

SPOTLIGHT

Controlling scissors during DNA repair of UV light-induced damage

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Nucleotide excision repair is essential for genome stability; however, enzymatic scissors that remove damage must be tightly regulated to prevent erroneous DNA cleavage. In this issue, Muniesa-Vargas et al. (<https://doi.org/10.1083/jcb.202602121>) used live-cell imaging to uncover how cutting DNA by XPG endonuclease is controlled during repair.

When ultraviolet (UV) radiation from sunlight exposure reacts with genomic DNA, it can produce UV photoproducts in the form of covalently linked pyrimidine dimers. If not repaired, these lesions can (a) be converted to mutations that promote carcinogenesis, or (b) impair DNA replication and transcription, causing cytotoxicity and degenerative diseases. To combat the deleterious effects of UV photoproducts, cells rely on nucleotide excision repair (NER), a process involving over 30 proteins to remove the damage and restore the DNA (1). Mutations that impair NER cause the genetic disorder xeroderma pigmentosum (XP), characterized by extreme sunlight sensitivity and a 2000-fold increase in skin cancers. In a study published in this issue of the *Journal of Cell Biology*, the Hannes Lans laboratory used live-cell imaging to uncover mechanisms that tightly regulate one of two critical NER enzymatic scissors, XPG, that cuts the DNA to remove the lesion (2).

Enzymatic scissors (endo- and exonucleases) involved in DNA repair must be tightly regulated to prevent erroneous DNA breaks that may provoke genomic alterations or impair DNA transactions (3). NER involves a precisely orchestrated series of steps initiated when the XPC complex recognizes the DNA lesion, followed by recruitment of TFIIH, which utilizes its XPB ATPase to open the DNA duplex and XPD helicase to scan and verify the lesion. In the resulting bubble, the single-stranded DNA (ssDNA) binding protein RPA binds the

undamaged strand through its interaction with the scaffold protein XPA. The scissors ERCC1-XPF and XPG are placed 5' and 3' to the lesion to incise the DNA strand. Cutting removes the damage and produces a 25- to 30-nucleotide ssDNA gap that is filled in by DNA polymerase and sealed with ligase to complete repair (3) (Fig. 1). Precise and coordinated timing of the two incisions is critical for preventing inappropriate buildup of ssDNA breaks as intermediates from incomplete repair processing. Therefore, the Lans laboratory sought to address two critical key questions regarding how XPG is (1) recruited to sites of UV damage in NER protein complexes, and (2) released from NER complexes following dual incision.

The authors monitored XPG dynamics in real time by fusing the endogenous protein with fluorescent protein mClover, avoiding potential artifacts from overexpressing exogenous proteins. They induced highly localized UV photoproducts by UV-C irradiation through micropores or by using a targeting UV-C laser and microscopy. XPG movement was visualized via the mClover tag and by using fluorescence recovery after photobleaching (FRAP). FRAP allows visualization of protein immobilization after UV-C irradiation as a readout of recruitment to DNA damage. Using these powerful techniques, the authors discovered that the TFIIH complex is essential for XPG recruitment to sites of UV damage, while XPA depletion reduced XPG recruitment,

consistent with its role in stabilizing the NER complex prior to incision of the DNA backbone. Two models for XPG recruitment to NER complexes have previously been proposed: one suggested that XPG and TFIIH form a constitutive complex (4), while other work was in line with the sequential arrival of TFIIH followed by XPG (5). With the endogenously tagged XPG cells, the current study found that TFIIH and XPG accumulate separately on the damaged DNA, reflective of a highly coordinated process of assembling different parts of the NER machinery in a precise order.

Next, the Lans laboratory tackled the question of XPG dissociation from the DNA during NER processing. Live-cell imaging revealed that XPG resides longer at the UV-damaged DNA sites than the upstream XPF scissors. This strongly supports prior evidence and models that XPF incises the DNA before XPG (6). PCNA, which partners with DNA polymerase to fill the gap, increases the rate of XPG dissociation, again supporting a coordinated timing of NER factors arising and departing. They also discovered that XPG incision activity promotes its dissociation from DNA, since catalytically dead mutants reside longer on DNA. XPG's timely departure after completing its role in NER avoids further erroneous cutting. However, even the XPG mutants dissociated, suggesting a "backup mechanism" for disassembly to complete NER. They tested a role of the 5' to 3' exonuclease EXO1 based on prior work showing this enzyme acts at NER-induced

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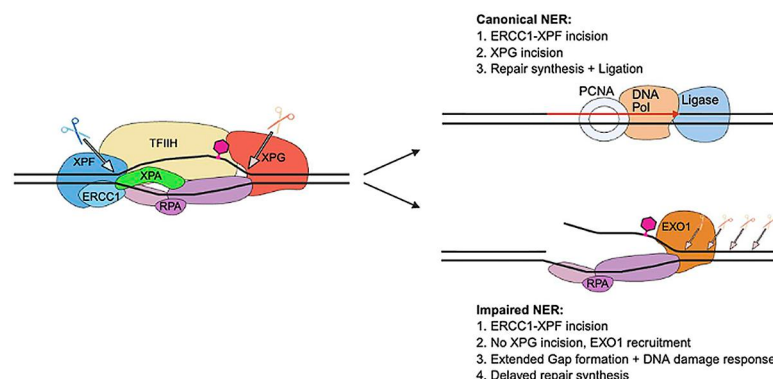


Figure 1. Canonical and impaired NER reactions. In normal NER, excision of the lesion-containing strand by the ERCC1-XPF and XPG exonucleases is coordinated with repair synthesis and ligation. If NER is impaired by inhibition of the 3' incision by XPG, EXO1 can resect the lesion-containing strand, leading to the formation of ssDNA gaps, DNA damage response activation, and eventually delayed repair synthesis and ligation. EXO1, exonuclease 1.

ssDNA gaps (7, 8). Indeed, their studies provide evidence that EXO1 can bind the damage sites following XPF incision 5' of the lesion and promote dissociation of both catalytically active and inactive XPG, while expanding the ssDNA gap (Fig. 1). These studies reveal a critical role of EXO1 as a backup when the canonical NER enzymatic XPG scissors stall or cannot complete its job.

DNA repair is essential for preserving the genome and protecting against environmental insults such as UV light, which can promote carcinogenesis or degenerative diseases. However, once initiated, NER must be completed in a timely and accurate manner, since the process involves cleaving the DNA strands, and breaks can often be more toxic than the initial lesion. Recent publications further support the perils of DNA breaks formed by faulty NER incision reactions. First, certain patient mutations in the XPD subunit of TFIID result in aberrant NER incisions and the formation of

persistent ssDNA breaks (9). This was attributed to a defect in coordinating the activities of XPD helicase with the XPG nuclease, consistent with new evidence showing that the interaction between XPG and XPD is critical for NER incision activity (10). In addition, the anticancer agent trabectedin is more active in NER-proficient cells due to the unique structural properties of the trabectedin-DNA lesion inhibiting XPG incision, again leading to persistent ssDNA breaks (11). (3) The neuronal toxicity of cisplatin has been attributed to incomplete DNA repair synthesis in NER. Incisions take place, but repair synthesis cannot be completed due to low nucleotide pools in postmitotic neurons (12). While this is not directly connected to the NER incision reactions, it demonstrates the perils of initiating and not completing NER.

These recent studies shine light on the importance of completing the NER reaction following damage incision. The current study from the Lans laboratory significantly

advances our understanding of how the NER enzymes that incise DNA are precisely regulated, and how the cell employs a non-NER enzyme, EXO1, to prevent aborted repair when the XPG incision is ineffective.

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