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Check, Check ...Triple Check: Multi-Step DNA Lesion Identification by Nucleotide Excision Repair

Jurgen A. Marteijn,[1] Jan H.J. Hoeijmakers,[1,*] and Wim Vermeulen[1]
[1]Department of Genetics, Cancer Genomics Netherlands, Erasmus University Medical Center, Wytemaweg 80, 3015 CN Rotterdam, The Netherlands
*Correspondence: j.hoeijmakers@erasmusmc.nl
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In this issue, Li et al. (2015) uncover roles for the XPB and XPD helicases and for XPA during damage verification in nucleotide excision repair, supporting a novel tripartite damage checking mechanism that combines extreme versatility with narrow specificity.

Damage to biomolecules by cellular metabolites and environmental agents is unavoidable. However, degradation and de novo synthesis are no option for damaged DNA, since the genome is unique, at the top of the informational hierarchy, and needs to remain intact lifelong. Therefore, DNA has to rely on self-encoded repair. The challenge of DNA repair is to identify any of a bewildering number of potential DNA lesions in an ocean of normal DNA conformations. Most repair systems have a set of lesion-specific proteins, each recognizing particular damage. Nucleotide excision repair (NER) is the exception; with only a limited set of damage-detection tools, it removes an extremely broad range of structurally unrelated DNA injuries (Figure 1). The enigmatic question is, therefore: *How does NER accomplish this?*

Although previously pieces of this puzzle have been uncovered, Li et al. (2015) "put the icing on the cake" by providing evidence for a more complete model for damage selection in global genome (GG-)NER, the NER sub-pathway that surveys the entire genome. Using highly purified factors and defined damage-containing model substrates, they identify a "tripartite checking mechanism" that combines initial, broad, non-specific sampling of abnormal DNA conformations with rigorous lesion-verification steps involving distinct, collaborating downstream NER factors.

Check 1: Base Pairing

The XPC complex is the major GG-NER initiator (Sugasawa et al., 1998). Structural analysis of the yeast XPC ortholog, RAD4, on a CPD lesion (Min and Pavletich, 2007) revealed the mode of action. The crux is that XPC ignores the lesion itself, but rather focuses on the damage-induced undamaged ssDNA opposite to the lesion, a structure common to all NER-targets that explains the broad substrate specificity. This non-specific XPC binding to small DNA bubbles comes at a price; XPC also binds DNA conformations, such as replication-induced mismatches or intercalating drugs not covalently bound to DNA. However, these are not processed by NER, implying that additional steps are required for lesion confirmation.

Check 2: Strand Threading

The next NER factor is the transcription/repair complex TFIIH, containing the 3′-5′ XPB and 5′-3′ XPD helicases (Compe and Egly, 2012). The helicase activity of yeast XPD, Rad3, is inhibited by bulky DNA lesions in the translocated strand (Naegeli et al., 1993), candidating this factor as lesion sensor. DNA bubble-bound XPC recruits TFIIH, which then scans for lesions in the 5′-3′ direction, mainly by XPD (Sugasawa et al., 2009). Li et al. (2015) used Helium sparging, counteracting oxidation of the delicate iron-sulfur cluster of XPD, to obtain recombinant TFIIH with improved enzymatic activities and showed that next to XPD also the XPB helicase is inhibited by bulky lesions to promote NER. This argues for a model in which XPC binds to helix distortions, after which bidirectional strand-threading of TFIIH verifies the presence of NER-inducing lesions.

Check 3: ssDNA Chemistry

Li et al. (2015) also showed that XPA promotes TFIIH-mediated lesion scanning directly and indirectly by stimulating the release of the CAK subcomplex from core TFIIH that inhibits the helicase function (Coin et al., 2008). This function comes on top of the previously reported specific affinity of XPA for chemically altered nucleotides in an ssDNA context (Camenisch et al., 2006).

Hence, the tripartite lesion selection in GG-NER is based on three principally distinct checks: (1) sensing of base-pairing disruptions by XPC; (2) TFIIH-mediated, XPA-stimulated strand threading for translocation-inhibiting injuries; (3) detection of chemically altered nucleotides by XPA (Figure 1). Moreover, XPA is a crucial NER organizer by positioning downstream NER factors through its multiple interactions, including ssDNA-binding RPA, which stabilizes the open NER intermediate, and the XPG and ERCC1/XPF endonucleases, which excise the damage-containing 22–30 base-pair oligonucleotide (see references in Marteijn et al., 2014).

The different steps involving numerous protein-protein interactions offers additional pathway control by post-translational modifications. For example, the RNF111-mediated XPC ubiquitylation promotes its release from the pre-incision complex to stimulate proper loading of the endonucleases and efficient NER (van Cuijk et al., 2015). This XPC ubiquitylation thus adds an extra layer of quality control: only when the pre-incision complex is properly configured, progression to incision is supported. This multi-faced quality

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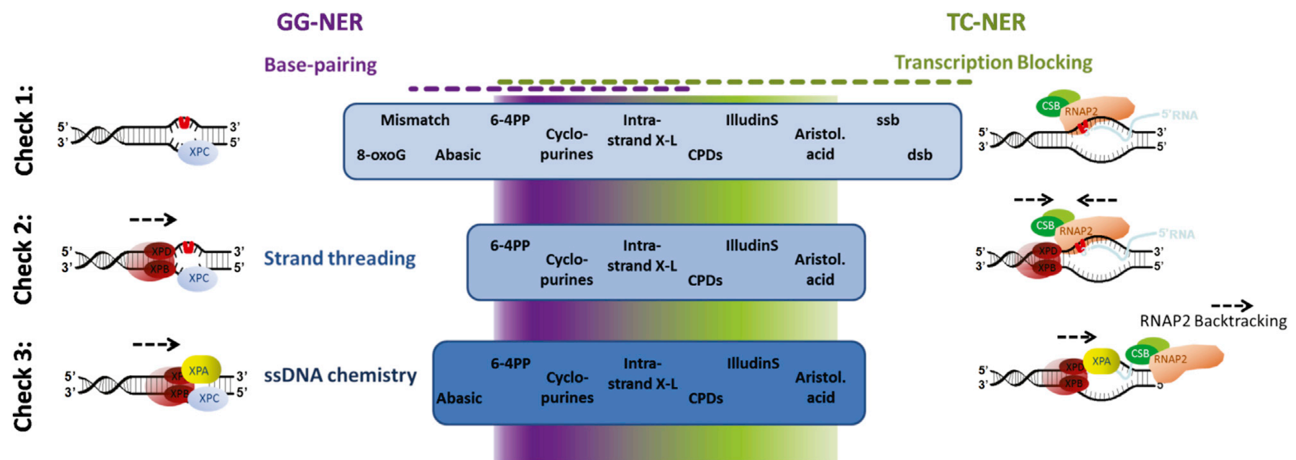

Figure 1. A Three-Step Damage Recognition and Verification Model of NER

NER removes the main UV lesions (cyclobutane pyrimidine dimers [CPD] and 6-4 photoproducts [6-4 pp]), cyclopurines from oxidative origin, different intra-strand crosslinks (e.g., Cis-Pt), numerous bulky chemical adducts, e.g., benzo[α]-pyrene and even completely artificial, man-made cholesterol adducts. Check 1: XPC detects indirectly DNA damage by probing the DNA for improper base-pairing everywhere in the genome (GG-NER). If helix distortions are detected, XPC binds not to the lesion itself, but on the opposite strand. By this indirect approach to detect DNA lesions, XPC recognizes also several non-NER substrates like mismatches (Sugasawa et al., 2009), showing the importance of additional damage verification steps. Recognition in TC-NER is achieved by RNA Polymerase 2 (RNAP2) that stalls at lesions. TC-NER detects a different subset of lesions than XPC. Check 2: Next, TFIIH verifies the DNA damage using strand threading in the 5′-3′ direction by XPD helicase, while XPB may unwind the undamaged opposite strand. RNAP2 completely covers the lesion on which it has been stalled and needs therefore most likely to backtrack to give TFIIH access to these damages. Check 3: In addition to its affinity for chemically altered nucleotides, XPA promotes TFIIH-mediated lesion scanning and thereby completes the damage-verifying process of NER to ensure that only true NER substrates are excised by the endonucleases.

control implies that the NER reaction can be aborted to secure that only true NER lesions are processed to the ‘‘point of no return’’, i.e., excision of the damage.

Why does TFIIH need two helicases? The XPB ATPase was suggested to load TFIIH (Oksenych et al., 2009) and initiate unwinding of the dsDNA, which is subsequently extended by XPD helicase. Although the XPD translocation appeared most important for lesion verification, it is likely that the XPB 3′-5′ helicase is needed to translocate on the complementary non-damaged strand simultaneously with XPD. The observed lesion-dependent inhibition of XPB helicase may be an indirect consequence of its connection to XPD, the co-translocation of which is anticipated to stall when the damaged strand gets stuck in the XPD ssDNA channel. Additional detailed structural studies on TFIIH and its interactions, such as those performed by the Ranish team (Lou et al., 2015), are required to gain insight on how TFIIH employs two helicases with opposite directionality to find lesions.

Despite the ingenious broad probing by XPC, some serious DNA injuries still escape detection by GG-NER as they do not cause sufficient base-pair disruption. Examples are CPDs and lesions in the minor groove induced by the mushroom and herbal toxins, Illudin-S, and aristolochic acid, respectively. These lesions however, block transcription (Figure 1) and thereby trigger the transcription-coupled (TC-) NER subpathway, to permit resumption of gene expression (see references in Marteijn et al., 2014). Sensing diverse lesions by TC-NER can be easily envisaged, since a damaged template likely stalls elongating RNA polymerases. Thus, strand threading by translocating RNA polymerases is a more rigorous quality check than XPC and explains why TC-NER can repair lesions not noticed by GG-NER (Figure 1). One consequence is that there is a category of lesions, which remains undetected in the non-transcribed compartment of the genome and likely accumulates in time. It will be important to find out to which extent this imperfection of GG-NER contributes to cancer and aging (Marteijn et al., 2014).

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