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NER additional players at the repair late stages

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Motivation and Aim: Nucleotide excision repair (NER) is the most versatile DNA repair pathway, which can remove diverse bulky DNA lesions destabilizing a DNA duplex. NER substrates are UV photoproducts, e.g., cyclobutane pyrimidine dimers (CPDs), pyrimidine-pyrimidone-(6-4)-photoproducts (6-4PPs), intrastrand crosslinks, and bulky adducts of DNA bases with reactive metabolites of some chemical carcinogens or chemotherapeutic agents. These kinds of lesions can be substrates for two NER sub-pathways—global genome NER (GG-NER) and transcription-coupled NER (TC-NER)—that overlap, except for the mode of DNA damage recognition. After the lesion has been recognized, a pre-incision complex is formed. Subsequent NER stages includes excision oligonucleotide containing lesion with ssDNA gap generating, gap filling and sealing the nick.

The DNA incision is first carried out by XPF–ERCC1 from the 5′ side to the damage site. Formed DNA intermediate contains long gap (about 30nt) with a free 3′-OH group, long flap bearing a lesion and an intact template. Next, replication machinery loads to start repair synthesis. Possible sets of replication machines: (DNA polymerase δ/ PCNA/ RFC1-RFC-p66), (DNA polymerase ε/ CTF18/RFC) or (DNA polymerase κ/ ubiquitinated PCNA/ XRCC1). Which set of replication factors are definitely recruited remains unknown, but generally it depends on the status of PCNA ubiquitination as well as on usage of distinct clamp loader complexes or XRCC1. Repair synthesis can proceed halfway through the gap. Then the XPG-made incision cleaves the flap and leaves a 5′-phosphate that is utilized in the nick-sealing reaction by DNA ligase I or by complex (DNA ligase IIIα/ XRCC1).

We have shown previously that XPA – the key NER protein factor – could stay in NER complex at the post-incision stages through its interaction with RPA, the major eukaryotic ssDNA-binding protein [1]. These data supported by the existed information that XPA interacts with PCNA. To further understand possible XPA involvement to the late NER stages, we analyze this protein interactions with two proteins that also could form stable complexes with PCNA: XRCC1 and FEN1.

The XRCC1 (X-ray cross complementing group 1) also plays important roles in the different repair pathways outside the NER. In general, XRCC1, same as XPA, is a scaffold protein so it has ability to interact with multiple protein partners. Furthermore, same as XPA, XRCC1 has intrinsic DNA-binding activity with unknown functional meaning. FEN1 (flap endonuclease 1) belongs to the same as XPG structure-specific nuclease family and key component of all replication machines where flap DNA structures could arise. To further explore possible FEN1 and XRCC1 involvement in an incision/resynthesis stages coordination we analyze protein-protein interactions between

XPA, FEN1, XRCC1 and PCNA and these protein factors interactions with DNA mimicking the late NER stages DNA intermediates.

Methods and Algorithms: We have used 60-mer DNA duplexes containing a 31 nt flap with the fluorescein substituted dUMP residue mimicking the bulky lesion and a 26, 10 or 3 nt gap. The structure containing a damaged flap and a 26 nt gap imitates the DNA intermediate arising in the NER process after damaged strand cleavage by XPF-ERCC1; DNA with the same flap and a 10 nt gap can be attributed to the intermediate of the subsequent partial gap filling. Protein-DNA interactions were analyzed by gel retardation and Flu-dUMP residue were used as a label for detection. Two methods were used to detect protein-protein interactions: a dot-blot analysis and detection complexes formed by fluorescently labeled protein (FAM-RPA, FAM-XPA or FAM-XRCC1). XPA and XRCC1 influence on the FEN1 enzymatic activity also analyzed.

Results: XRCC1 binds to DNA containing damaged flap and gap and Y-type structures with nano molar range of Kd. Thus, we performed gel retardation experiments with these DNAs and bands corresponded to ternary complexes XRCC1-XPA-DNA or XRCC1-FEN1-DNA were detected. The XRCC1-XPA-DNA ternary complex detected with all DNA structures except one: duplex containing 5'-protruding 31nt ss tail. XPA-FEN1-DNA ternary complex also registered with all DNA structures used in experiments. As expected, FEN1 effectively binds and catalyze flap incision of DNA containing flaps and different size gaps. XRCC1 and XPA are slightly inhibit FEN1 catalysis at concentration corresponded to the ternary complex formation. Using dot blot analysis and experiments with fluorescently labeled proteins, XPA-FEN1 and XPA-XRCC1 complexes existence confirmed.

Conclusion: Our findings expand protein participants of the last NER stages and allow us to propose XPA-FEN1-XRCC1-PCNA complex formation. Functional role of this complex stay to be unknown and we assume that future experiments will shed the light on it.

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References

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