Scientists achieve a new understanding of the nucleotide excision repair process

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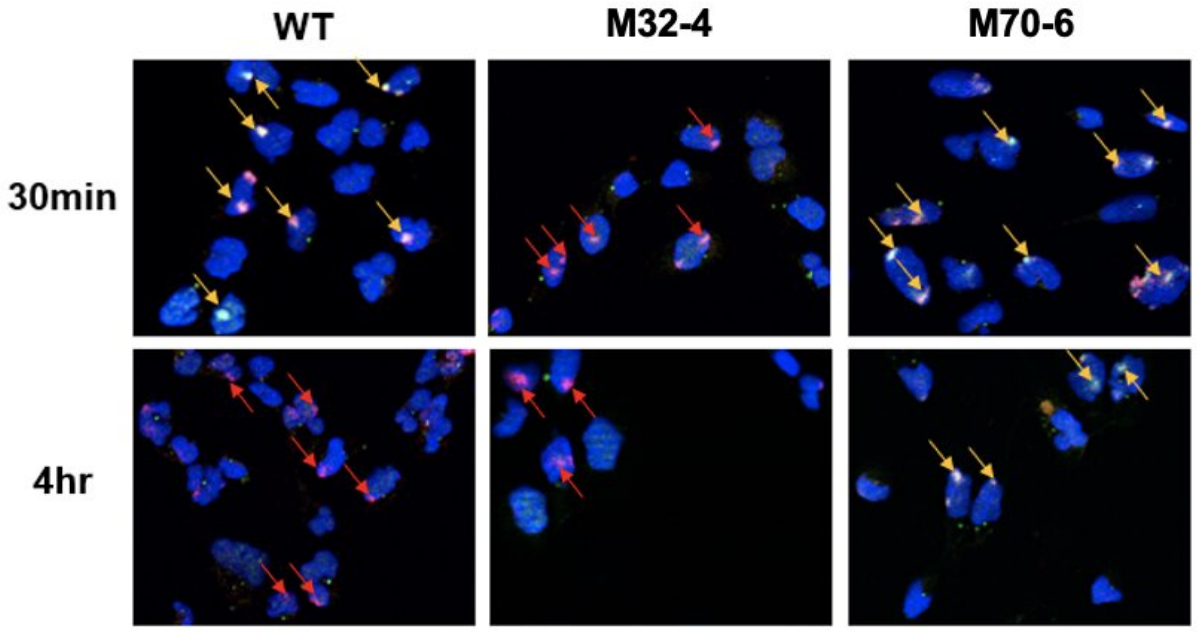


Distinct role of the interactions of RPA32 and RPA70 with XPA in NER. Cells were irradiated with UV and the colocalization of XPA with UV damage sites was visualized by fluorescence microscopy. DNA damage is highlighted by red arrows and co-localization of XPA with UV DNA is indicated by yellow arrows. In XPA-RPA32 mutant cells (M32-4), reduced XPA recruitment to DNA damage was observed. In XPA-RPA70 mutant cells (M70-6), XPA remained bound at damaged sites for an extended time. This indicates that RPA32 interaction with XPA is required for recruitment of XPA to UV-induced damage, while RPA70 interaction with XPA is important for positioning of XPA for completion of NER. Credit: Institute for Basic Science

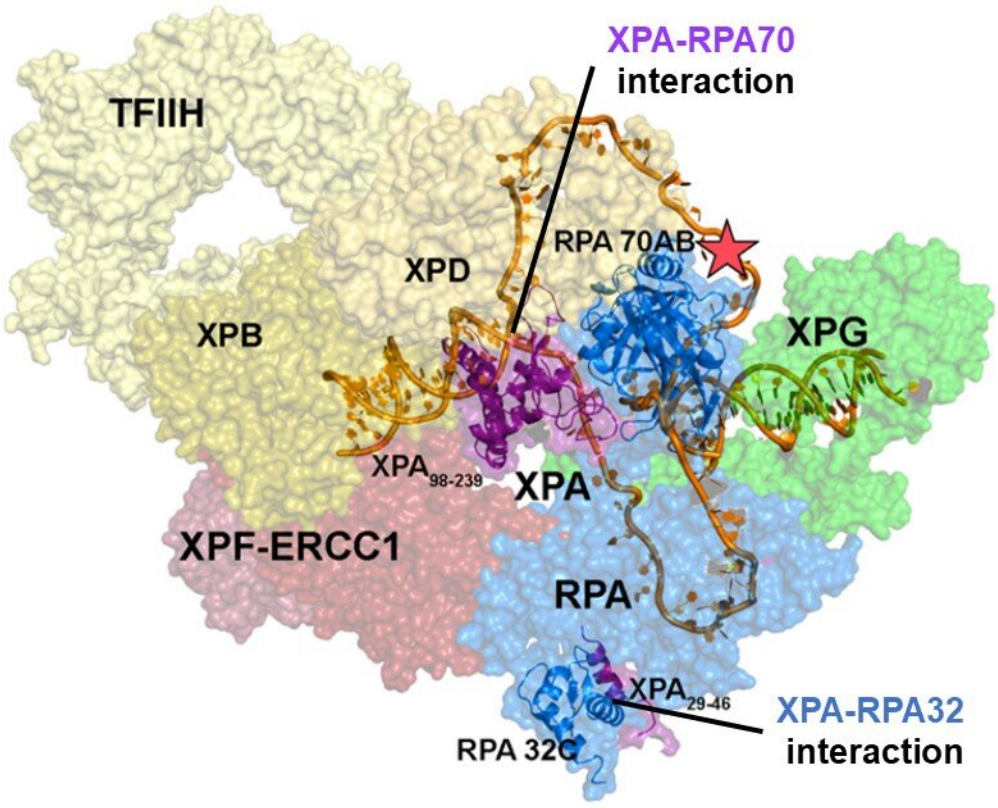
Nucleotide excision repair (NER) is a major conserved DNA repair pathway, which repairs various types of damage in the genome, such as those induced by ultraviolet light and environmental agents. Dysfunction in this pathway can be detrimental to human health. For example, individuals with defects in NER suffer from xeroderma pigmentosum, a disease characterized by an extreme disposition to sunlight-induced skin cancer due to an inability to repair UV-damaged DNA.

While NER thus counteracts cancer formation, it is also important for antitumor therapy. Many antitumor agents, such as cisplatin, induce damage to DNA that can be repaired by NER. In this context, NER is a drug target of interest to improve cancer therapy outcomes. At the molecular level, NER is a highly complex and dynamic molecular machine, involving over 30 proteins that assemble at DNA lesions to excise the damage and replace it with intact DNA. This process is guided by protein-protein and protein-DNA interactions.

A team of researchers led by the Associate Director Orlando D. Schärer and graduate student Kim Mihyun of the Center for Genomic Integrity within the Institute for Basic Science, South Korea explored these interactions. The team found that two key proteins in NER, namely xeroderma pigmentosum protein A (XPA) and replication protein A (RPA) proteins, are required for organizing the pre-incision complex in NER.

The two proteins XPA and RPA are responsible for the organization of the NER complex after it has found the damage in DNA. The present study compared mutant variants of these two proteins to investigate how the two proteins engage in a pivotal interaction for the NER pathway. Specifically, it was discovered that two interaction interfaces between XPA and RPA are critical for NER and have distinct roles in the pathway. The interaction of XPA with RPA32 is crucial for the initial association of XPA with DNA damage, whereas the interaction between

XPA and RPA70 is important for the completion of NER.



Structure of the NER pre-incision complex. The interaction of XPA and RPA70 is localized in the center of the NER complex, while the interaction site of XPA and RPA32 is at the periphery. The interactions between XPA and RPA70 stabilize the pre-incision complex and constricts the DNA to assume a U-shape, which appears to be the active form of the complex, allowing it to remove the DNA damage. Credit: Institute for Basic Science

Integrative structural studies of an XPA-RPA-DNA complex revealed how the interactions of the two proteins shape the NER complex and

trigger excision of the damage. The interaction of XPA and RPA32 occurs at the periphery of the complex, where it facilitates the initial assembly of the proteins at the site of damage. The interaction between XPA and RPA70 is located at the heart of the NER complex and forces the DNA into a U-shape. This allows the two ss/dsDNA junctions to become localized in close proximity, allowing for the NER complex to incise the DNA to remove the damage.

Schärer stated that their "study revealed a surprising new model of the NER complex and how the interaction between XPA and RPA shapes its architecture. Disruption of the interaction between XPA and RPA inhibits NER, and our study provides a blueprint for how this interaction may be targeted by small molecules to improve cancer therapy. We are continuing to pursue follow-up research together with our long-term collaborator on this project, Prof. Walter Chazin at Vanderbilt University."

This research was published in *Proceedings of the National Academy of Sciences*.

More information: Mihyun Kim et al, Two interaction surfaces between XPA and RPA organize the preincision complex in nucleotide excision repair, *Proceedings of the National Academy of Sciences* (2022). DOI: 10.1073/pnas.2207408119

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