from taking part in DNA replication and other DNA metabolic processes, which is an important issue given that insufficient RPA protein levels may contribute to genomic instability (61,100).

Nucleolytic degradation of excised oligonucleotides—The nucleases that degrade the excised oligonucleotides are not known, and it is possible that there are multiple nucleases that act in a redundant manner to break down these products of nucleotide excision repair. It is clear that there must be at least one nuclease responsible for converting the primary, full-length excised oligomers (24–32 nt) that are bound to TFIIH to the slightly smaller products (18–20 nt) that are bound to RPA. Whether this limited degradation occurs on DNAs bound to RPA or on transiently protein-free DNA molecules is not known. However, we note that one of the major DNA binding modes of RPA includes DNA in this size range (101,102), and thus the length of the oligonucleotides bound to RPA could simply represent a footprint of RPA on the excised oligonucleotide that limits further degradation of the DNA. Furthermore, the generation of 18- to 20-nt-long oligomers from the larger DNAs may not be unique to excision repair and damaged DNA processing because addition of a random 27-mer oligonucleotide to cell-free extract was shown to generate the same size products as found in a bona fide nucleotide excision repair event (56).

The enzymes responsible for this limited degradation or trimming of RPA-bound oligonucleotides are unlikely to be either of the two nucleases (XPF and XPG) that comprise the core nucleotide excision repair machinery because excised oligonucleotides generated in a fully reconstituted reaction comprising only the six core repair factors (which contain XPF and XPG) did not show any nucleolytic processing (56). Thus, there must be other nucleases present in cells that are responsible for this initial degradation. Though the 5’→3’ exonuclease Exo1 and 3’→5’ exonuclease Trex1 are possible candidates, the pattern of post-excision oligonucleotide processing is not apparently affected by loss of either of these nucleases from cells or cell-free extracts (56) (Hu and Kemp, unpublished).

There are a number of other possible nuclease candidates that could be explored in the future using UV-irradiated cells and gene-targeting knockdown methods. One challenge with this experimental approach, however, is that the nuclease that degrades the excised oligonucleotides may also facilitate the turnover or recycling of TFIIH or RPA during repair and thus impact the overall rate of excision repair. Thus, loss of a candidate nuclease could in principle affect both the degradation and the generation of excision oligonucleotides. Nonetheless, there is experimental evidence that various nucleases contribute to the cellular response to UV. For example, loss of the 3’→5’ exonuclease Trex2 has been shown to slow nucleotide excision repair rate and lead to increased UV- and UV mimetic-induced skin carcinogenesis (103,104). Similarly, loss of the 3’→5’ exonuclease/nucleoside diphosphate kinase is associated with a reduced rate of UV photoproduct repair and elevated carcinogenesis in experimental models (105,106). Though these studies have not demonstrated a direct role for these nucleases in nucleotide excision repair, future work should explore roles for these and other nucleases in repair and excised oligonucleotide degradation.

It should also be noted that several studies employing tritiated thymidine-containing genomic DNA, trichloroacetic acid precipitation, and high performance liquid chromatography to follow the fate of excised, UV photoproduct-containing oligonucleotides described the detection and isolation of small, pyrimidine dimer-containing oligonucleotides 6- to 7-nt and 3- to 4-nt in length in UV-irradiated human cells (107,108). Oligonucleotides

in this size range are not efficiently retained using the in vivo excision assay (10,53,54) because the ability to precipitate oligonucleotides in ethanol drops significantly with oligonucleotides less than ~16 nt in length (109). Nonetheless, these alternative methods demonstrate the existence of additional intermediates in the post-dual incision processing of excised oligonucleotides that may be useful for characterizing the enzymes that degrade the excised oligonucleotide products of repair. Indeed, such approaches have revealed the existence of an enzyme that appears to hydrolyze the interpyrimidine phosphodiester bond in excised cyclobutane dimers (107,108,110).

Lastly, the ultimate fate of the UV-damaged nucleotides is also an important, unanswered question (12). This issue is particularly important for cyclobutane pyrimidine dimers (CPDs) because the cyclobutane ring between the C5-C5 and C6-C6 bonds is resistant to direct photoreversal by UV wavelengths present in sunlight (>300 nm) and to non-enzymatic degradation by extreme heat or pH. Though *E. coli* photolyase is able to repair CPDs within short trithymidylates in vitro (111,112), it is much less active than on longer oligonucleotides. Furthermore, there are no known mammalian enzymes that are able to metabolize and breakdown CPDs into simpler constituents, and thus the identification of such enzymes is a worthwhile pursuit.

Localization of excised oligonucleotides in the cell—The subcellular localization of the excised DNA oligonucleotide products of repair is an important issue given that the presence of DNA in the cytosol following infection or other cellular pathologies is associated with the induction of innate immune signaling that can contribute to autoimmune disorders (113,114). Early work following the fate of excised, radiolabeled thymidine in UV-irradiated human cells demonstrated that the excision products were retained in cells and not appreciably released from cells into the culture medium (16,115). In vitro studies with defined DNA substrates and purified repair proteins or cell-free extracts showed that more than 90% of the excised oligonucleotides dissociate from immobilized, duplex DNA into the soluble fraction of the reaction following the dual incisions (24,56). Translating these in vitro findings to subcellular localization in vivo is potentially more difficult because biochemical fractionation methods may disrupt the integrity of cellular architecture. Nonetheless, when UV-irradiated cells are lysed under conditions that keep the nucleus and nuclear membrane largely intact and then centrifuged, nearly all of the excised oligonucleotides remain within the nuclear pellet (90). These findings suggest that the primary and partially degraded excised oligonucleotides do not reach the cytosol of the cell under normal cellular conditions. Moreover, though the cGAS-STING (stimulator of interferon genes)-dependent innate immune signaling pathway is robustly activated by cytosolic DNA (114), a recent study did not find evidence for excision repair-dependent activation of this pathway in UV-irradiated cells (116). Nonetheless, it remains formally possible that under specific cellular conditions, excised oligonucleotides may contact and activate innate immune sensors of cytosolic DNA. Thus, the excised oligonucleotide products of repair could, in principle, contribute to the pathological effects of sunlight UV in autoimmune disorders such as lupus.

Though this recent report showed that essentially all of the excised oligonucleotides remain in the nucleus following UV (90), the primary and partially degraded excised

oligonucleotides were found to exhibit different biochemical properties with regards to their extractability from chromatin and nuclear matrix. Though both classes of excision products can be readily solubilized and separated from the bulk chromatin fraction of UV-irradiated cells using an isotonic buffer containing a non-ionic detergent (10,54,90), the two classes show different degrees of extractability with hypotonic buffers. The primary, TFIIH-bound oligonucleotides are largely resistant to extraction under these conditions (90), which suggests that ionic strength impacts TFIIH-excised oligonucleotide solubility. Experiments showing that the primary excised oligonucleotide products of repair are not recoverable from cells that are fixed with formaldehyde prior to cell lysis suggest that the TFIIH-bound excised oligonucleotides may be associated with chromatin (90). In contrast, the partially degraded, RPA-bound excised oligonucleotides are readily solubilized when cells are lysed in a hypotonic buffer containing a non-ionic detergent and can be recovered from formaldehyde-crosslinked cells. Immunoblot analyses of total TFIIH and RPA protein distribution in the same sub-cellular fractions demonstrated that the excised oligonucleotide-bound forms of TFIIH and RPA have unique biochemical properties that are distinct from the total, excised oligonucleotide-free protein. Though the physiological relevance of these biochemical properties remains to be determined, the unique biochemical solubility of the TFIIH- and RPA-excised oligonucleotide complexes allows for the easy, differential isolation of the two excision product species from UV-irradiated cells.

It will also be important to determine whether the full-length or degraded excised oligonucleotide products of nucleotide excision repair are ever released from UV-irradiated cells. Though a modest amount radiolabeled thymidine can be detected in cell culture medium at late time points following exposure of human cells to high doses of UV (16), it is not known whether this apparent release of damage-containing DNA from cells is dependent on repair and part of an active export process or simply a result of cell death. Similarly, ELISA and ^{32}P-postlabeling methods have detected the presence of CPDs in human urine, including after exposure to solar UV radiation (117–121). It will therefore be interesting to determine whether these CPD-containing species are bona fide products of nucleotide excision repair and can be used as a biomarker for DNA repair capacity.

Excised oligonucleotides and cell signaling—Interestingly, recent studies of double-strand break repair and base excision repair suggest that the excised products of DNA repair may activate intracellular signaling pathways. For example, during DNA end resection at double-strand breaks, small exonucleolytically generated oligonucleotides 4- to 12-nt in length associate with the MRN (Mre11-Rad50-Nbs1) nuclease complex to help amplify ATM kinase signaling (122). Similarly, OGG1 (8-oxoguanine glycosylase), which removes 8-oxoguanine residues from DNA during base excision repair, remains in a tight complex with 8-oxoguanine following excision. This protein-nucleotide base complex then serves as a nucleotide exchange factor for the Ras family of signaling proteins to induce various immune and inflammatory responses (123–127). Thus it will be interesting to determine whether the excised, UV photoproduct-containing oligonucleotide products of nucleotide excision repair may similarly serve a signaling function in the cell, either alone or in complex with additional proteins. Indeed, artificial ssDNA oligonucleotides with lengths similar to the excised oligonucleotide products of nucleotide excision repair impart

sequence-specific binding activity to the tumor suppressor and transcriptional regulator p53 (128).

INTERPLAY BETWEEN GAP FILLING AND EXCISED OLIGONUCLEOTIDE PROCESSING

Excision gap filling and excised oligonucleotide processing have thus far been considered as two independent events with different processing steps during nucleotide excision repair. The demonstration that purified human damage recognition and incision factors are sufficient for the dual incision event in vitro (22,23) proved unequivocally that DNA repair synthesis is not necessary for the dual incision event to take place. Nonetheless, whether the gap filling machinery impacts the function and catalytic nature of the six core human excision repair factors has not been thoroughly studied.

Interestingly, there is strong biochemical data showing that the efficiency and turnover of the *E. coli* nucleotide excision repair system is affected by gap filling proteins. In the *E. coli* excision repair system, UV photoproducts are removed from DNA by the *uvr*A, *uvr*B, and *uvr*C gene products, which are necessary and sufficient for the dual incision reaction (129,130). However, genetic studies indicated that addition factors were required for maximal photoproduct removal in *E. coli* in vivo (131–134). In vitro studies showed that both the 12-mer oligonucleotide dual incision product of excision repair and the UvrC nuclease remain bound to the undamaged strand of DNA following the dual incision event and then require the action of the UvrD helicase for displacement (135–138). Similarly, gap filling by DNA Polymerase I is necessary to release UvrB from the post-incision complex (135). Thus, additional post-excision factors are required for the *E. coli* excision repair machinery to function in a catalytic manner. Though the proteins responsible for excision repair are not conserved between bacteria and humans, the general phenomenon that the post-incision gap filling steps of excision repair may affect the catalytic nature of the dual incision machinery has potentially important implications for the human nucleotide excision repair system.

Along these lines, independent methods that have quantified excision repair capacity in UV-irradiated cells in vivo have shown that the maximum number of excision gaps and excised oligonucleotides that are present in UV-irradiated cells are approximately equal at roughly $1–2 \times 10^5$ per cell (53,90,139,140). Thus, neither reaction product accumulates in the cell in vivo relative to the other product, which argues that the post-excision processing of the excised oligonucleotides and excision gaps could be coordinated. Consistent with this notion, the similar kinetics of excision and repair synthesis in cell-free excision repair systems further indicated that the two processes may be coupled (30,141). Moreover, there appear to be structural and functional links between the incision events and gap filling. For example, the presence of a PCNA-interaction motif (PIP box) in XPG (142) has long hinted that XPG may play a role in facilitating the resynthesis step of excision repair. In vitro biochemical studies with recombinant proteins and defined DNA substrates indeed further confirmed this hypothesis (141). Recent data with cultured human cells in vivo also propose that XPG must be ubiquitinated and degraded to allow for gap filling to take place (143).

Interestingly, there is also evidence that repair synthesis can begin before both incision events have taken place. Though the 5' incision by XPF-ERCC1 is required to generate a 3'-hydroxyl for DNA polymerase to act on, in vitro and in vivo experiments with an XPG mutant that lacks nuclease activity but retains other functions in pre-incision complex formation have shown that nucleotides are incorporated into excision gaps when XPG is unable to make the 3' incision (144). Thus, though the dual incision product is a stable, bone fide intermediate of excision repair both in vitro and in vivo, the kinetics of DNA polymerization within the excision gap relative to the two incision events may be complex and influenced by a variety of factors.

Similar to the *E. coli* excision repair system, in vitro approaches with mammalian cell-free systems have indicated that the efficiency of the mammalian nucleotide excision repair system may be affected by gap filling processes. For example, the inclusion of dNTPs in cell-free extract-based in vitro excision reactions, which are necessary for gap filling DNA synthesis to take place, has been shown to lead to the production of a greater number of excised oligonucleotides (9,145). Moreover, an early application of an in vitro assay for monitoring the release of thymine dimers from DNA in the form of oligonucleotides 24- to 32-nt in length discovered PCNA as a factor that promoted nucleotide excision repair (145). Similarly, the addition of the PCNA-interacting protein p21 to in vitro excision reactions, which prevents PCNA from binding to and recruiting DNA polymerases, partially inhibited both the generation of the dual incision product and gap filling DNA synthesis (146). The subsequent purification of excision repair proteins and optimization of in vitro reaction conditions in the mid-1990s proved that the six core excision repair factors (RPA, XPA, TFIIH, XPC, XPF-ERCC1, and XPG) were sufficient to carry out the dual incision reaction (22,23), and thus the role for gap filling in improving the efficiency of the reaction by promoting the turnover of one or more factors was not further considered. However, given the unexpectedly stable and tight binding of excised oligonucleotides to TFIIH (56), it is expected that there are likely one or more factors that facilitate the release of excised oligonucleotides from TFIIH and RPA to promote the recycling of these proteins for new rounds of repair. Thus, it is formally possible that one or more gap filling proteins may impact the stability of the TFIIH-excised oligonucleotide complex.

Furthermore, there is also evidence with UV-irradiated, cultured human cells that gap filling may affect the rate of repair in vivo. It has been recognized for more than 35 years that the treatment of UV-irradiated, non-replicating cells with compounds that block gap filling, such as nucleotide analogs and ribonucleotide reductase and DNA polymerase inhibitors, slow or inhibit the rate of removal of UV photoproducts from the genome (60,61,147–151). However, a limitation of many of these previous studies is that the experimental methods that were employed may not fully or accurately monitor repair. For example, early assays of repair in vivo involved monitoring the release of radiolabeled thymine dimers from the acid-precipitable fraction of cells (14–16). Such assays detect only small oligonucleotides less than approximately 10- to 12-nt in length (109). Thus, the generation of primary and partially degraded excision products 18- to 32-nt in length, which are now known to be bound to TFIIH and RPA, respectively, would not be detected as bona fide repair events with this classical assay for excision repair. Similarly, though fluorescence microscopy is frequently used to monitor the release of UV photoproducts from genomic DNA, fixation

steps may crosslink the TFIIH-excised oligonucleotide products of repair to chromatin and prevent the detection of these repair events (90). Thus, the full-length excised oligonucleotide products of repair are not detected as repair events with either of these repair methodologies, and this issue is potentially problematic when attempting to study how treatments with inhibitors of gap filling affect the post-excision processing of excised oligonucleotides.

To address this issue, a recent analysis used more stringent assays of repair, including immuno-slot blotting with lesion-specific antibodies and the in vivo excision assay (90), to examine how the inhibition of gap filling affects UV photoproduct removal. Consistent with the consensus view that gap filling is important for repair rate, this work found that inhibiting either repair synthesis or ligation slowed the rate of removal of UV photoproducts from genomic DNA (90). Interestingly, this slower rate of removal of damage from genomic DNA was correlated with an accumulation of excised, damage-containing oligonucleotides that remained in complex with RPA in UV-irradiated cells when gap filling processes were inhibited. Interestingly, recent fluorescence microcopy analyses of excision repair protein movement in the nuclei of UV-irradiated cells has similarly suggested that RPA turnover and localization to new damage sites requires gap filling processes (61). These results support a consensus view that gap filling indeed influences the post-excision processing of excised oligonucleotides and the turnover of repair proteins, with a specific effect on RPA. However, given that prolonged inhibition of gap filling in UV-irradiated quiescent cells stimulates an apoptotic form of cell death (90), additional work is necessary to characterize this mechanism further and to rule out any indirect effects of apoptotic or other cell death processes on the various steps of nucleotide excision repair.

CONCLUSIONS

The efficient removal of UV photoproducts from genomic DNA by the nucleotide excision repair system is important to prevent transcription and replication stress, which lead to mutagenesis, genomic instability, and cell death in UV-irradiated cells. The post-excision steps of repair, which include gap filling and excised oligonucleotide processing, are now recognized as playing important roles in repair efficiency and the cellular response to UV. Thus, defects in these post-excision steps of repair may lead to genomic instability and human diseases associated with UV exposure, such as cancer, aging and autoimmunity. Continued investigation of these post-excision steps of repair will therefore provide a comprehensive understanding of the nucleotide excision repair mechanism and may facilitate the development of new strategies and therapies for minimizing UV light-induced human disease.

Acknowledgments

This work was supported by National Institutes of Health grants R01GM32833 and R21ES024425 (to Aziz Sancar).

REFERENCES

1. Reardon JT, Sancar A. Nucleotide excision repair. Prog. Nucleic Acid Res. Mol. Biol. 2005; 79:183–235. [PubMed: 16096029]

2. Cleaver JE, Lam ET, Revet I. Disorders of nucleotide excision repair: the genetic and molecular basis of heterogeneity. Nat. Rev. Genet. 2009; 10:756–768. [PubMed: 19809470]
3. Marteijn JA, Lans H, Vermeulen W, Hoeijmakers JH. Understanding nucleotide excision repair and its roles in cancer and ageing. Nat. Rev. Mol. Cell Biol. 2014; 15:465–481. [PubMed: 24954209]
4. Scharer OD. Nucleotide excision repair in eukaryotes. Cold Spring Harb Perspect. Biol. 2013; 5:a012609. [PubMed: 24086042]
5. Huang JC, Svoboda DL, Reardon JT, Sancar A. Human nucleotide excision nuclease removes thymine dimers from DNA by incising the 22nd phosphodiester bond 5' and the 6th phosphodiester bond 3' to the photodimer. Proc. Natl. Acad. Sci. U. S. A. 1992; 89:3664–3668. [PubMed: 1314396]
6. Edenberg H, Hanawalt P. Size of repair patches in the DNA of ultraviolet-irradiated HeLa cells. Biochim. Biophys. Acta. 1972; 272:361–372. [PubMed: 5049469]
7. Guzder SN, Habraken Y, Sung P, Prakash L, Prakash S. Reconstitution of yeast nucleotide excision repair with purified Rad proteins, replication protein A, and transcription factor TFIIH. J. Biol. Chem. 1995; 270:12973–12976. [PubMed: 7768886]
8. Reardon JT, Thompson LH, Sancar A. Rodent UV-sensitive mutant cell lines in complementation groups 6–10 have normal general excision repair activity. Nucleic Acids Res. 1997; 25:1015–1021. [PubMed: 9023113]
9. Svoboda DL, Taylor JS, Hearst JE, Sancar A. DNA repair by eukaryotic nucleotide excision nuclease. Removal of thymine dimer and psoralen monoadduct by HeLa cell-free extract and of thymine dimer by Xenopus laevis oocytes. J. Biol. Chem. 1993; 268:1931–1936. [PubMed: 8420966]
10. Hu J, Choi JH, Gaddameedhi S, Kemp MG, Reardon JT, Sancar A. Nucleotide excision repair in human cells: fate of the excised oligonucleotide carrying DNA damage in vivo. J. Biol. Chem. 2013; 288:20918–20926. [PubMed: 23749995]
11. Canturk F, Karaman M, Selby CP, Kemp MG, Kulaksiz-Erkmen G, Hu J, Li W, Lindsey-Boltz LA, Sancar A. Nucleotide excision repair by dual incisions in plants. Proc. Natl. Acad. Sci. U. S. A. 2016; 113:4706–4710. [PubMed: 27071131]
12. Kemp MG, Sancar A. DNA excision repair: where do all the dimers go? Cell. Cycle. 2012; 11:2997–3002. [PubMed: 22825251]
13. Novarina D, Amara F, Lazzaro F, Plevani P, Muzi-Falconi M. Mind the gap: keeping UV lesions in check. DNA Repair (Amst). 2011; 10:751–759. [PubMed: 21602108]
14. Boyce RP, Howard-Flanders P. Release of Ultraviolet Light-Induced Thymine Dimers from Dna in E. Coli K-12. Proc. Natl. Acad. Sci. U. S. A. 1964; 51:293–300. [PubMed: 14124327]
15. Setlow RB, Carrier WL. The Disappearance of Thymine Dimers from Dna: an Error-Correcting Mechanism. Proc. Natl. Acad. Sci. U. S. A. 1964; 51:226–231. [PubMed: 14124320]
16. Regan JD, Trosko JE, Carrier WL. Evidence for excision of ultraviolet-induced pyrimidine dimers from the DNA of human cells in vitro. Biophys. J. 1968; 8:319–325. [PubMed: 5759918]
17. Rasmussen RE, Painter RB. Evidence for Repair of Ultra-Violet Damaged Deoxyribonucleic Acid in Cultured Mammalian Cells. Nature. 1964; 203:1360–1362. [PubMed: 14207310]
18. Pettijohn D, Hanawalt P. Evidence for Repair-Replication of Ultraviolet Damaged Dna in Bacteria. J. Mol. Biol. 1964; 9:395–410. [PubMed: 14202275]
19. Cleaver JE. Defective repair replication of DNA in xeroderma pigmentosum. Nature. 1968; 218:652–656. [PubMed: 5655953]
20. Setlow RB, Regan JD, German J, Carrier WL. Evidence that xeroderma pigmentosum cells do not perform the first step in the repair of ultraviolet damage to their DNA. Proc. Natl. Acad. Sci. U. S. A. 1969; 64:1035–1041. [PubMed: 5264135]
21. Aboussekhra A, Biggerstaff M, Shivji MK, Vilpo JA, Moncollin V, Podust VN, Protic M, Hubscher U, Egly JM, Wood RD. Mammalian DNA nucleotide excision repair reconstituted with purified protein components. Cell. 1995; 80:859–868. [PubMed: 7697716]
22. Mu D, Park CH, Matsunaga T, Hsu DS, Reardon JT, Sancar A. Reconstitution of human DNA repair excision nuclease in a highly defined system. J. Biol. Chem. 1995; 270:2415–2418. [PubMed: 7852297]

23. Evans E, Moggs JG, Hwang JR, Egly JM, Wood RD. Mechanism of open complex and dual incision formation by human nucleotide excision repair factors. EMBO J. 1997; 16:6559–6573. [PubMed: 9351836]
24. Mu D, Hsu DS, Sancar A. Reaction mechanism of human DNA repair excision nuclease. J. Biol. Chem. 1996; 271:8285–8294. [PubMed: 8626523]
25. Mu D, Wakasugi M, Hsu DS, Sancar A. Characterization of reaction intermediates of human excision repair nuclease. J. Biol. Chem. 1997; 272:28971–28979. [PubMed: 9360969]
26. Araujo SJ, Tirode F, Coin F, Pospiech H, Syvaoja JE, Stucki M, Hubscher U, Egly JM, Wood RD. Nucleotide excision repair of DNA with recombinant human proteins: definition of the minimal set of factors, active forms of TFIIH, and modulation by CAK. Genes Dev. 2000; 14:349–359. [PubMed: 10673506]
27. Reardon JT, Sancar A. Recognition and repair of the cyclobutane thymine dimer, a major cause of skin cancers, by the human excision nuclease. Genes Dev. 2003; 17:2539–2551. [PubMed: 14522951]
28. Sugasawa K, Okamoto T, Shimizu Y, Masutani C, Iwai S, Hanaoka F. A multistep damage recognition mechanism for global genomic nucleotide excision repair. Genes Dev. 2001; 15:507–521. [PubMed: 11238373]
29. Volker M, Mone MJ, Karmakar P, van Hoffen A, Schul W, Vermeulen W, Hoeijmakers JH, van Driel R, van Zeeland AA, Mullenders LH. Sequential assembly of the nucleotide excision repair factors in vivo. Mol. Cell. 2001; 8:213–224. [PubMed: 11511374]
30. Riedl T, Hanaoka F, Egly JM. The comings and goings of nucleotide excision repair factors on damaged DNA. EMBO J. 2003; 22:5293–5303. [PubMed: 14517266]
31. Li CL, Golebiowski FM, Onishi Y, Samara NL, Sugasawa K, Yang W. Tripartite DNA Lesion Recognition and Verification by XPC, TFIIH, and XPA in Nucleotide Excision Repair. Mol. Cell. 2015; 59:1025–1034. [PubMed: 26384665]
32. Reardon JT, Sancar A. Thermodynamic cooperativity and kinetic proofreading in DNA damage recognition and repair. Cell. Cycle. 2004; 3:141–144. [PubMed: 14712076]
33. Sugasawa K. Molecular mechanisms of DNA damage recognition for mammalian nucleotide excision repair. DNA Repair (Amst). 2016; 44:110–117. [PubMed: 27264556]
34. Fuss JO, Tainer JA. XPB and XPD helicases in TFIIH orchestrate DNA duplex opening and damage verification to coordinate repair with transcription and cell cycle via CAK kinase. DNA Repair (Amst). 2011; 10:697–713. [PubMed: 21571596]
35. Compe E, Egly JM. TFIIH: when transcription met DNA repair. Nat. Rev. Mol. Cell Biol. 2012; 13:343–354. [PubMed: 22572993]
36. Wakasugi M, Sancar A. Order of assembly of human DNA repair excision nuclease. J. Biol. Chem. 1999; 274:18759–18768. [PubMed: 10373492]
37. Bohr VA, Smith CA, Okumoto DS, Hanawalt PC. DNA repair in an active gene: removal of pyrimidine dimers from the DHFR gene of CHO cells is much more efficient than in the genome overall. Cell. 1985; 40:359–369. [PubMed: 3838150]
38. Mellon I, Bohr VA, Smith CA, Hanawalt PC. Preferential DNA repair of an active gene in human cells. Proc. Natl. Acad. Sci. U. S. A. 1986; 83:8878–8882. [PubMed: 3466163]
39. Mellon I, Spivak G, Hanawalt PC. Selective removal of transcription-blocking DNA damage from the transcribed strand of the mammalian DHFR gene. Cell. 1987; 51:241–249. [PubMed: 3664636]
40. Tornaletti S, Hanawalt PC. Effect of DNA lesions on transcription elongation. Biochimie. 1999; 81:139–146. [PubMed: 10214918]
41. Selby CP, Drapkin R, Reinberg D, Sancar A. RNA polymerase II stalled at a thymine dimer: footprint and effect on excision repair. Nucleic Acids Res. 1997; 25:787–793. [PubMed: 9016630]
42. Donahue BA, Fuchs RP, Reines D, Hanawalt PC. Effects of aminofluorene and acetylaminofluorene DNA adducts on transcriptional elongation by RNA polymerase II. J. Biol. Chem. 1996; 271:10588–10594. [PubMed: 8631860]
43. Donahue BA, Yin S, Taylor JS, Reines D, Hanawalt PC. Transcript cleavage by RNA polymerase II arrested by a cyclobutane pyrimidine dimer in the DNA template. Proc. Natl. Acad. Sci. U. S. A. 1994; 91:8502–8506. [PubMed: 8078911]

44. Hanawalt PC, Spivak G. Transcription-coupled DNA repair: two decades of progress and surprises. Nat. Rev. Mol. Cell Biol. 2008; 9:958–970. [PubMed: 19023283]
45. Troelstra C, van Gool A, de Wit J, Vermeulen W, Bootsma D, Hoeijmakers JH. ERCC6, a member of a subfamily of putative helicases, is involved in Cockayne's syndrome and preferential repair of active genes. Cell. 1992; 71:939–953. [PubMed: 1339317]
46. van Hoffen A, Natarajan AT, Mayne LV, van Zeeland AA, Mullenders LH, Venema J. Deficient repair of the transcribed strand of active genes in Cockayne's syndrome cells. Nucleic Acids Res. 1993; 21:5890–5895. [PubMed: 8290349]
47. Venema J, Mullenders LH, Natarajan AT, van Zeeland AA, Mayne LV. The genetic defect in Cockayne syndrome is associated with a defect in repair of UV-induced DNA damage in transcriptionally active DNA. Proc. Natl. Acad. Sci. U. S. A. 1990; 87:4707–4711. [PubMed: 2352945]
48. Selby CP, Sancar A. Cockayne syndrome group B protein enhances elongation by RNA polymerase II. Proc. Natl. Acad. Sci. U. S. A. 1997; 94:11205–11209. [PubMed: 9326587]
49. Selby CP, Sancar A. Human transcription-repair coupling factor CSB/ERCC6 is a DNA-stimulated ATPase but is not a helicase and does not disrupt the ternary transcription complex of stalled RNA polymerase II. J. Biol. Chem. 1997; 272:1885–1890. [PubMed: 8999876]
50. Huang JC, Sancar A. Determination of minimum substrate size for human excinuclease. J. Biol. Chem. 1994; 269:19034–19040. [PubMed: 8034661]
51. Hu J, Adar S, Selby CP, Lieb JD, Sancar A. Genome-wide analysis of human global and transcription-coupled excision repair of UV damage at single-nucleotide resolution. Genes Dev. 2015; 29:948–960. [PubMed: 25934506]
52. Adar S, Hu J, Lieb JD, Sancar A. Genome-wide kinetics of DNA excision repair in relation to chromatin state and mutagenesis. Proc. Natl. Acad. Sci. U. S. A. 2016; 113:E2124–E2133. [PubMed: 27036006]
53. Choi JH, Gaddameedhi S, Kim SY, Hu J, Kemp MG, Sancar A. Highly specific and sensitive method for measuring nucleotide excision repair kinetics of ultraviolet photoproducts in human cells. Nucleic Acids Res. 2014; 42:e29. [PubMed: 24271390]
54. Choi JH, Kim SY, Kim SK, Kemp MG, Sancar A. An Integrated Approach for Analysis of the DNA Damage Response in Mammalian Cells: Nucleotide Excision Repair, DNA Damage Checkpoint, and Apoptosis. J. Biol. Chem. 2015; 290:28812–28821. [PubMed: 26438822]
55. Bowman KK, Smith CA, Hanawalt PC. Excision-repair patch lengths are similar for transcription-coupled repair and global genome repair in UV-irradiated human cells. Mutat. Res. 1997; 385:95–105. [PubMed: 9447231]
56. Kemp MG, Reardon JT, Lindsey-Boltz LA, Sancar A. Mechanism of release and fate of excised oligonucleotides during nucleotide excision repair. J. Biol. Chem. 2012; 287:22889–22899. [PubMed: 22573372]
57. Nishida C, Reinhard P, Linn S. DNA repair synthesis in human fibroblasts requires DNA polymerase delta. J. Biol. Chem. 1988; 263:501–510. [PubMed: 3335506]
58. Shivji MK, Podust VN, Hubscher U, Wood RD. Nucleotide excision repair DNA synthesis by DNA polymerase epsilon in the presence of PCNA, RFC, and RPA. Biochemistry. 1995; 34:5011–5017. [PubMed: 7711023]
59. Ogi T, Lehmann AR. The Y-family DNA polymerase kappa (pol kappa) functions in mammalian nucleotide-excision repair. Nat. Cell Biol. 2006; 8:640–642. [PubMed: 16738703]
60. Moser J, Kool H, Giakzidis I, Caldecott K, Mullenders LH, Fousteri MI. Sealing of chromosomal DNA nicks during nucleotide excision repair requires XRCC1 and DNA ligase III alpha in a cell-cycle-specific manner. Mol. Cell. 2007; 27:311–323. [PubMed: 17643379]
61. Overmeer RM, Moser J, Volker M, Kool H, Tomkinson AE, van Zeeland AA, Mullenders LH, Fousteri M. Replication protein A safeguards genome integrity by controlling NER incision events. J. Cell Biol. 2011; 192:401–415. [PubMed: 21282463]
62. Ogi T, Limsirichaikul S, Overmeer RM, Volker M, Takenaka K, Cloney R, Nakazawa Y, Niimi A, Miki Y, Jaspers NG, Mullenders LH, Yamashita S, Fousteri MI, Lehmann AR. Three DNA polymerases, recruited by different mechanisms, carry out NER repair synthesis in human cells. Mol. Cell. 2010; 37:714–727. [PubMed: 20227374]

63. Overmeer RM, Gourdin AM, Giglia-Mari A, Kool H, Houtsmuller AB, Siegal G, Fousteri MI, Mullenders LH, Vermeulen W. Replication factor C recruits DNA polymerase delta to sites of nucleotide excision repair but is not required for PCNA recruitment. Mol. Cell. Biol. 2010; 30:4828–4839. [PubMed: 20713449]
64. Teo IA, Broughton BC, Day RS, James MR, Karran P, Mayne LV, Lehmann AR. A biochemical defect in the repair of alkylated DNA in cells from an immunodeficient patient (46BR). Carcinogenesis. 1983; 4:559–564. [PubMed: 6850987]
65. Bentley DJ, Harrison C, Ketchen AM, Redhead NJ, Samuel K, Waterfall M, Ansell JD, Melton DW. DNA ligase I null mouse cells show normal DNA repair activity but altered DNA replication and reduced genome stability. J. Cell. Sci. 2002; 115:1551–1561. [PubMed: 11896201]
66. Giannattasio M, Follonier C, Tourriere H, Puddu F, Lazzaro F, Pasero P, Lopes M, Plevani P, Muzi-Falconi M. Exo1 competes with repair synthesis, converts NER intermediates to long ssDNA gaps, and promotes checkpoint activation. Mol. Cell. 2010; 40:50–62. [PubMed: 20932474]
67. Sertic S, Pizzi S, Cloney R, Lehmann AR, Marini F, Plevani P, Muzi-Falconi M. Human exonuclease 1 connects nucleotide excision repair (NER) processing with checkpoint activation in response to UV irradiation. Proc. Natl. Acad. Sci. U. S. A. 2011; 108:13647–13652. [PubMed: 21808022]
68. Lindsey-Boltz LA, Kemp MG, Reardon JT, DeRocco V, Iyer RR, Modrich P, Sancar A. Coupling of human DNA excision repair and the DNA damage checkpoint in a defined in vitro system. J. Biol. Chem. 2014; 289:5074–5082. [PubMed: 24403078]
69. Cimprich KA, Cortez D. ATR: an essential regulator of genome integrity. Nat. Rev. Mol. Cell Biol. 2008; 9:616–627. [PubMed: 18594563]
70. Sancar A, Lindsey-Boltz LA, Unsal-Kacmaz K, Linn S. Molecular mechanisms of mammalian DNA repair and the DNA damage checkpoints. Annu. Rev. Biochem. 2004; 73:39–85. [PubMed: 15189136]
71. Byun TS, Pacek M, Yee MC, Walter JC, Cimprich KA. Functional uncoupling of MCM helicase and DNA polymerase activities activates the ATR-dependent checkpoint. Genes Dev. 2005; 19:1040–1052. [PubMed: 15833913]
72. Lupardus PJ, Byun T, Yee MC, Hekmat-Nejad M, Cimprich KA. A requirement for replication in activation of the ATR-dependent DNA damage checkpoint. Genes Dev. 2002; 16:2327–2332. [PubMed: 12231621]
73. Zou L, Elledge SJ. Sensing DNA damage through ATRIP recognition of RPA-ssDNA complexes. Science. 2003; 300:1542–1548. [PubMed: 12791985]
74. Cortez D, Guntuku S, Qin J, Elledge SJ. ATR and ATRIP: partners in checkpoint signaling. Science. 2001; 294:1713–1716. [PubMed: 11721054]
75. Nam EA, Cortez D. ATR signalling: more than meeting at the fork. Biochem. J. 2011; 436:527–536. [PubMed: 21615334]
76. Choi JH, Lindsey-Boltz LA, Sancar A. Cooperative activation of the ATR checkpoint kinase by TopBP1 and damaged DNA. Nucleic Acids Res. 2009; 37:1501–1509. [PubMed: 19139065]
77. Choi JH, Lindsey-Boltz LA, Sancar A. Reconstitution of a human ATR-mediated checkpoint response to damaged DNA. Proc. Natl. Acad. Sci. U. S. A. 2007; 104:13301–13306. [PubMed: 17686975]
78. Unsal-Kacmaz K, Makhov AM, Griffith JD, Sancar A. Preferential binding of ATR protein to UV-damaged DNA. Proc. Natl. Acad. Sci. U. S. A. 2002; 99:6673–6678. [PubMed: 12011431]
79. Hanasoge S, Ljungman M. H2AX phosphorylation after UV irradiation is triggered by DNA repair intermediates and is mediated by the ATR kinase. Carcinogenesis. 2007; 28:2298–2304. [PubMed: 17615256]
80. Marini F, Nardo T, Giannattasio M, Minuzzo M, Stefanini M, Plevani P, Falconi MM. DNA nucleotide excision repair-dependent signaling to checkpoint activation. Proc. Natl. Acad. Sci. U. S. A. 2006; 103:17325–17330. [PubMed: 17088560]
81. Matsumoto M, Yaginuma K, Igarashi A, Imura M, Hasegawa M, Iwabuchi K, Date T, Mori T, Ishizaki K, Yamashita K, Inobe M, Matsunaga T. Perturbed gap-filling synthesis in nucleotide excision repair causes histone H2AX phosphorylation in human quiescent cells. J. Cell. Sci. 2007; 120:1104–1112. [PubMed: 17327276]

82. Marti TM, Hefner E, Feeney L, Natale V, Cleaver JE. H2AX phosphorylation within the G1 phase after UV irradiation depends on nucleotide excision repair and not DNA double-strand breaks. Proc. Natl. Acad. Sci. U. S. A. 2006; 103:9891–9896. [PubMed: 16788066]

83. Vrouwe MG, Pines A, Overmeer RM, Hanada K, Mullenders LH. UV-induced photolesions elicit ATR-kinase-dependent signaling in non-cycling cells through nucleotide excision repair-dependent and -independent pathways. J. Cell. Sci. 2011; 124:435–446. [PubMed: 21224401]

84. Kemp MG, Sancar A. ATR Kinase Inhibition Protects Non-cycling Cells from the Lethal Effects of DNA Damage and Transcription Stress. J. Biol. Chem. 2016; 291:9330–9342. [PubMed: 26940878]

85. Derheimer FA, O'Hagan HM, Krueger HM, Hanasoge S, Paulsen MT, Ljungman M. RPA and ATR link transcriptional stress to p53. Proc. Natl. Acad. Sci. U. S. A. 2007; 104:12778–12783. [PubMed: 17616578]

86. Wakasugi M, Sasaki T, Matsumoto M, Nagaoka M, Inoue K, Inobe M, Horibata K, Tanaka K, Matsunaga T. Nucleotide excision repair-dependent DNA double-strand break formation and ATM signaling activation in mammalian quiescent cells. J. Biol. Chem. 2014; 289:28730–28737. [PubMed: 25164823]

87. Bakkenist CJ, Kastan MB. Initiating cellular stress responses. Cell. 2004; 118:9–17. [PubMed: 15242640]

88. Kitagawa R, Kastan MB. The ATM-dependent DNA damage signaling pathway. Cold Spring Harb. Symp. Quant. Biol. 2005; 70:99–109. [PubMed: 16869743]

89. Jette N, Lees-Miller SP. The DNA-dependent protein kinase: A multifunctional protein kinase with roles in DNA double strand break repair and mitosis. Prog. Biophys. Mol. Biol. 2015; 117:194–205. [PubMed: 25550082]

90. Kemp MG, Gaddameedhi S, Choi JH, Hu J, Sancar A. DNA repair synthesis and ligation affect the processing of excised oligonucleotides generated by human nucleotide excision repair. J. Biol. Chem. 2014; 289:26574–26583. [PubMed: 25107903]

91. Reardon JT, Sancar A. Molecular anatomy of the human excision nuclease assembled at sites of DNA damage. Mol. Cell. Biol. 2002; 22:5938–5945. [PubMed: 12138203]

92. Winkler GS, Araujo SJ, Fiedler U, Vermeulen W, Coin F, Egly JM, Hoeijmakers JH, Wood RD, Timmers HT, Weeda G. TFIIH with inactive XPD helicase functions in transcription initiation but is defective in DNA repair. J. Biol. Chem. 2000; 275:4258–4266. [PubMed: 10660593]

93. Coin F, Bergmann E, Tremeau-Bravard A, Egly JM. Mutations in XPB and XPD helicases found in xeroderma pigmentosum patients impair the transcription function of TFIIH. EMBO J. 1999; 18:1357–1366. [PubMed: 10064601]

94. Naegeli H, Bardwell L, Friedberg EC. Inhibition of Rad3 DNA helicase activity by DNA adducts and abasic sites: implications for the role of a DNA helicase in damage-specific incision of DNA. Biochemistry. 1993; 32:613–621. [PubMed: 8380702]

95. Naegeli H, Bardwell L, Friedberg EC. The DNA helicase and adenosine triphosphatase activities of yeast Rad3 protein are inhibited by DNA damage. A potential mechanism for damage-specific recognition. J. Biol. Chem. 1992; 267:392–398. [PubMed: 1309743]

96. Mathieu N, Kaczmarek N, Naegeli H. Strand- and site-specific DNA lesion demarcation by the xeroderma pigmentosum group D helicase. Proc. Natl. Acad. Sci. U. S. A. 2010; 107:17545–17550. [PubMed: 20876134]

97. Mathieu N, Kaczmarek N, Ruthemann P, Luch A, Naegeli H. DNA quality control by a lesion sensor pocket of the xeroderma pigmentosum group D helicase subunit of TFIIH. Curr. Biol. 2013; 23:204–212. [PubMed: 23352696]

98. Kuper J, Wolski SC, Michels G, Kisker C. Functional and structural studies of the nucleotide excision repair helicase XPD suggest a polarity for DNA translocation. EMBO J. 2012; 31:494–502. [PubMed: 22081108]

99. Pugh RA, Wu CG, Spies M. Regulation of translocation polarity by helicase domain 1 in SF2B helicases. EMBO J. 2012; 31:503–514. [PubMed: 22081110]

100. Toledo LI, Altmeyer M, Rask MB, Lukas C, Larsen DH, Povlsen LK, Bekker-Jensen S, Mailand N, Bartek J, Lukas J. ATR prohibits replication catastrophe by preventing global exhaustion of RPA. Cell. 2013; 155:1088–1103. [PubMed: 24267891]

101. Fanning E, Klimovich V, Nager AR. A dynamic model for replication protein A (RPA) function in DNA processing pathways. Nucleic Acids Res. 2006; 34:4126–4137. [PubMed: 16935876]
102. Wold MS. Replication protein A: a heterotrimeric, single-stranded DNA-binding protein required for eukaryotic DNA metabolism. Annu. Rev. Biochem. 1997; 66:61–92. [PubMed: 9242902]
103. Parra D, Manils J, Castellana B, Vina-Vilaseca A, Moran-Salvador E, Vazquez-Villoldo N, Tarancon G, Borras M, Sancho S, Benito C, Ortega S, Soler C. Increased Susceptibility to Skin Carcinogenesis in TREX2 Knockout Mice. Cancer Res. 2009; 69:6676–6684. [PubMed: 19654293]
104. Manils J, Gomez D, Salla-Martret M, Fischer H, Fye JM, Marzo E, Marruecos L, Serrano I, Salgado R, Rodrigo JP, Garcia-Pedrero JM, Serafin AM, Canas X, Benito C, Toll A, Forcales SV, Perrino FW, Eckhart L, Soler C. Multifaceted role of TREX2 in the skin defense against UV-induced skin carcinogenesis. Oncotarget. 2015; 6:22375–22396. [PubMed: 26090614]
105. Jarrett SG, Novak M, Dabernat S, Daniel JY, Mellon I, Zhang Q, Harris N, Ciesielski MJ, Fenstermaker RA, Kovacic D, Slominski A, Kaetzel DM. Metastasis suppressor NM23-H1 promotes repair of UV-induced DNA damage and suppresses UV-induced melanomagenesis. Cancer Res. 2012; 72:133–143. [PubMed: 22080566]
106. Jarrett SG, Novak M, Harris N, Merlino G, Slominski A, Kaetzel DM. NM23 deficiency promotes metastasis in a UV radiation-induced mouse model of human melanoma. Clin. Exp. Metastasis. 2013; 30:25–36. [PubMed: 22699362]
107. Weinfeld M, Gentner NE, Johnson LD, Paterson MC. Photoreversal-dependent release of thymidine and thymidine monophosphate from pyrimidine dimer-containing DNA excision fragments isolated from ultraviolet-damaged human fibroblasts. Biochemistry. 1986; 25:2656–2664. [PubMed: 3521740]
108. Galloway AM, Liuzzi M, Paterson MC. Metabolic processing of cyclobutyl pyrimidine dimers and (6–4) photoproducts in UV-treated human cells. Evidence for distinct excision-repair pathways. J. Biol. Chem. 1994; 269:974–980. [PubMed: 8288650]
109. Cleaver JE, Boyer HW. Solubility and dialysis limits of DNA oligonucleotides. Biochim. Biophys. Acta. 1972; 262:116–124. [PubMed: 4336092]
110. Famulski KS, Liuzzi M, Bashir S, Mirzayans R, Paterson MC. Purification and characterization of a novel human acidic nuclease/intra-cyclobutyl-pyrimidine-dimer-DNA phosphodiesterase. Biochem. J. 2000; 345(Pt 3):583–593. [PubMed: 10642517]
111. Jorns MS, Sancar GB, Sancar A. Identification of oligothymidylates as new simple substrates for Escherichia coli DNA photolyase and their use in a rapid spectrophotometric enzyme assay. Biochemistry. 1985; 24:1856–1861. [PubMed: 3893539]
112. Liuzzi M, Weinfeld M, Paterson MC. Enzymatic analysis of isomeric trithymidylates containing ultraviolet light-induced cyclobutane pyrimidine dimers. I. Nuclease P1-mediated hydrolysis of the intradimer phosphodiester linkage. J. Biol. Chem. 1989; 264:6355–6363. [PubMed: 2539379]
113. O'Neill LA. Immunology. Sensing the dark side of DNA. Science. 2013; 339:763–764. [PubMed: 23413341]
114. Cai X, Chiu YH, Chen ZJ. The cGAS-cGAMP-STING pathway of cytosolic DNA sensing and signaling. Mol. Cell. 2014; 54:289–296. [PubMed: 24766893]
115. La Belle M, Linn S. In vivo excision of pyrimidine dimers is mediated by a DNA N-glycosylase in Micrococcus luteus but not in human fibroblasts. Photochem. Photobiol. 1982; 36:319–324. [PubMed: 7146108]
116. Kemp MG, Lindsey-Boltz LA, Sancar A. UV Light Potentiates STING (Stimulator of Interferon Genes)-dependent Innate Immune Signaling through Deregulation of ULK1 (Unc51-like Kinase 1). J. Biol. Chem. 2015; 290:12184–12194. [PubMed: 25792739]
117. Ahmad J, Cooke MS, Hussieni A, Evans MD, Patel K, Burd RM, Bleiker TO, Hutchinson PE, Lunec J. Urinary thymine dimers and 8-oxo-2'-deoxyguanosine in psoriasis. FEBS Lett. 1999; 460:549–553. [PubMed: 10556533]
118. Cooke MS, Evans MD, Burd RM, Patel K, Barnard A, Lunec J, Hutchinson PE. Induction and excretion of ultraviolet-induced 8-oxo-2'-deoxyguanosine and thymine dimers in vivo: implications for PUVA. J. Invest. Dermatol. 2001; 116:281–285. [PubMed: 11180005]

119. Kotova N, Hemminki K, Segerback D. Urinary thymidine dimer as a marker of total body burden of UV-inflicted DNA damage in humans. Cancer Epidemiol. Biomarkers Prev. 2005; 14:2868–2872. [PubMed: 16365002]
120. Le Curieux F, Hemminki K. Cyclobutane thymidine dimers are present in human urine following sun exposure: quantitation using 32P–postlabeling and high-performance liquid chromatography. J. Invest. Dermatol. 2001; 117:263–268. [PubMed: 11511303]
121. Liljendahl TS, Kotova N, Segerback D. Quantification of ultraviolet radiation-induced DNA damage in the urine of Swedish adults and children following exposure to sunlight. Biomarkers. 2012; 17:634–641. [PubMed: 22889379]
122. Jazayeri A, Balestrini A, Garner E, Haber JE, Costanzo V. Mre11-Rad50-Nbs1-dependent processing of DNA breaks generates oligonucleotides that stimulate ATM activity. EMBO J. 2008; 27:1953–1962. [PubMed: 18596698]
123. Boldogh I, Hajas G, Aguilera-Aguirre L, Hegde ML, Radak Z, Bacsi A, Sur S, Hazra TK, Mitra S. Activation of ras signaling pathway by 8-oxoguanine DNA glycosylase bound to its excision product, 8-oxoguanine. J. Biol. Chem. 2012; 287:20769–20773. [PubMed: 22568941]
124. German P, Szaniszlo P, Hajas G, Radak Z, Bacsi A, Hazra TK, Hegde ML, Ba X, Boldogh I. Activation of cellular signaling by 8-oxoguanine DNA glycosylase-1-initiated DNA base excision repair. DNA Repair (Amst). 2013; 12:856–863. [PubMed: 23890570]
125. Hajas G, Bacsi A, Aguilera-Aguirre L, Hegde ML, Tapas KH, Sur S, Radak Z, Ba X, Boldogh I. 8-Oxoguanine DNA glycosylase-1 links DNA repair to cellular signaling via the activation of the small GTPase Rac1. Free Radic. Biol. Med. 2013; 61C:384–394.
126. Aguilera-Aguirre L, Bacsi A, Radak Z, Hazra TK, Mitra S, Sur S, Brasier AR, Ba X, Boldogh I. Innate inflammation induced by the 8-oxoguanine DNA glycosylase-1-KRAS-NF-kappaB pathway. J. Immunol. 2014; 193:4643–4653. [PubMed: 25267977]
127. Ba X, Aguilera-Aguirre L, Rashid QT, Bacsi A, Radak Z, Sur S, Hosoki K, Hegde ML, Boldogh I. The role of 8-oxoguanine DNA glycosylase-1 in inflammation. Int. J. Mol. Sci. 2014; 15:16975–16997. [PubMed: 25250913]
128. Jayaraman J, Prives C. Activation of p53 sequence-specific DNA binding by short single strands of DNA requires the p53 C-terminus. Cell. 1995; 81:1021–1029. [PubMed: 7600571]
129. Sancar A, Rupp WD. A novel repair enzyme: UVRABC excision nuclease of Escherichia coli cuts a DNA strand on both sides of the damaged region. Cell. 1983; 33:249–260. [PubMed: 6380755]
130. Lin JJ, Sancar A. (A)BC excinuclease: the Escherichia coli nucleotide excision repair enzyme. Mol. Microbiol. 1992; 6:2219–2224. [PubMed: 1406264]
131. Youngs DA, Smith KC. Evidence for the control by exrA and polA genes of two branches of the uvr gene-dependent excision repair pathway in Escherichia coli K-12. J. Bacteriol. 1973; 116:175–182. [PubMed: 4583206]
132. Van Sluis CA, Mattern IE, Paterson MC. Properties of uvrE mutants of Escherichia coli K12. I. Effects of UV irradiation on DNA metabolism. Mutat. Res. 1974; 25:273–279. [PubMed: 4612354]
133. Tang MS, Smith KC. The effects of lexA101, recB21, recF143 and uvrD3 mutations on liquid-holding recovery in ultraviolet-irradiated Escherichia coli K12 recA56. Mutat. Res. 1981; 80:15–25. [PubMed: 7010131]
134. Kuemmerle NB, Masker WE. Effect of the uvrD mutation on excision repair. J. Bacteriol. 1980; 142:535–546. [PubMed: 6991479]
135. Orren DK, Selby CP, Hearst JE, Sancar A. Post-incision steps of nucleotide excision repair in Escherichia coli. Disassembly of the UvrBC-DNA complex by helicase II and DNA polymerase I. J. Biol. Chem. 1992; 267:780–788. [PubMed: 1530937]
136. Husain I, Van Houten B, Thomas DC, Abdel-Monem M, Sancar A. Effect of DNA polymerase I and DNA helicase II on the turnover rate of UvrABC excision nuclease. Proc. Natl. Acad. Sci. U. S. A. 1985; 82:6774–6778. [PubMed: 2931721]
137. Van Houten B, Gamper H, Hearst JE, Sancar A. Analysis of sequential steps of nucleotide excision repair in Escherichia coli using synthetic substrates containing single psoralen adducts. J. Biol. Chem. 1988; 263:16553–16560. [PubMed: 3053693]

138. Caron PR, Kushner SR, Grossman L. Involvement of helicase II (uvrD gene product) and DNA polymerase I in excision mediated by the uvrABC protein complex. Proc. Natl. Acad. Sci. U. S. A. 1985; 82:4925–4929. [PubMed: 3161077]

139. Snyder RD, Carrier WL, Regan JD. Application of arabinofuranosyl cytosine in the kinetic analysis and quantitation of DNA repair in human cells after ultraviolet irradiation. Biophys. J. 1981; 35:339–350. [PubMed: 7272442]

140. Erixon K, Ahnstrom G. Single-strand breaks in DNA during repair of UV-induced damage in normal human and xeroderma pigmentosum cells as determined by alkaline DNA unwinding and hydroxylapatite chromatography: effects of hydroxyurea, 5-fluorodeoxyuridine and 1-beta-D-arabinofuranosylcytosine on the kinetics of repair. Mutat. Res. 1979; 59:257–271. [PubMed: 35744]

141. Mocquet V, Laine JP, Riedl T, Yajin Z, Lee MY, Egly JM. Sequential recruitment of the repair factors during NER: the role of XPG in initiating the resynthesis step. EMBO J. 2008; 27:155–167. [PubMed: 18079701]

142. Gary R, Ludwig DL, Cornelius HL, MacInnes MA, Park MS. The DNA repair endonuclease XPG binds to proliferating cell nuclear antigen (PCNA) and shares sequence elements with the PCNA-binding regions of FEN-1 and cyclin-dependent kinase inhibitor p21. J. Biol. Chem. 1997; 272:24522–24529. [PubMed: 9305916]

143. Han C, Wani G, Zhao R, Qian J, Sharma N, He J, Zhu Q, Wang QE, Wani AA. Cdt2-mediated XPG degradation promotes gap-filling DNA synthesis in nucleotide excision repair. Cell. Cycle. 2015; 14:1103–1115. [PubMed: 25483071]

144. Staresincic L, Fagbemi AF, Enzlin JH, Gourdin AM, Wijgers N, Dunand-Sauthier I, Giglia-Mari G, Clarkson SG, Vermeulen W, Scharer OD. Coordination of dual incision and repair synthesis in human nucleotide excision repair. EMBO J. 2009; 28:1111–1120. [PubMed: 19279666]

145. Nichols AF, Sancar A. Purification of PCNA as a nucleotide excision repair protein. Nucleic Acids Res. 1992; 20:2441–2446. [PubMed: 1352873]

146. Pan ZQ, Reardon JT, Li L, Flores-Rozas H, Legerski R, Sancar A, Hurwitz J. Inhibition of nucleotide excision repair by the cyclin-dependent kinase inhibitor p21. J. Biol. Chem. 1995; 270:22008–22016. [PubMed: 7665622]

147. Dunn WC, Regan JD. Inhibition of DNA excision repair in human cells by arabinofuranosyl cytosine: effect on normal and xeroderma pigmentosum cells. Mol. Pharmacol. 1979; 15:367–374. [PubMed: 470933]

148. Snyder RD, Regan JD. Aphidicolin inhibits repair of DNA in UV-irradiated human fibroblasts. Biochem. Biophys. Res. Commun. 1981; 99:1088–1094. [PubMed: 6789826]

149. Snyder RD. Consequences of the depletion of cellular deoxynucleoside triphosphate pools on the excision-repair process in cultured human fibroblasts. Mutat. Res. 1988; 200:193–199. [PubMed: 3292906]

150. Snyder RD, Regan JD. Differential responses of log and stationary phase human fibroblasts to inhibition of DNA repair by aphidicolin. Biochim. Biophys. Acta. 1982; 697:229–234. [PubMed: 6809050]

151. Downes CS, Collins AR. Effects of DNA replication inhibitors on UV excision repair in synchronised human cells. Nucleic Acids Res. 1982; 10:5357–5368. [PubMed: 7145704]

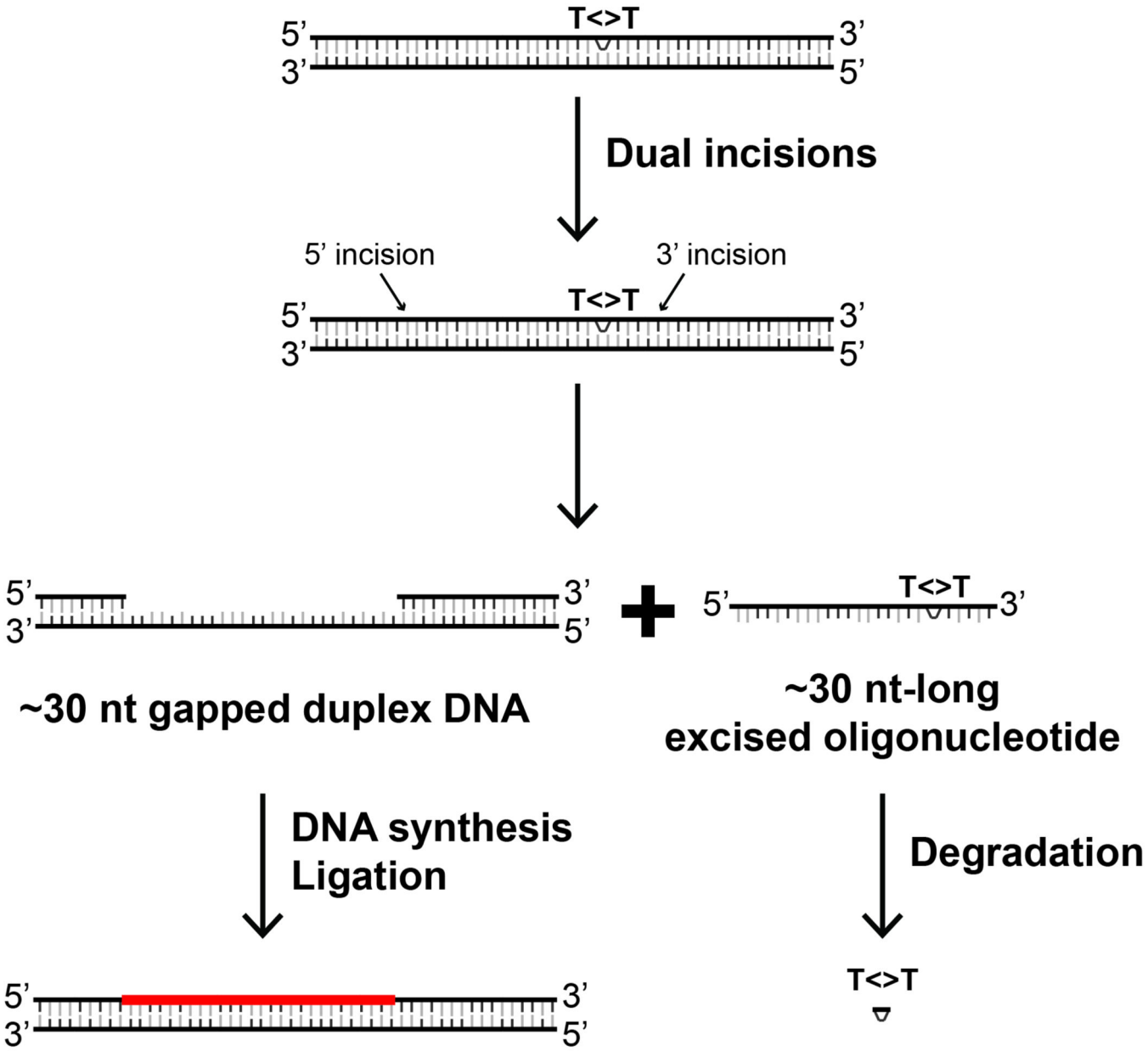


Figure 1. Simple schematic of eukaryotic nucleotide excision repair of UV-induced DNA damage

UV induces the formation of UV photoproducts in DNA, including a representative thymine dimer indicated in the figure. Two nucleolytic incision events take place ~20 ± 5 phosphodiester bonds 5’ and 6 ± 3 nt phosphodiester bonds 3’ of the UV photoproduct to generate an ~30-nt-long gapped DNA duplex and a 30-nt-long damage-containing DNA oligonucleotide. Completion of the DNA repair reaction requires DNA repair synthesis and ligation to fill in the gap and degradation of the excised oligonucleotide.

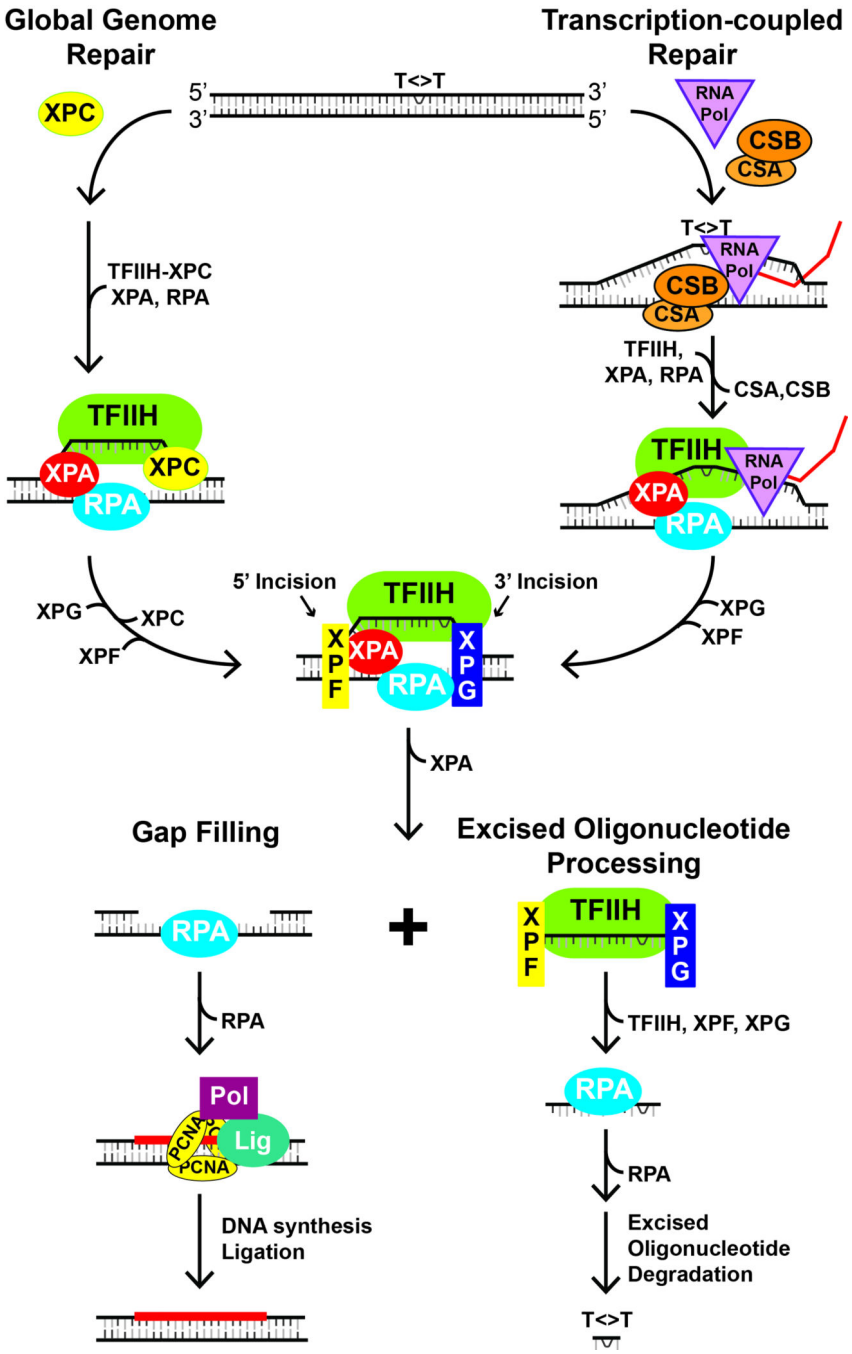


Figure 2. Model of the global genomic and transcription-coupled repair pathways of human nucleotide excision repair

UV photoproducts and other bulky DNA adducts are recognized by either the XPC-dependent global genomic repair pathway or the CSA/CSB- and RNA polymerase-dependent transcription-coupled repair pathway. In the general or global genomic repair pathway, XPC, XPA, and RPA recognize the damage in a cooperative manner that is dependent on TFIIH, which subsequently unwinds the DNA duplex around the lesion to generate a bubble structure that is targeted by the XPF and XPG nucleases. A similar intermediate is also ultimately generated in the transcription-coupled repair pathway, though

the damage recognition step requires RNA polymerase stalling at the lesion and the recruitment of the CSA and CSB proteins. Regardless of the mode of damage recognition, the dual incisions by XPF and XPG generate a single-stranded DNA gap and an excised, damage-containing DNA oligonucleotide that remains in complex with TFIIH. The gap is filled in by a DNA polymerase and then the remaining nick sealed by a DNA ligase. The damage-containing oligonucleotide dissociates from TFIIH and becomes bound to RPA before undergoing further degradation.

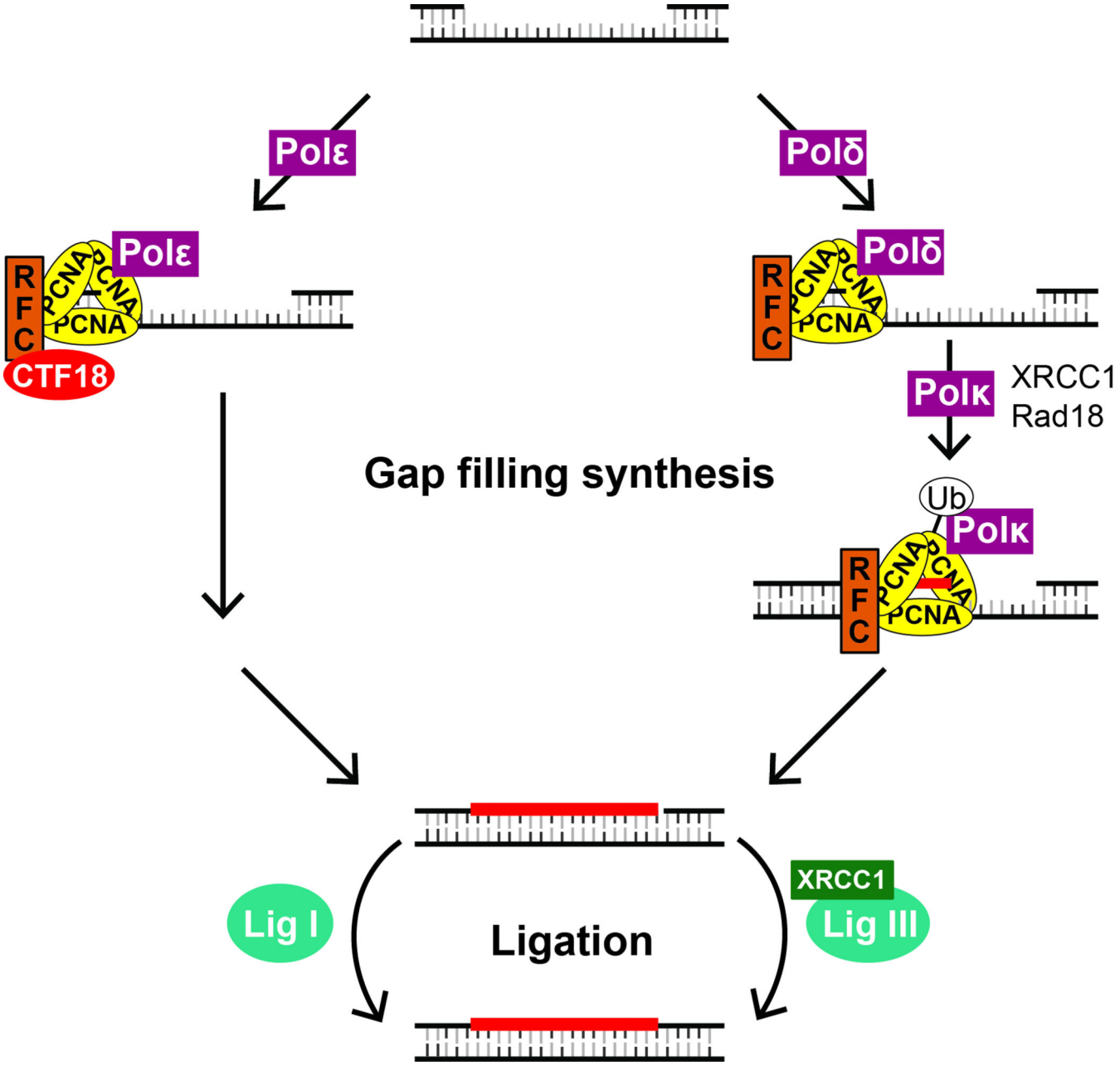


Figure 3. Schematic of the gap filling processes of human nucleotide excision repair

RPA is thought to remain bound to the single-stranded DNA excision gap along with XPG, which contains a domain that may facilitate the recruitment of the DNA polymerase clamp protein PCNA to the gap. PCNA is then loaded onto the primer-template junction by either the canonical RFC complex or the CTF18-RFC complex and may undergo ubiquitination by Rad18. Depending on the particular RFC that was utilized and the ubiquitination status of PCNA, Polymerases ε, δ, or κ are then recruited to gap to carry out gap filling DNA synthesis. Polymerases δ and κ carry out approximately half of repair synthesis as part the same pathway, whereas polymerase ε functions independently to carry out the remaining

gap filling. The remaining nick is sealed by either DNA Ligase I (Lig I) or the XRCC1-Lig III complex.

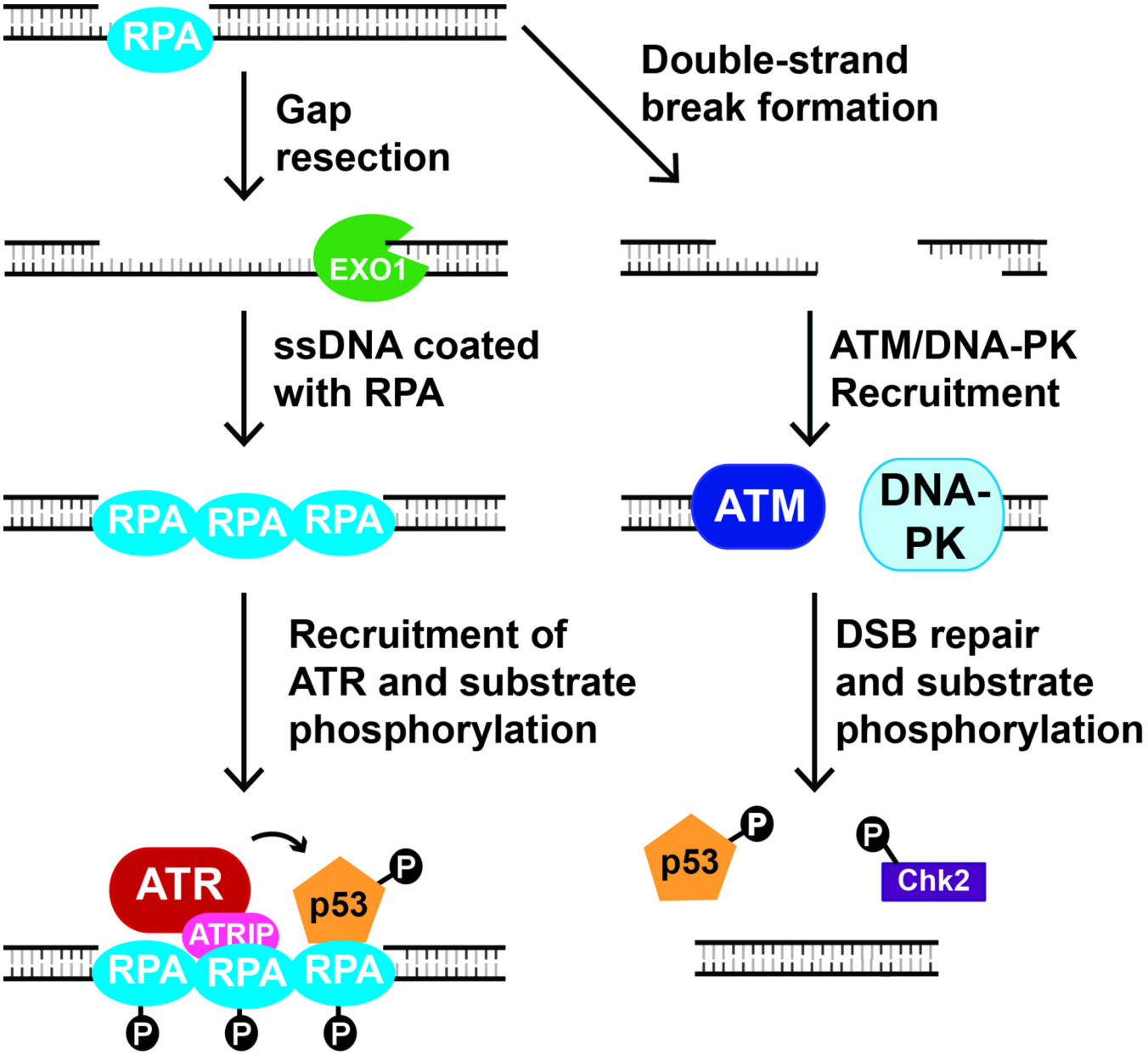


Figure 4. Nucleotide excision repair-dependent activation of DNA damage response kinases following UV

Under some circumstances, the post-excision gap may be enlarged by Exonuclease I (Exo I). The extended region of single-stranded DNA is thought be then become bound by the single-stranded DNA-binding protein RPA, which facilitates the recruitment of the DNA damage checkpoint kinase ATR to the enlarged gap. ATR then phosphorylates its substrates, including the tumor suppressor protein p53. In a mechanism that remains to be fully elucidated, excision gaps in non-cycling, non-replicating cells may also give rise to the formation of double-strand breaks, which are targeted for repair and damage signaling by ATM and DNA-PK.

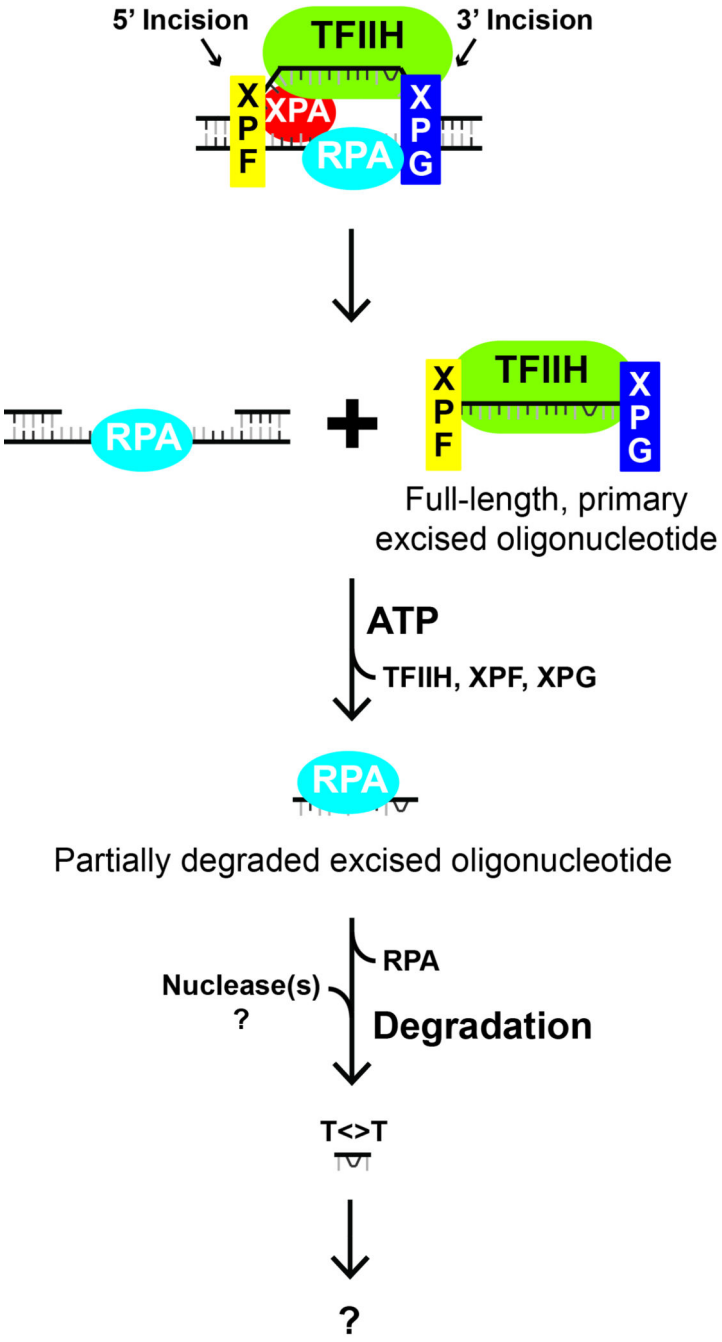


Figure 5. Post-excision processing of the excised, damage-containing DNA oligonucleotide products of human nucleotide excision repair

Following the dual incisions by XPF and XPG, the UV photoproduct damage is released in the form of an ~30-nt-long oligonucleotide in a tight complex with the repair factor TFIIH. XPG, and to a lesser extent XPF, can also be observed to associate with the TFIIH-excised oligonucleotide complex (10). In an ATP-dependent but ATP hydrolysis-independent manner, the full-length, primary excised oligonucleotides dissociate from TFIIH and become bound by RPA (56). Once bound to RPA, the excised oligonucleotides undergo a limited

amount of nucleolytic degradation before being released and undergoing further degradation. The ultimate fate of the damaged/adducted nucleotides is unknown.