



REGULATION OF DUAL INCISION AND REPAIR SYNTHESIS IN HUMAN NUCLEOTIDE EXCISION REPAIR

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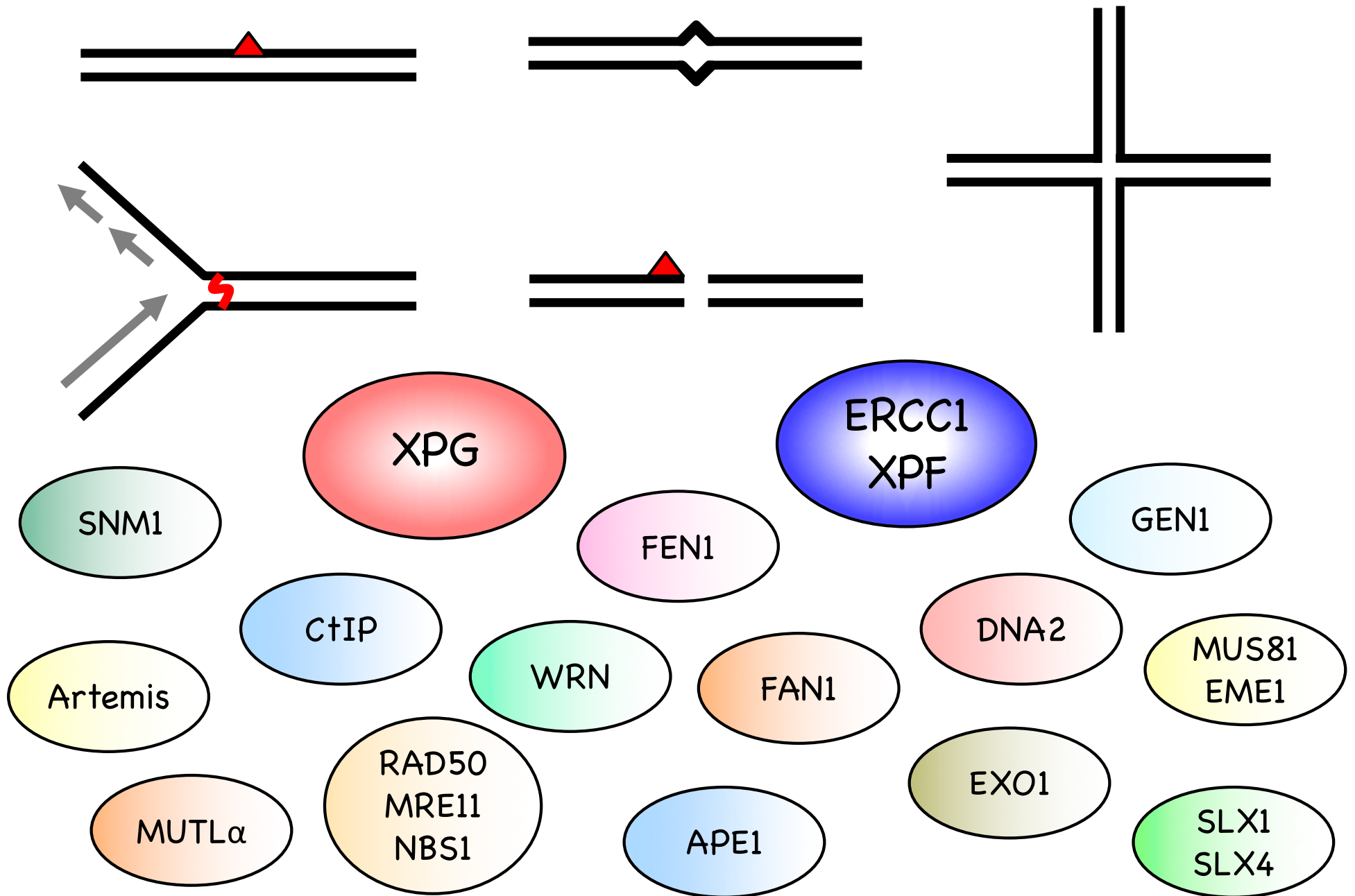
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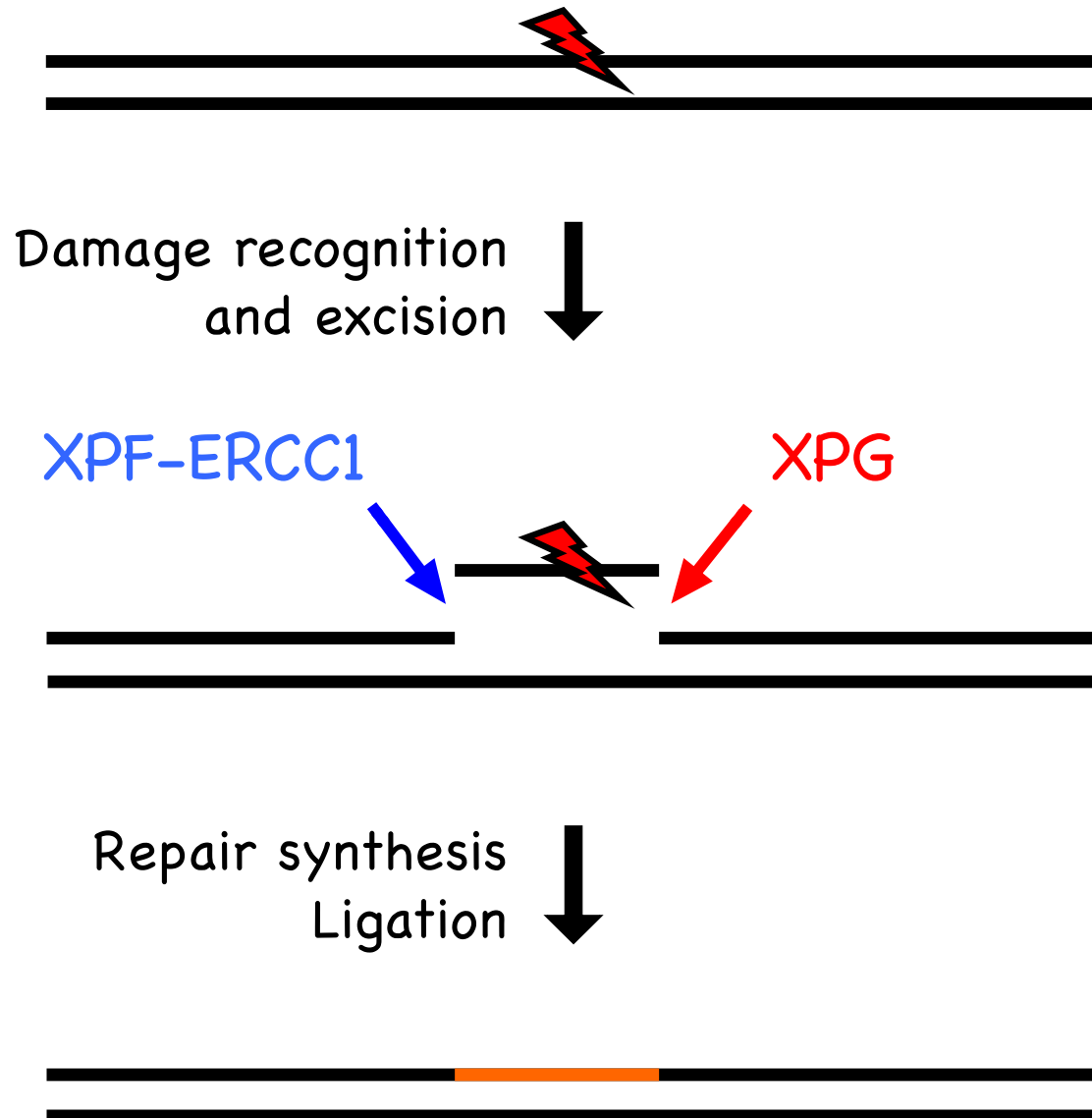
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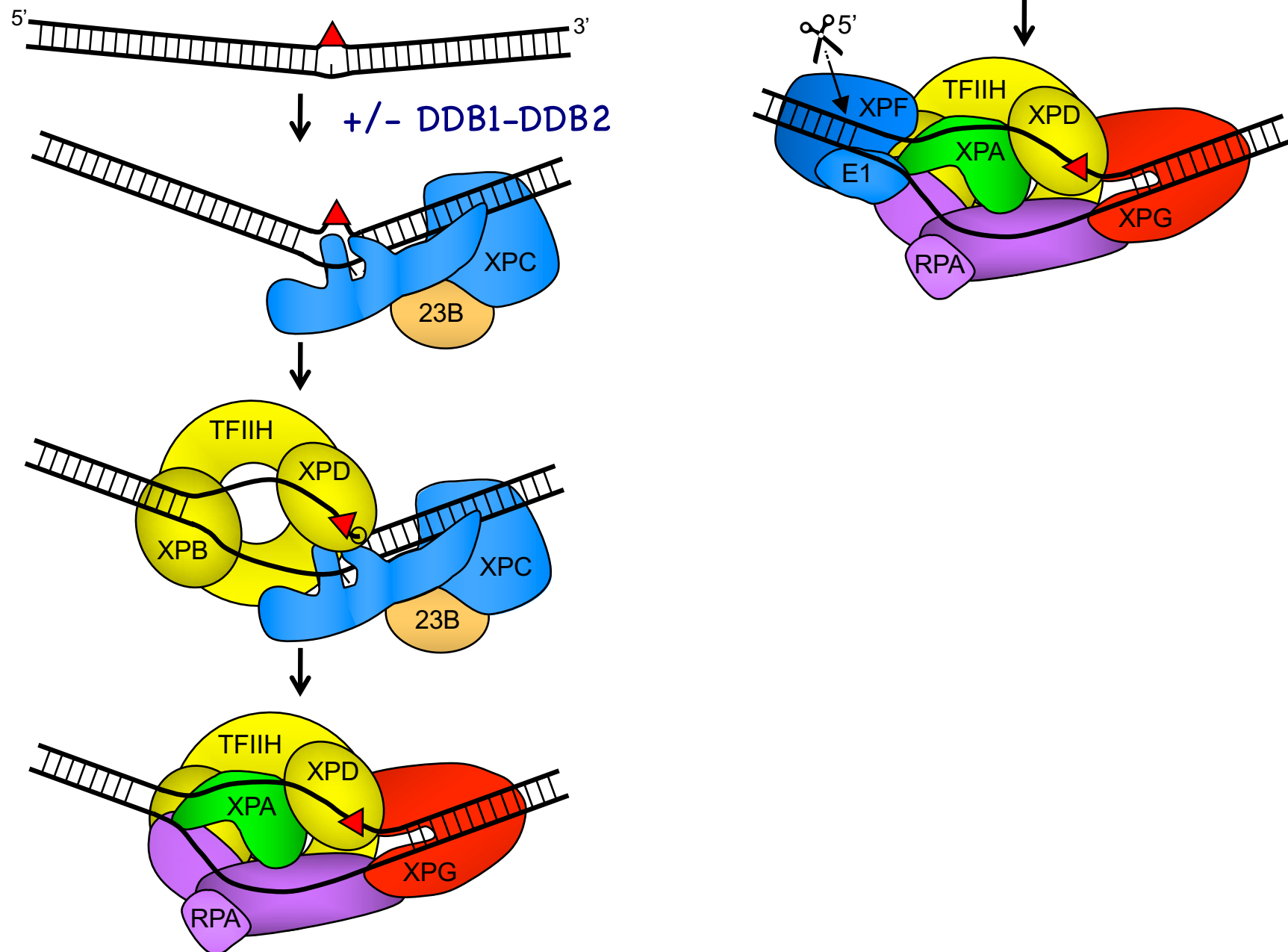
EXO- AND ENDONUCLEASES IN DNA REPAIR



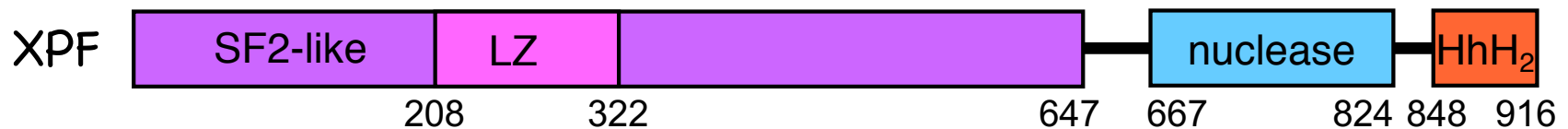
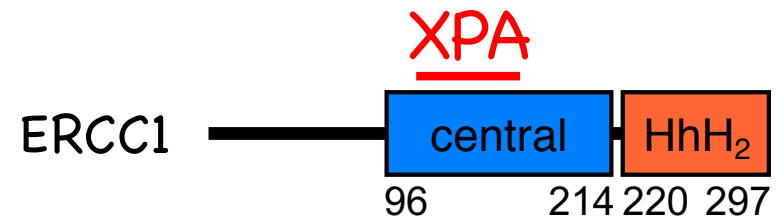
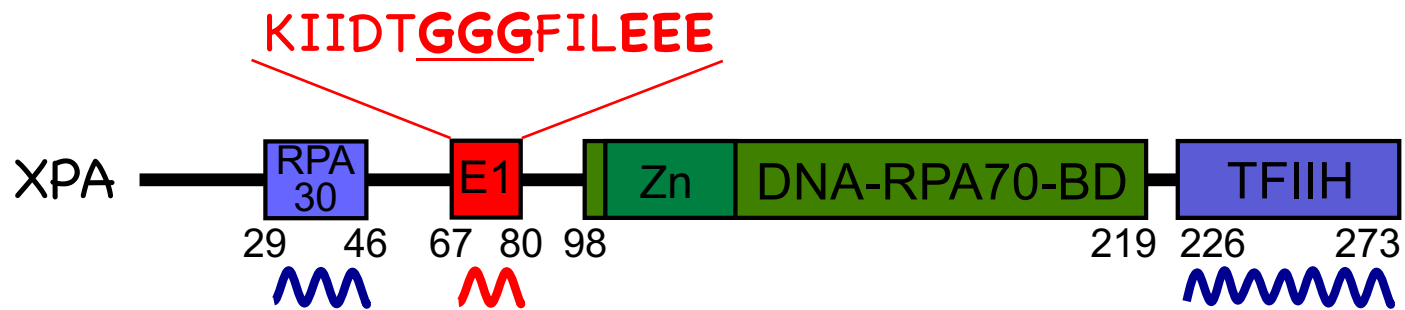
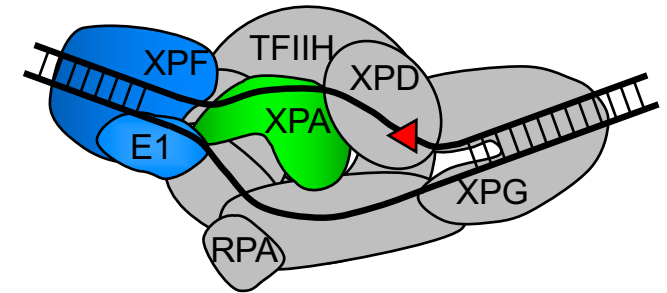
NUCLEOTIDE EXCISION REPAIR (NER)



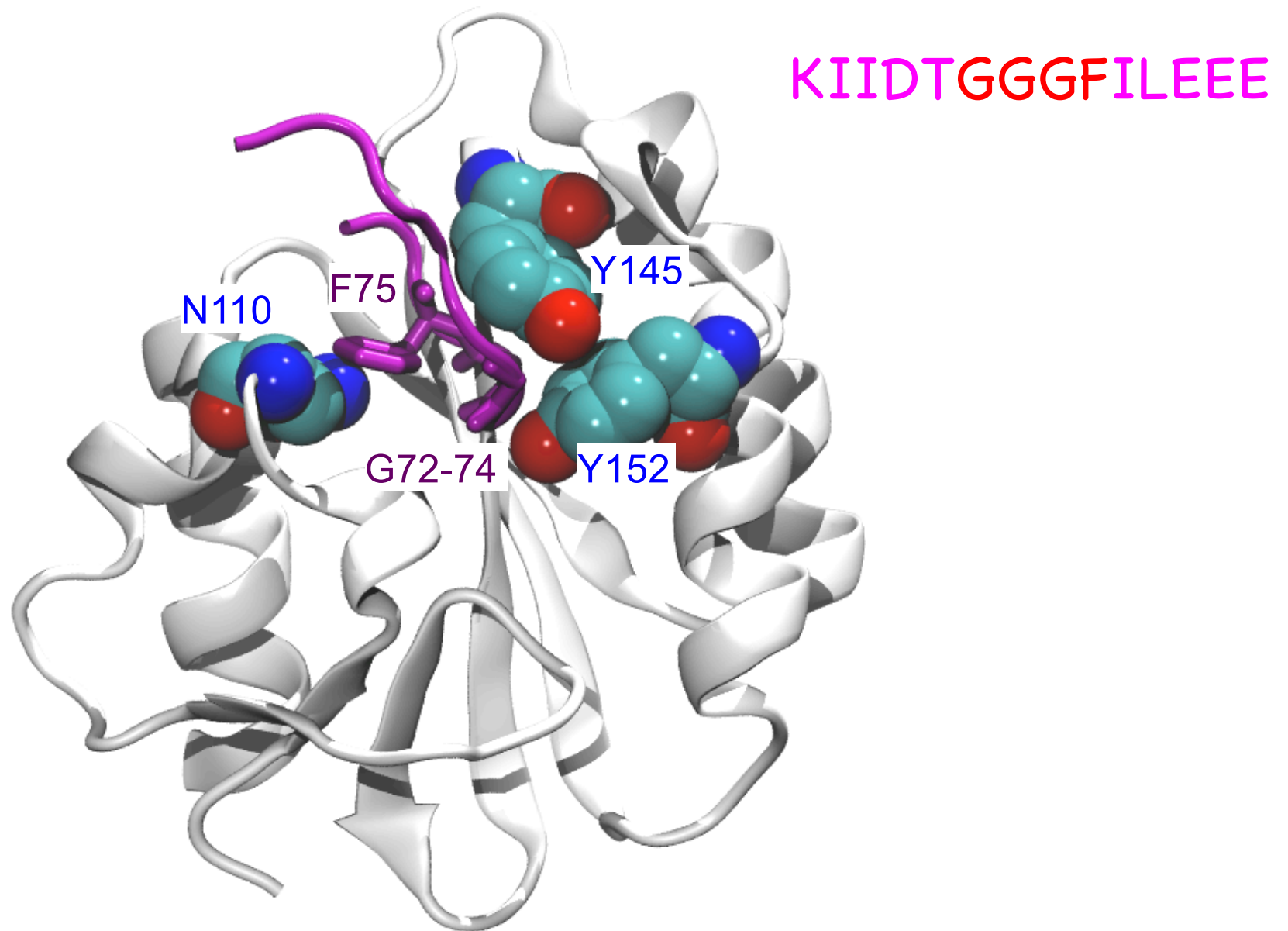
A MODEL FOR NUCLEOTIDE EXCISION REPAIR



THE XPA-ERCC1-XPF INTERACTION NETWORK

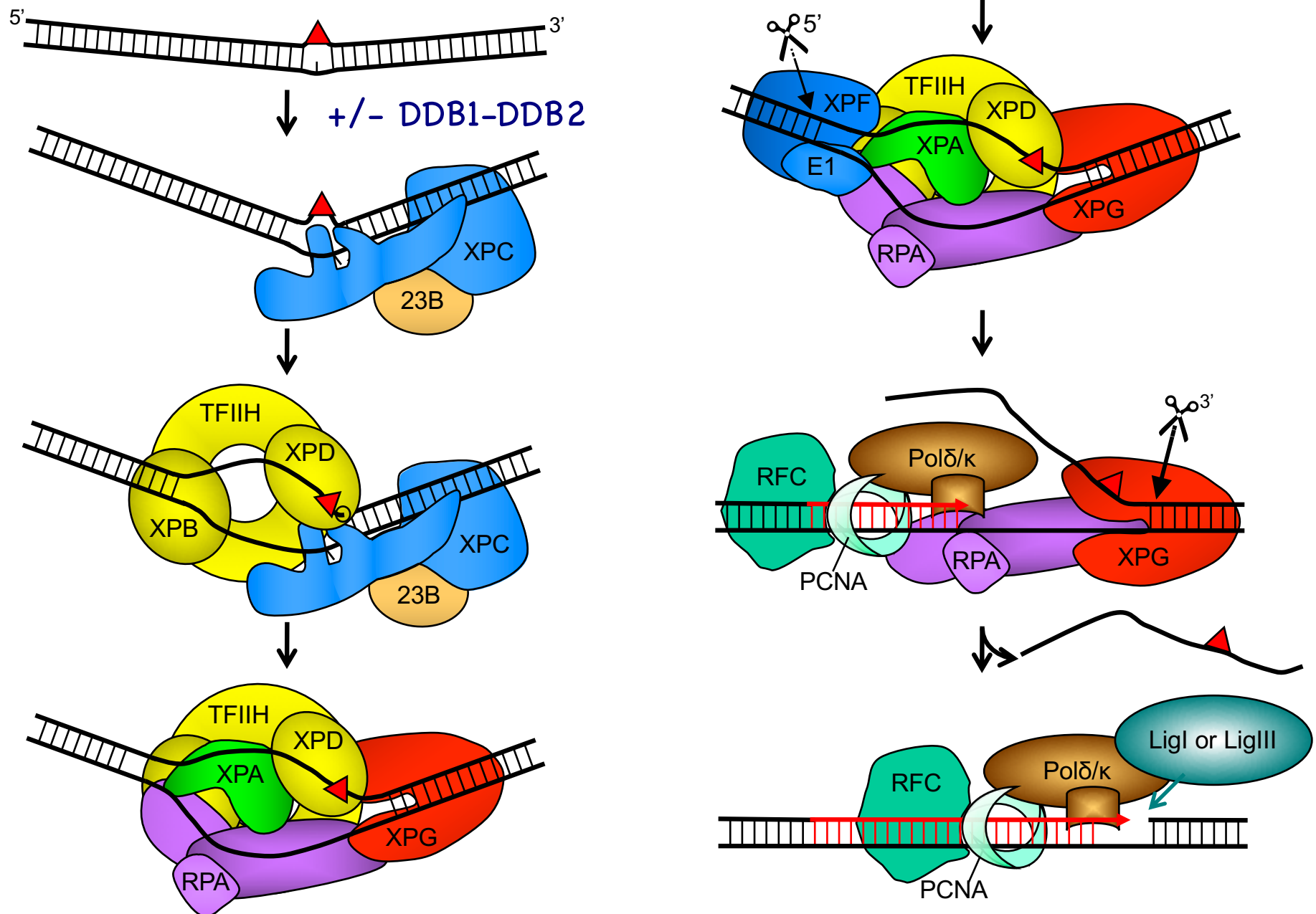


A SPECIFIC XPA BINDING POCKET IN ERCC1



Tsodikov et al. (2007) *Embo J.*, 26, 4768-4776;
Orelli et al. (2010) *JBC*, 285, 3705-3712

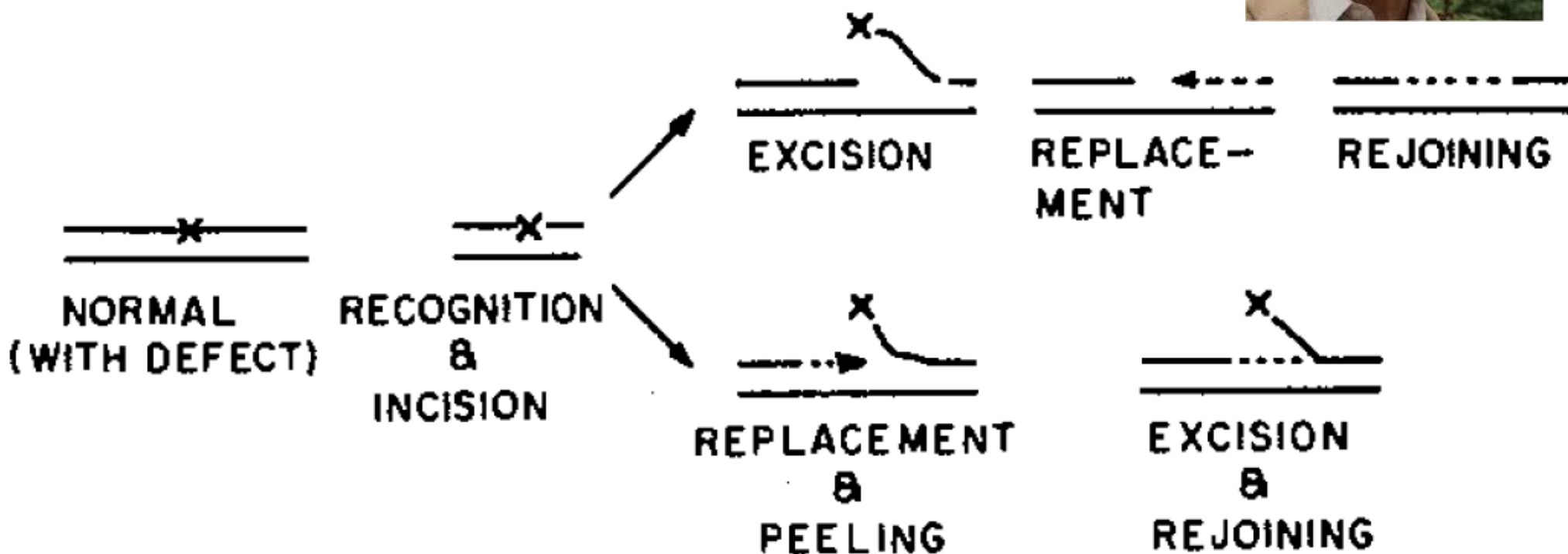
A MODEL FOR NUCLEOTIDE EXCISION REPAIR



A CUT AND PATCH MECHANISM FOR NER

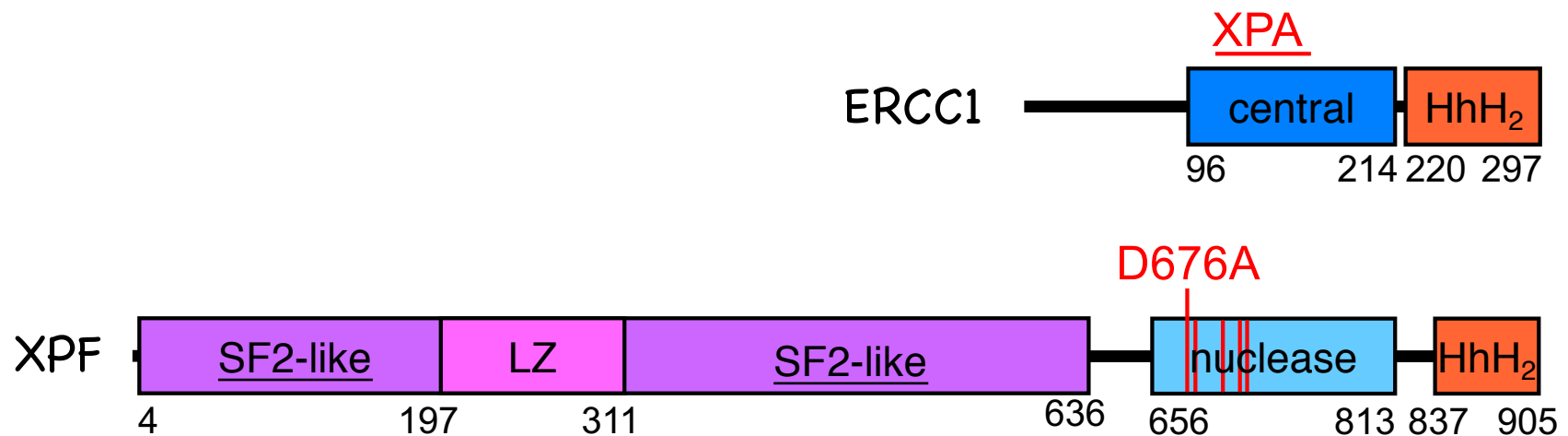
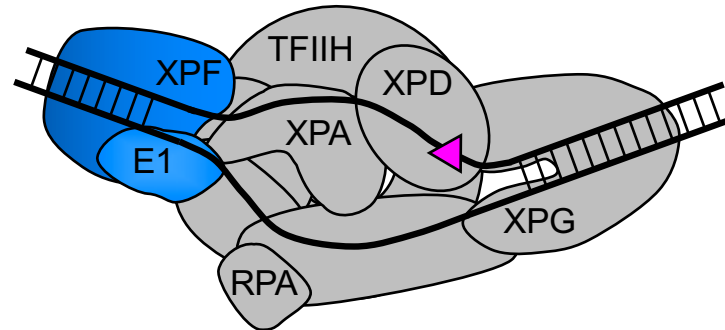


NONCONSERVATIVE REPAIR REPLICATION

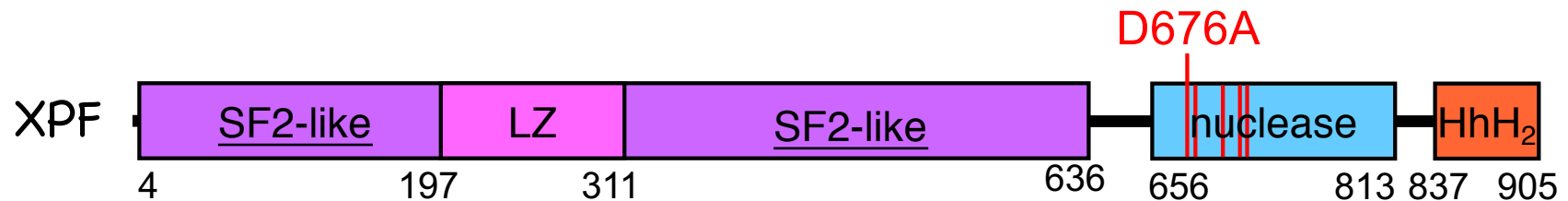
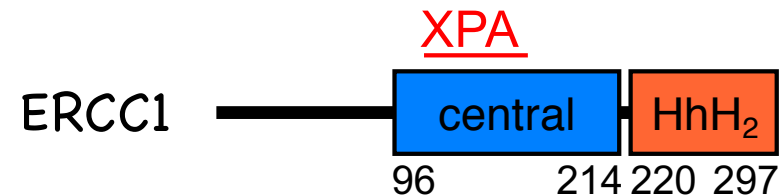
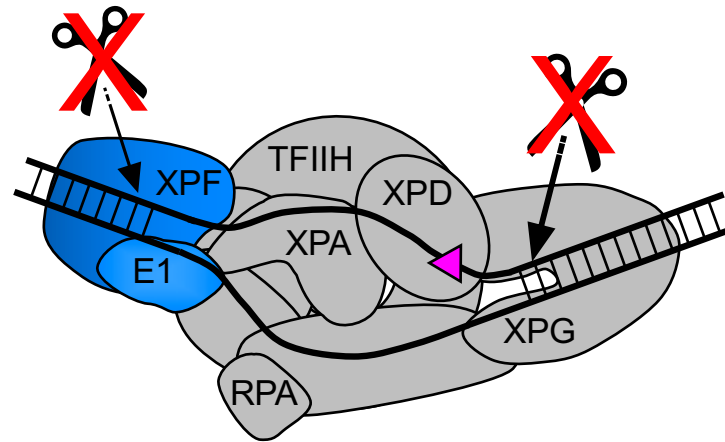


Hanawalt PC (1966) Repair replication in the bacterial genome. In *Genetical Aspects of Radiosensitivity: Mechanisms of Repair* Vol.24, pp 97-104. Vienna: IAEA

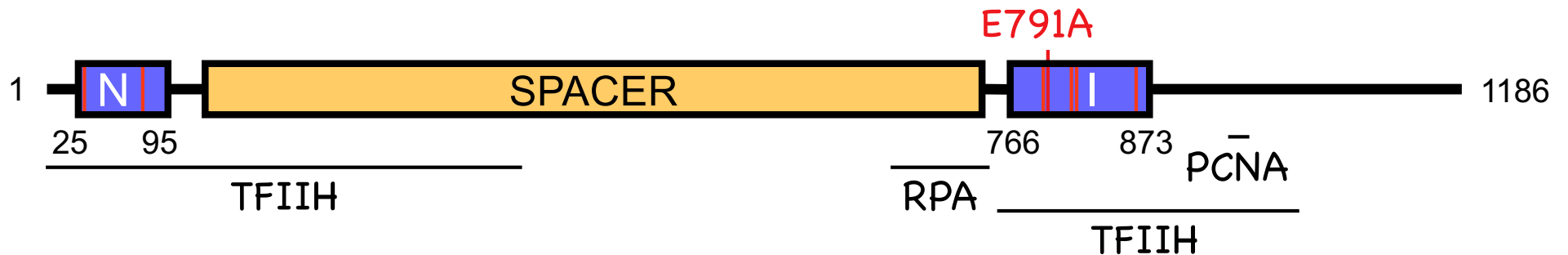
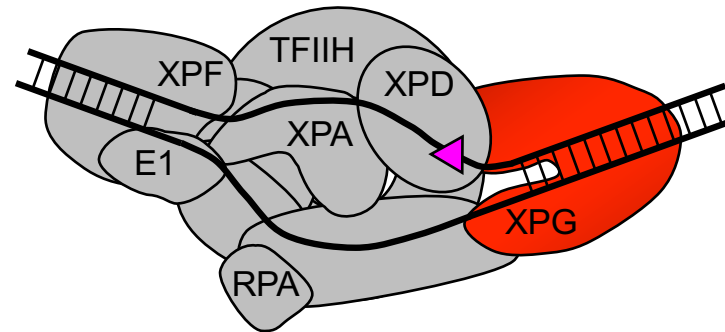
ERCC1-XPF AND ITS ROLE IN NER



XPF-D676A DOES NOT SUPPORT 3' INCISION BY XPG

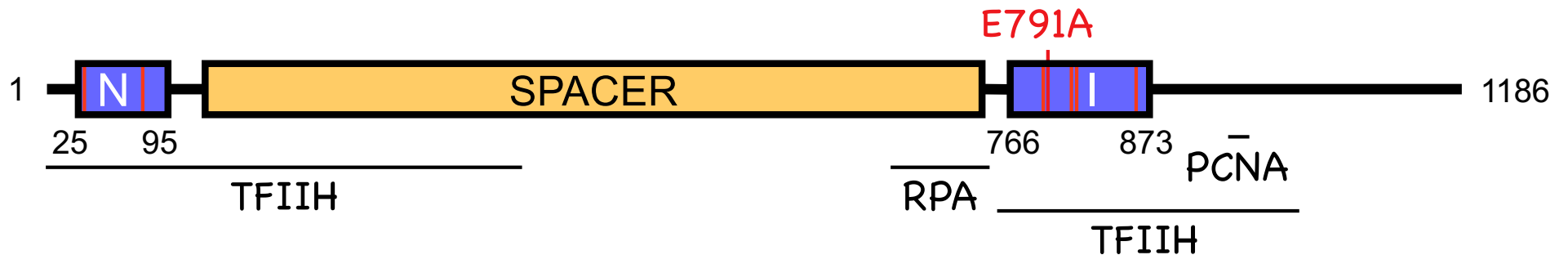
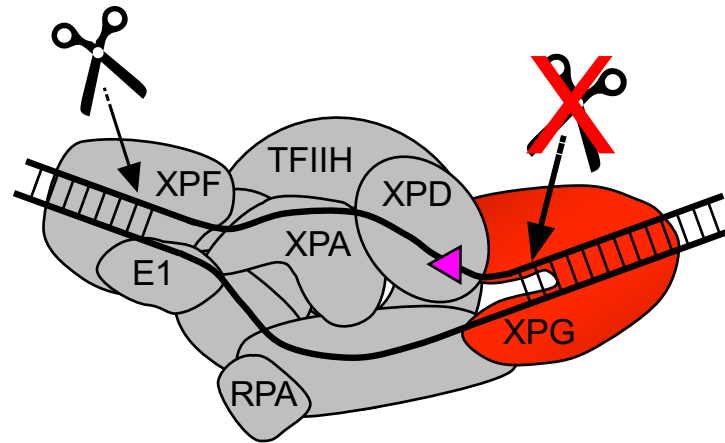


XPG AND ITS ROLE IN NER

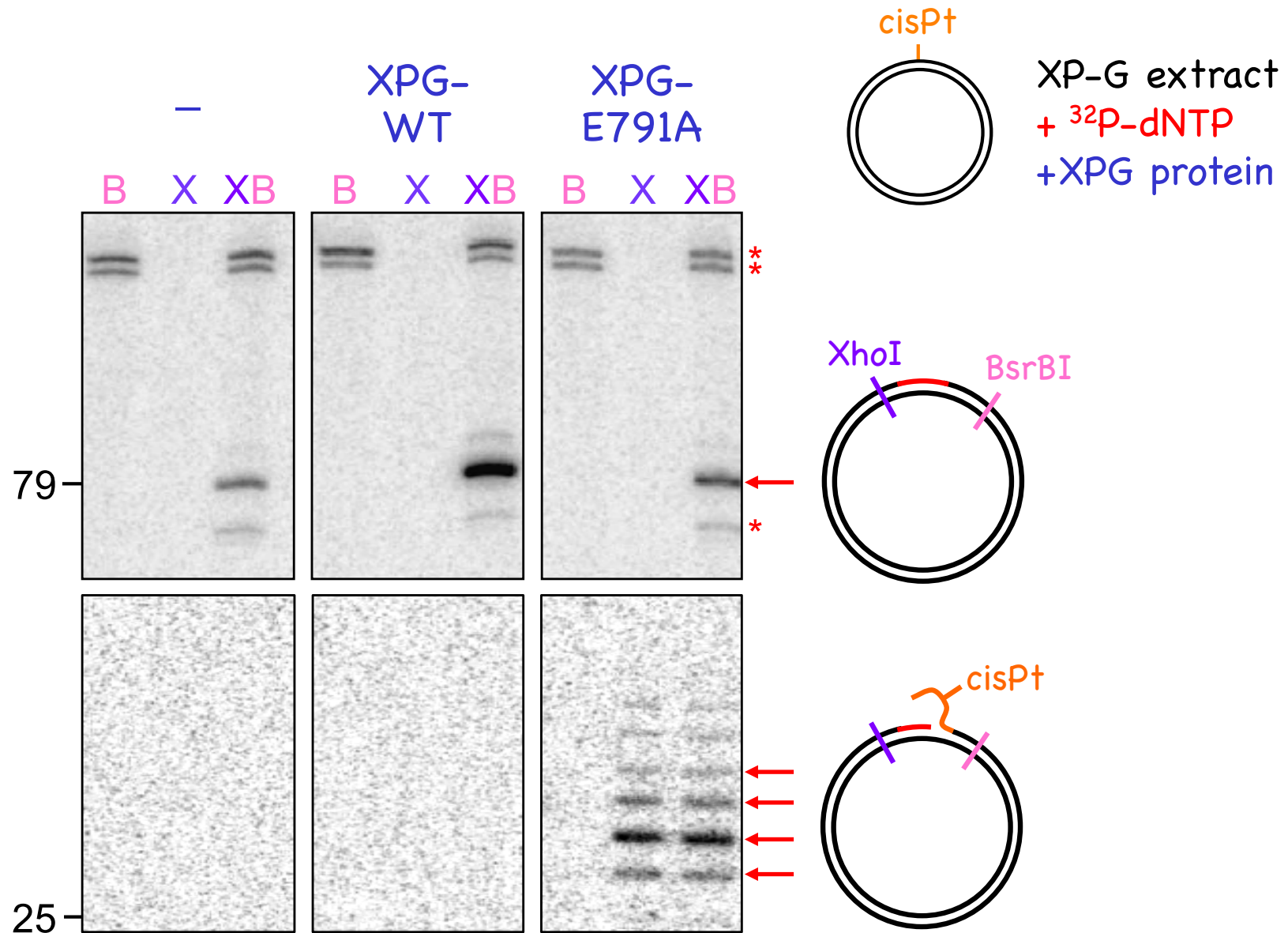


Constantinou et al. **J. Biol. Chem.** 274, 5637 (1999)
Dunand-Sauthier et al. **J. Biol. Chem.** 280, 703 (2005)

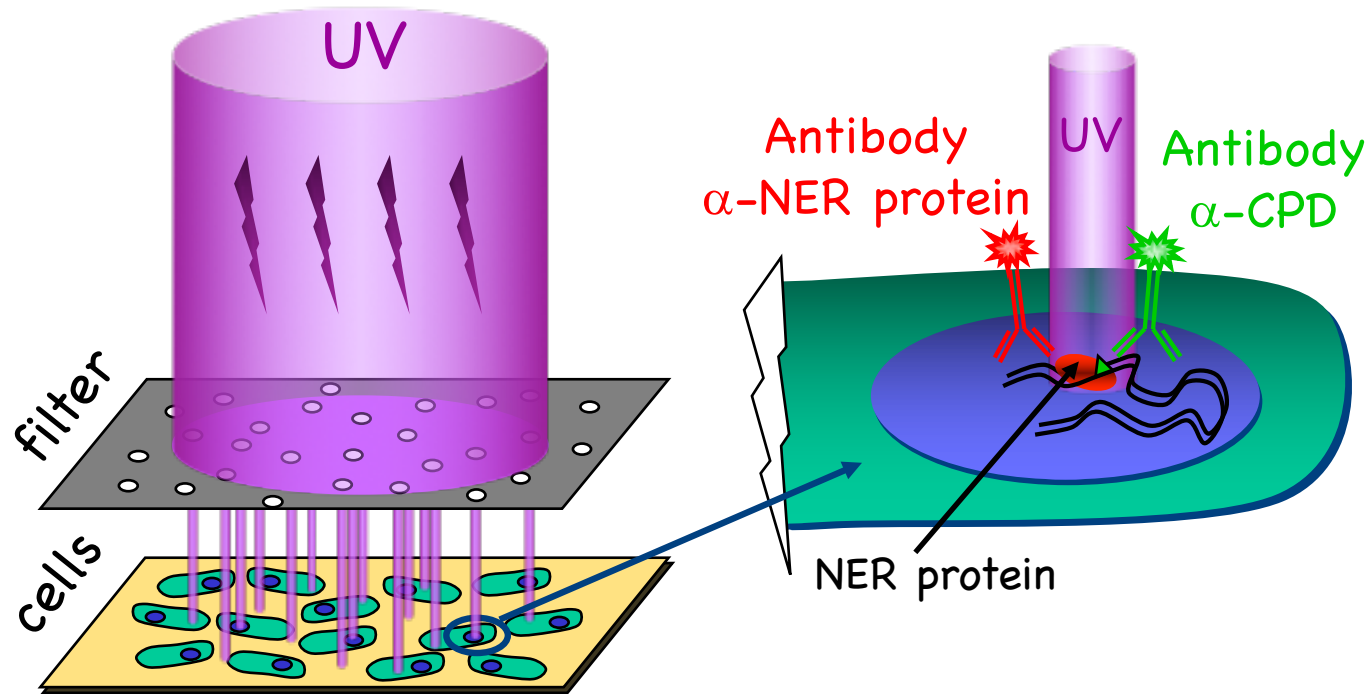
XPG-E791A SUPPORTS 5' INCISION BY XPF



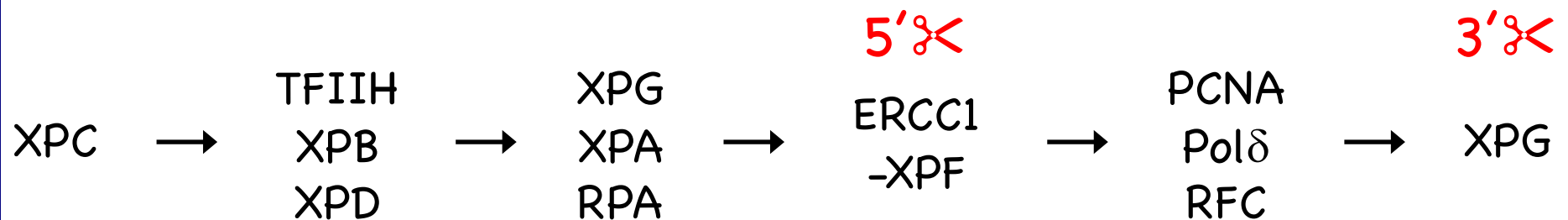
REPAIR SYNTHESIS IS INITIATED BEFORE XPG INCISION



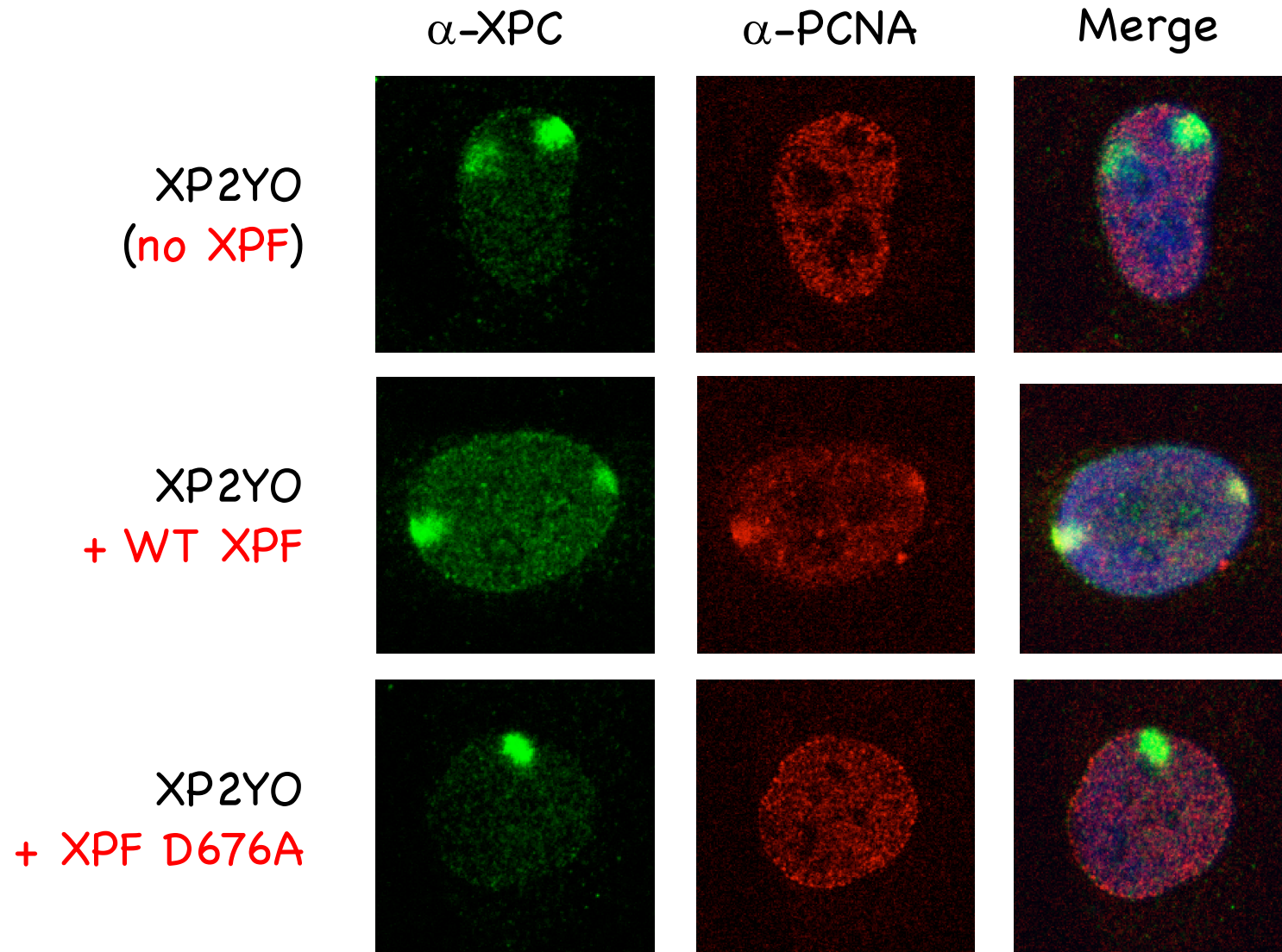
OBSERVING NER AT SITES OF LOCAL UV DAMAGE



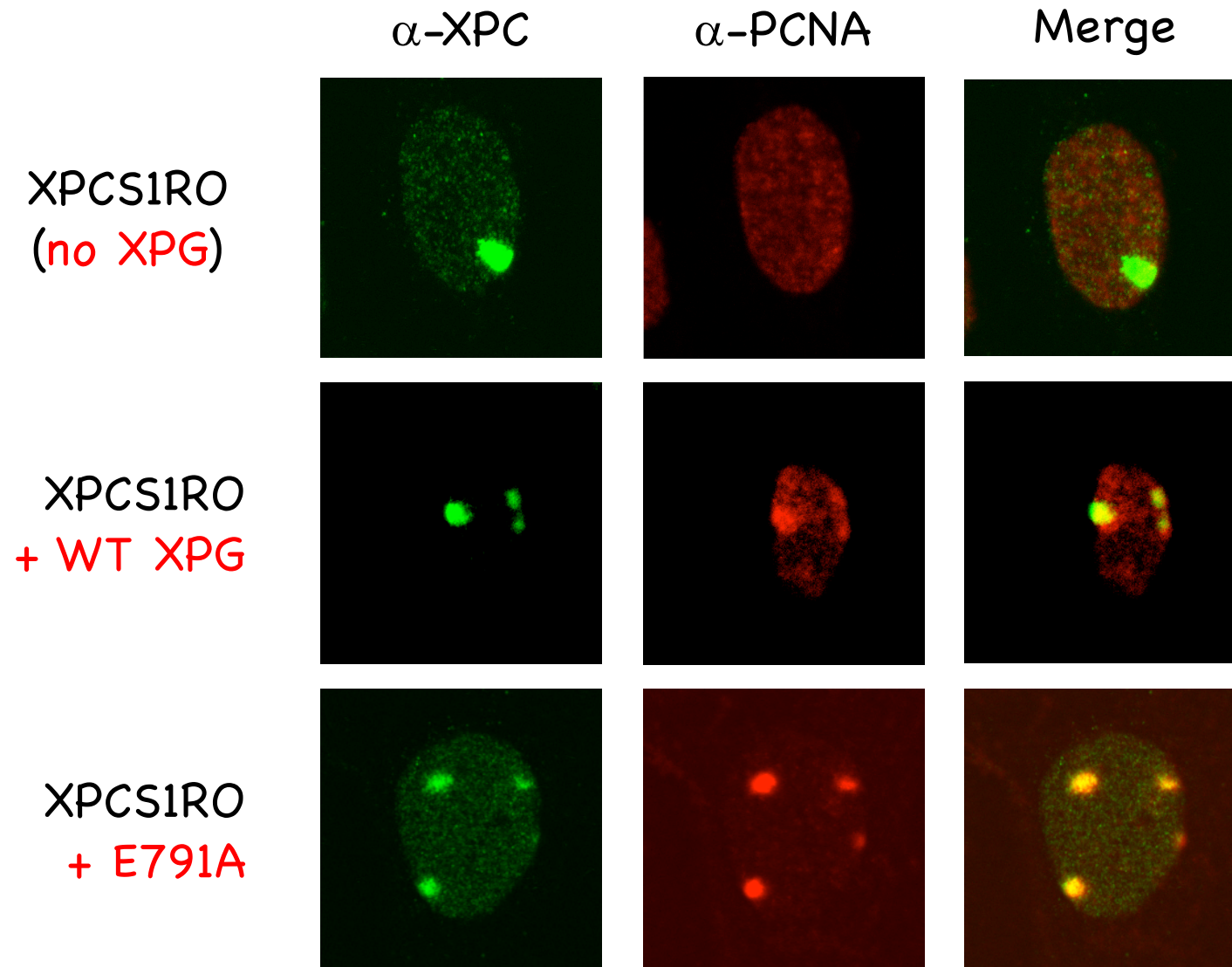
NER TIMELINE



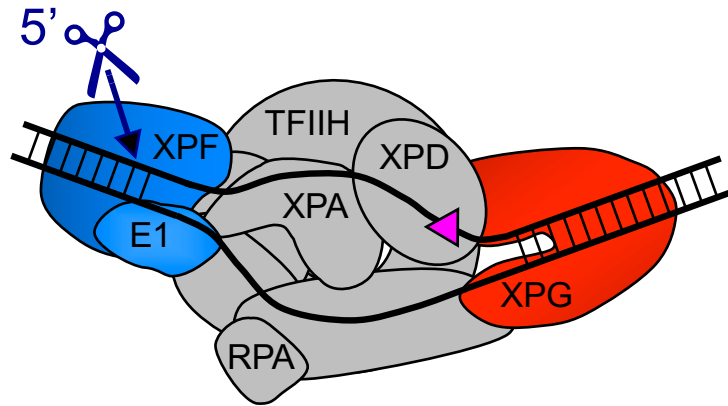
WT XPF IS REQUIRED FOR RECRUITMENT OF PCNA



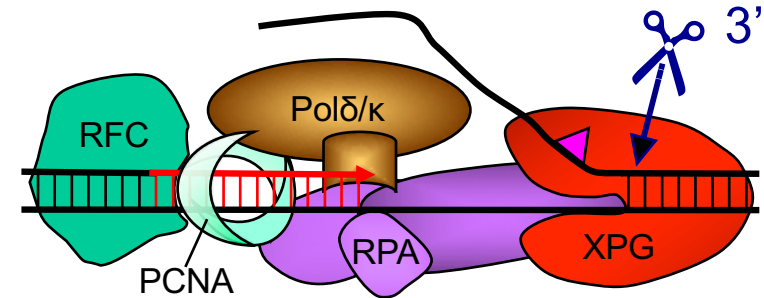
PCNA IS AT UV SITES IN XPG-E791A CELLS



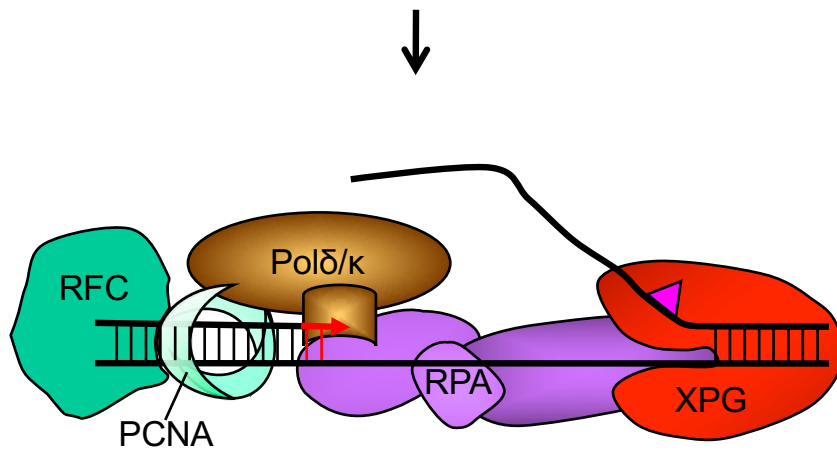
FROM DUAL INCISION TO REPAIR SYNTHESIS



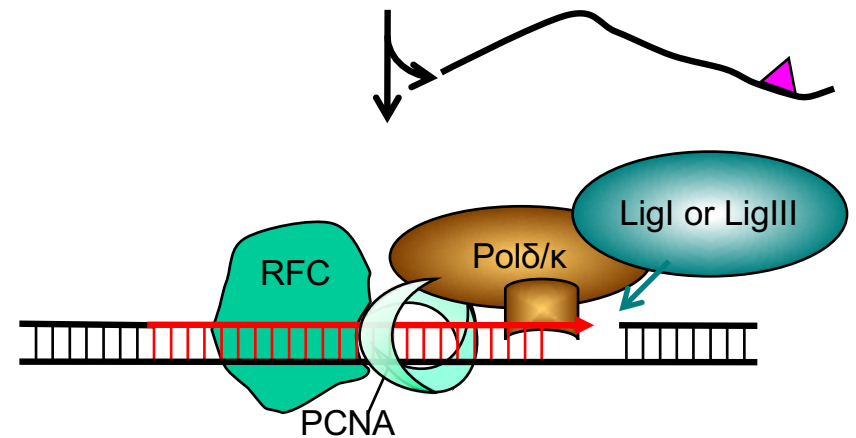
5' Incision by XPF



3' Incision by XPG



Initiation of repair synthesis



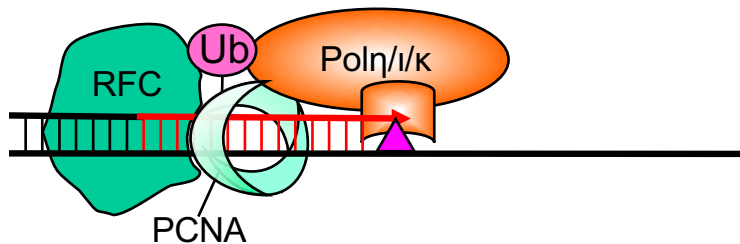
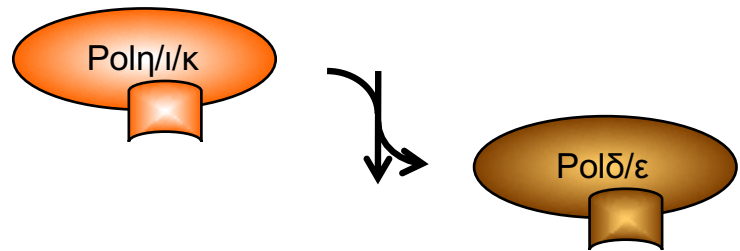
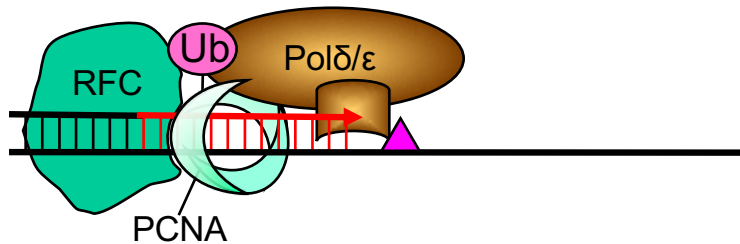
Completion of repair synthesis

PCNA- AND UBIQUITIN-BINDING DOMAINS IN XPG

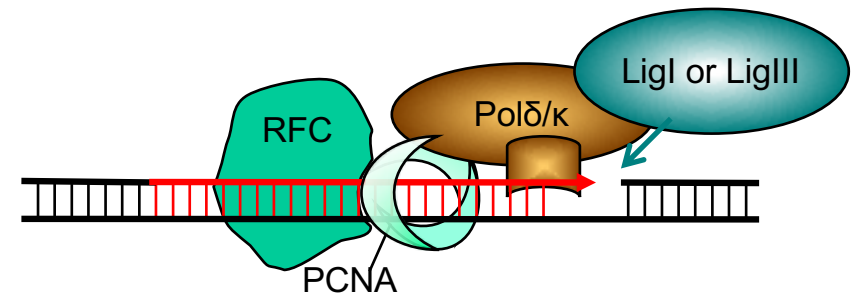
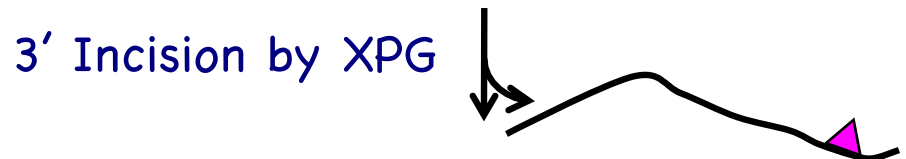
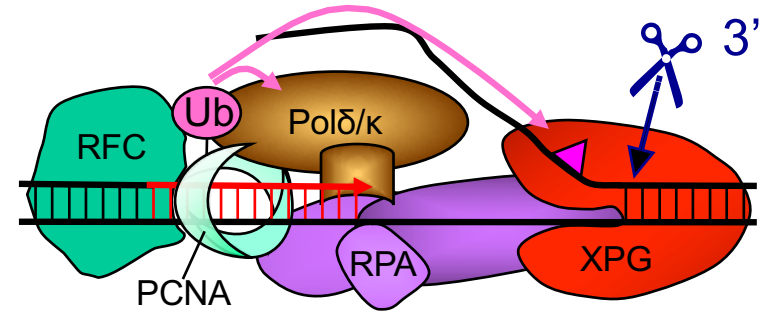


PARALLELS BETWEEN TLS AND NER REPAIR SYNTHESIS?

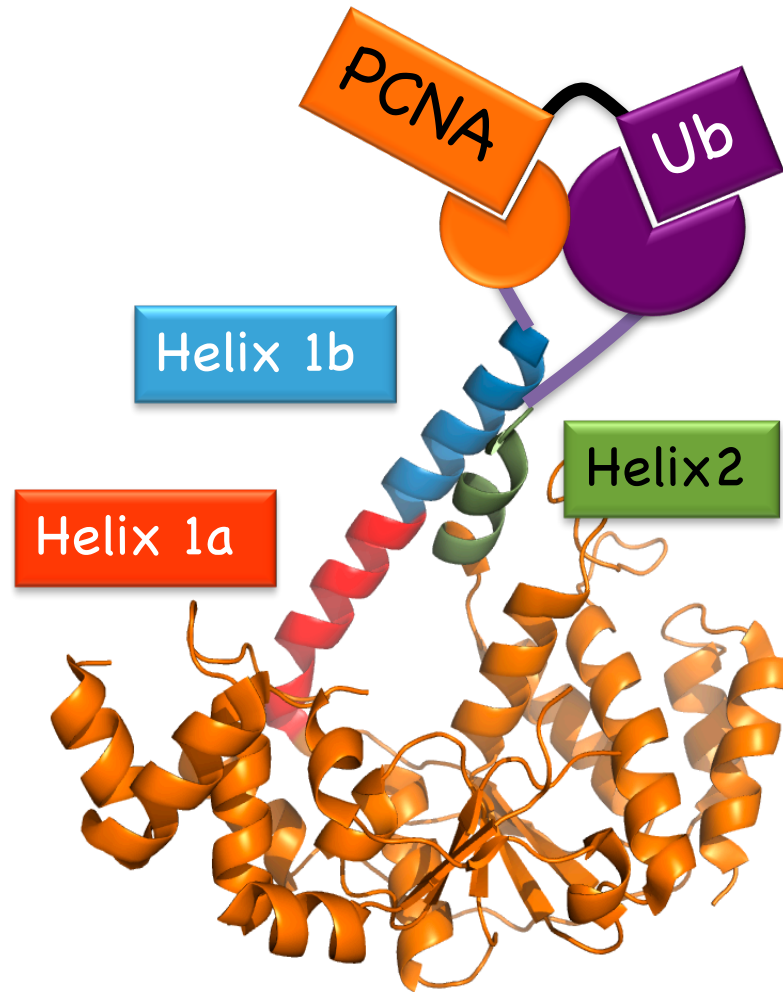
Translesion synthesis



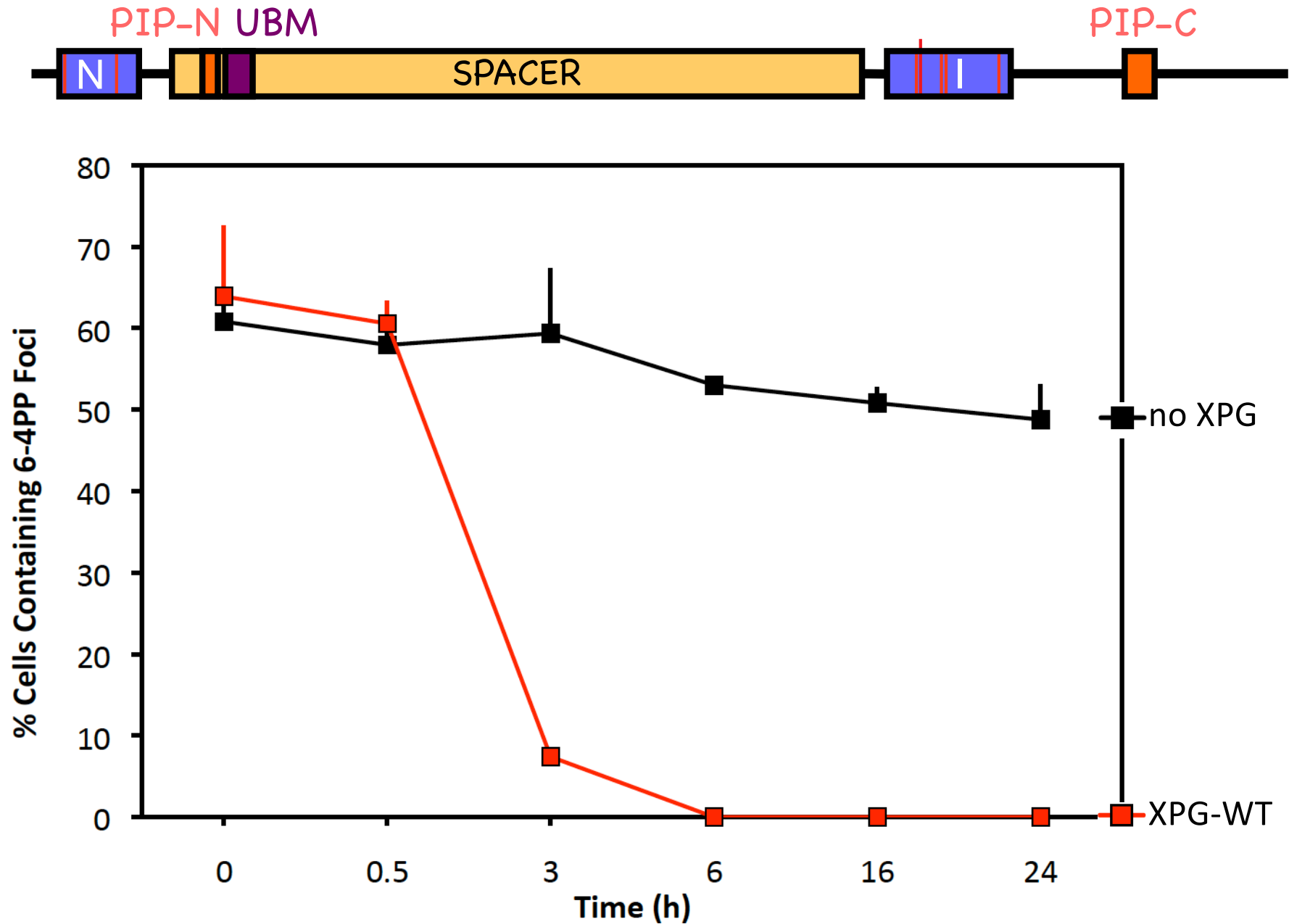
NER incision/repair synthesis



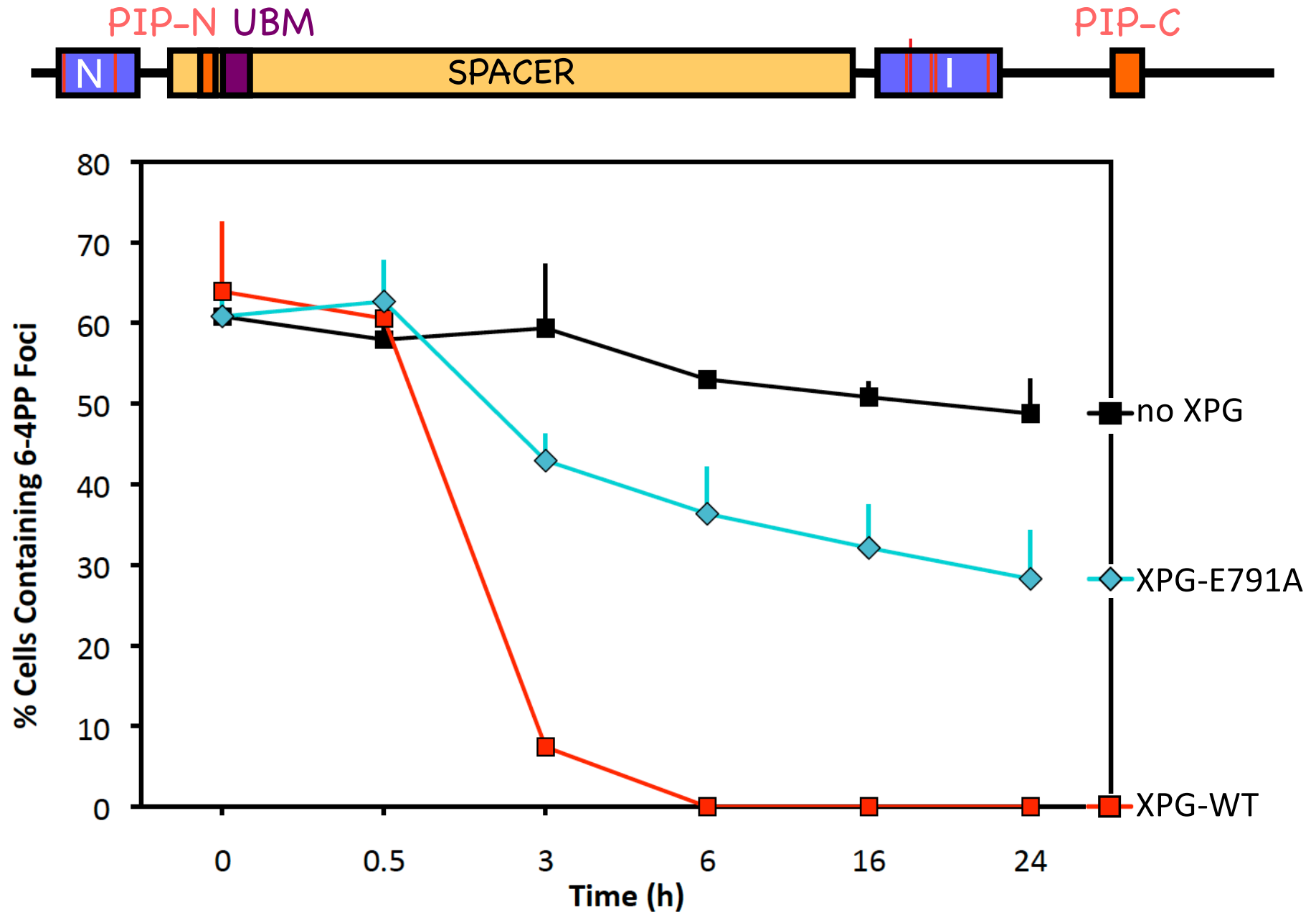
THE UBM & PIPN DOMAINS MAY INFLUENCE INCISION



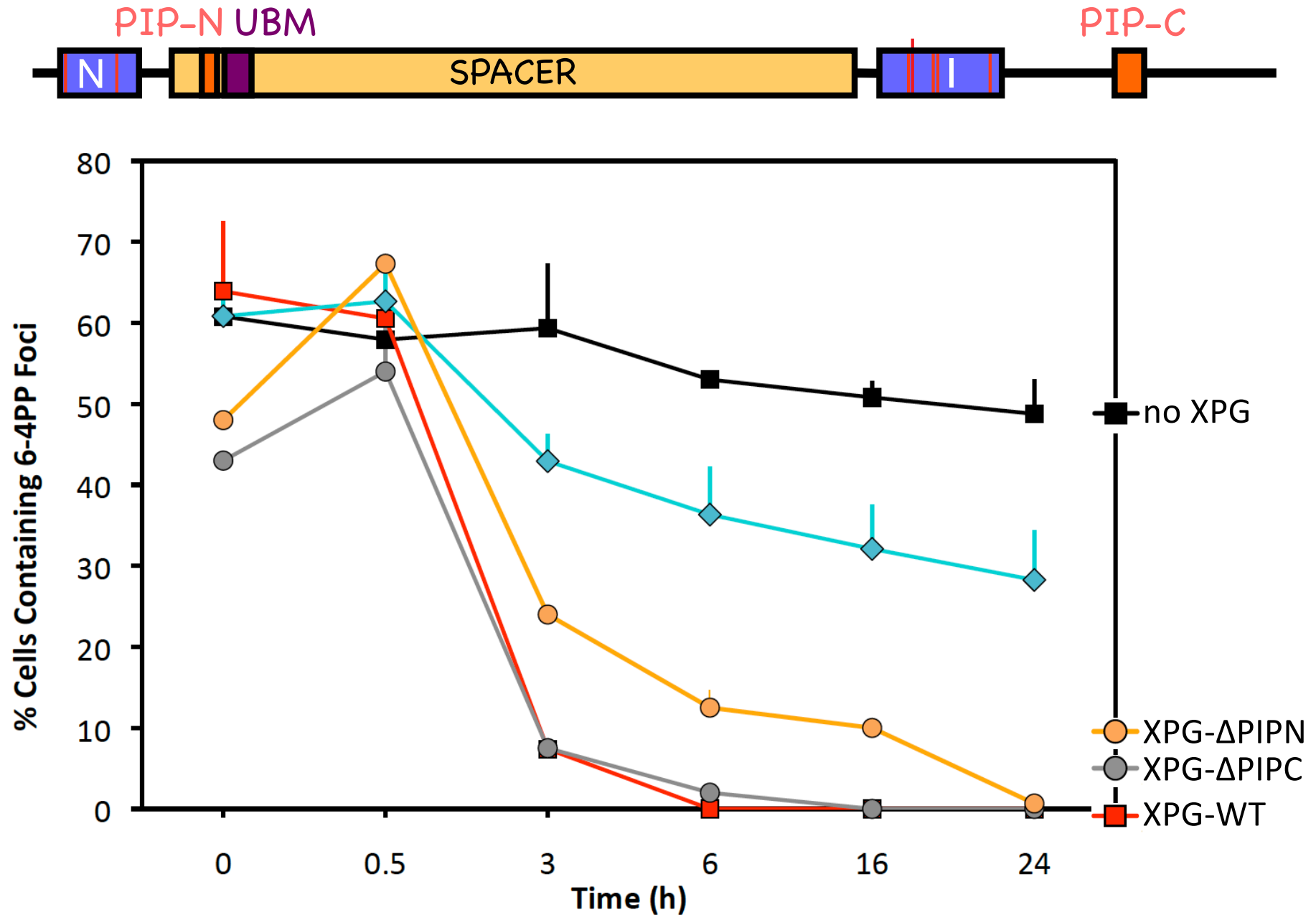
MUTATIONS IN UBM & PIPN, BUT NOT PIPC AFFECT NER



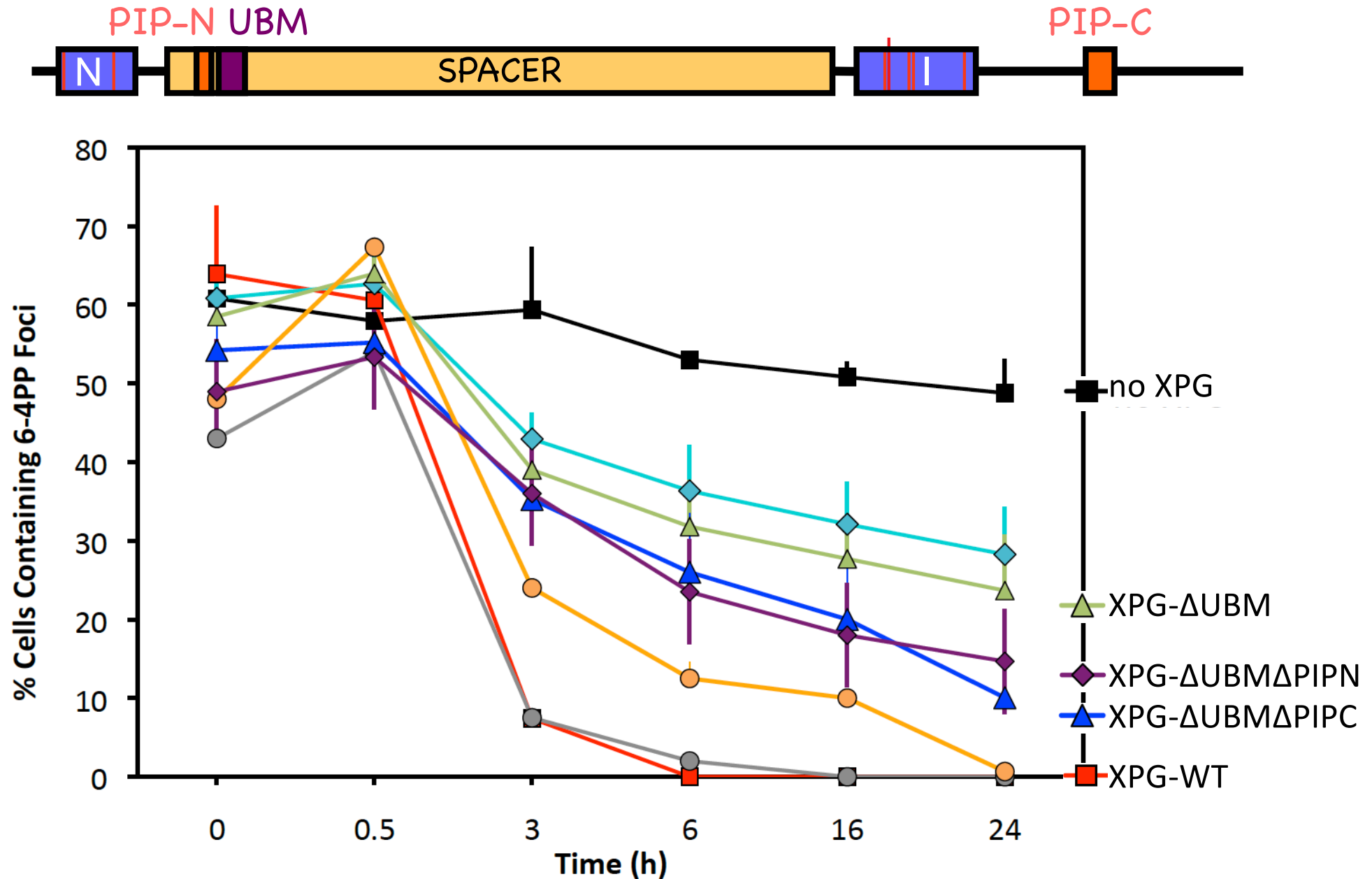
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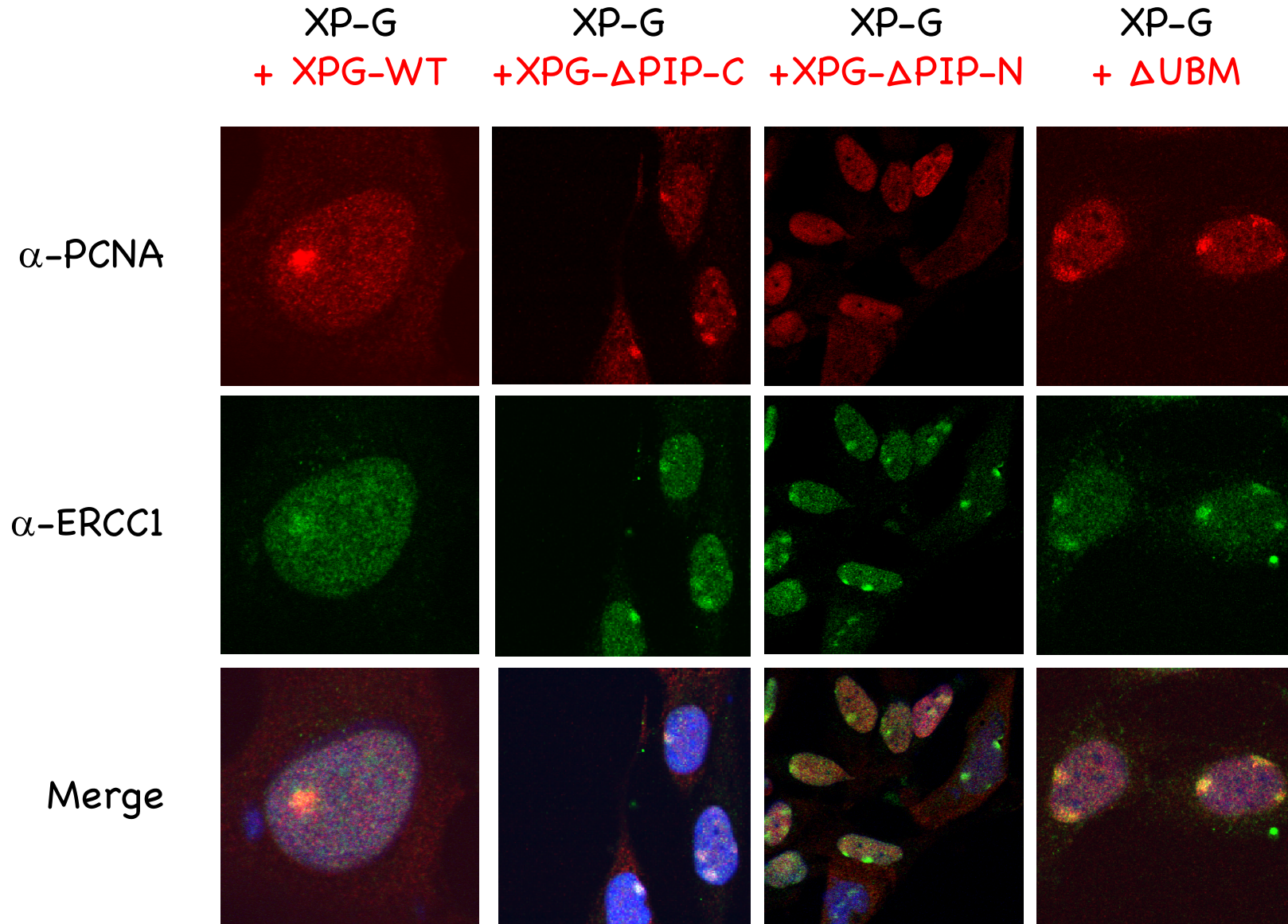
MUTATIONS IN UBM & PIPN, BUT NOT PIPC AFFECT NER



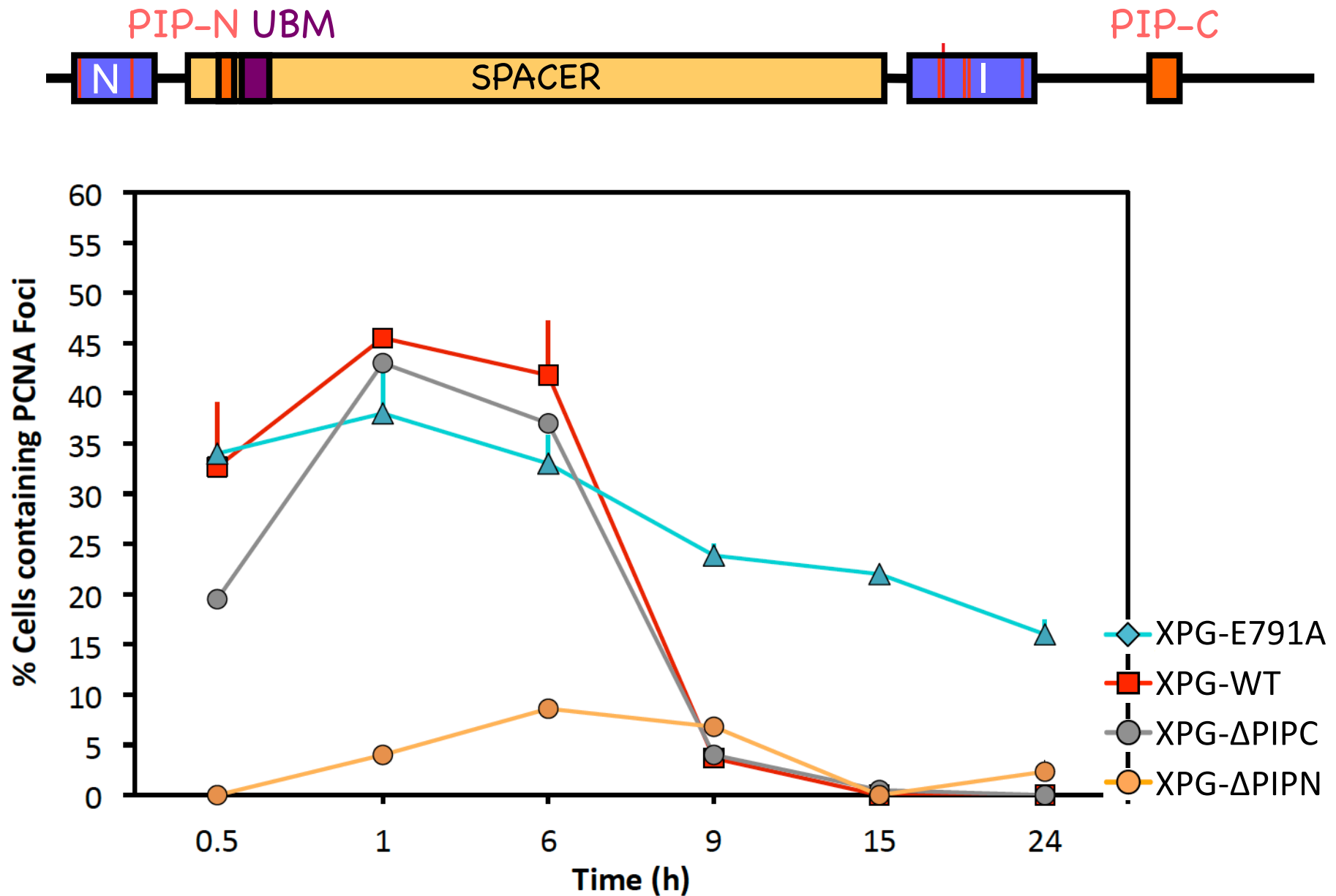
MUTATIONS IN UBM & PIPN, BUT NOT PIPC AFFECT NER



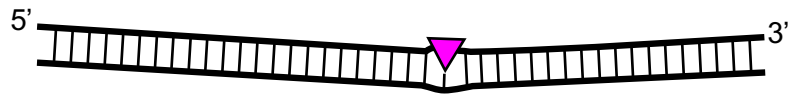
PIP_N, BUT NOT PIP_C IS REQUIRED FOR THE RECRUITMENT OF PCNA TO SITES OF UV DAMAGE



RECRUITMENT OF PCNA TO SITES OF UV DAMAGE

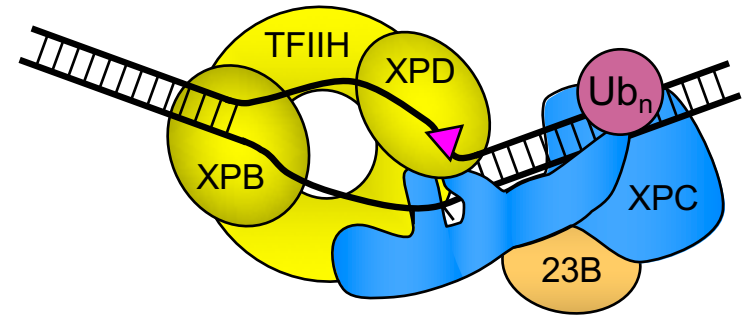
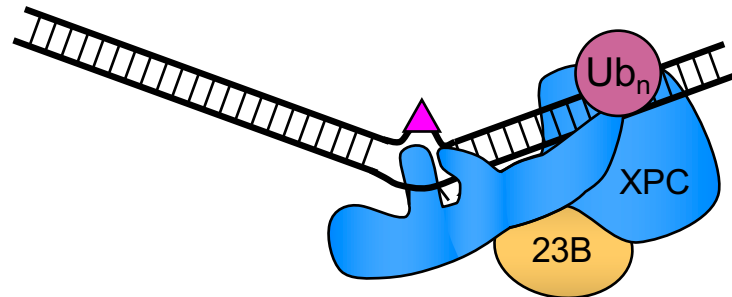
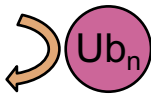


COULD XPG-UBM INTERACT WITH UB-XPC?



DDB1/DDB2
ROC4/CUL4A

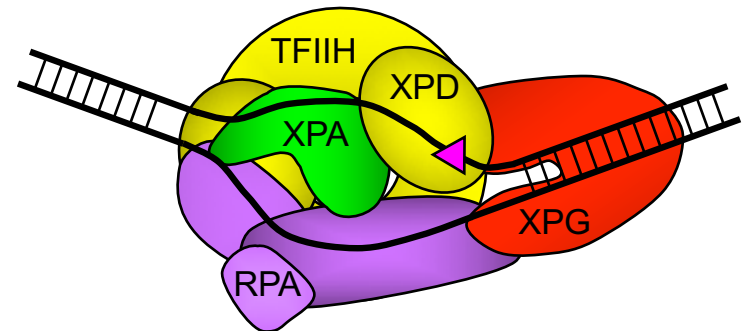
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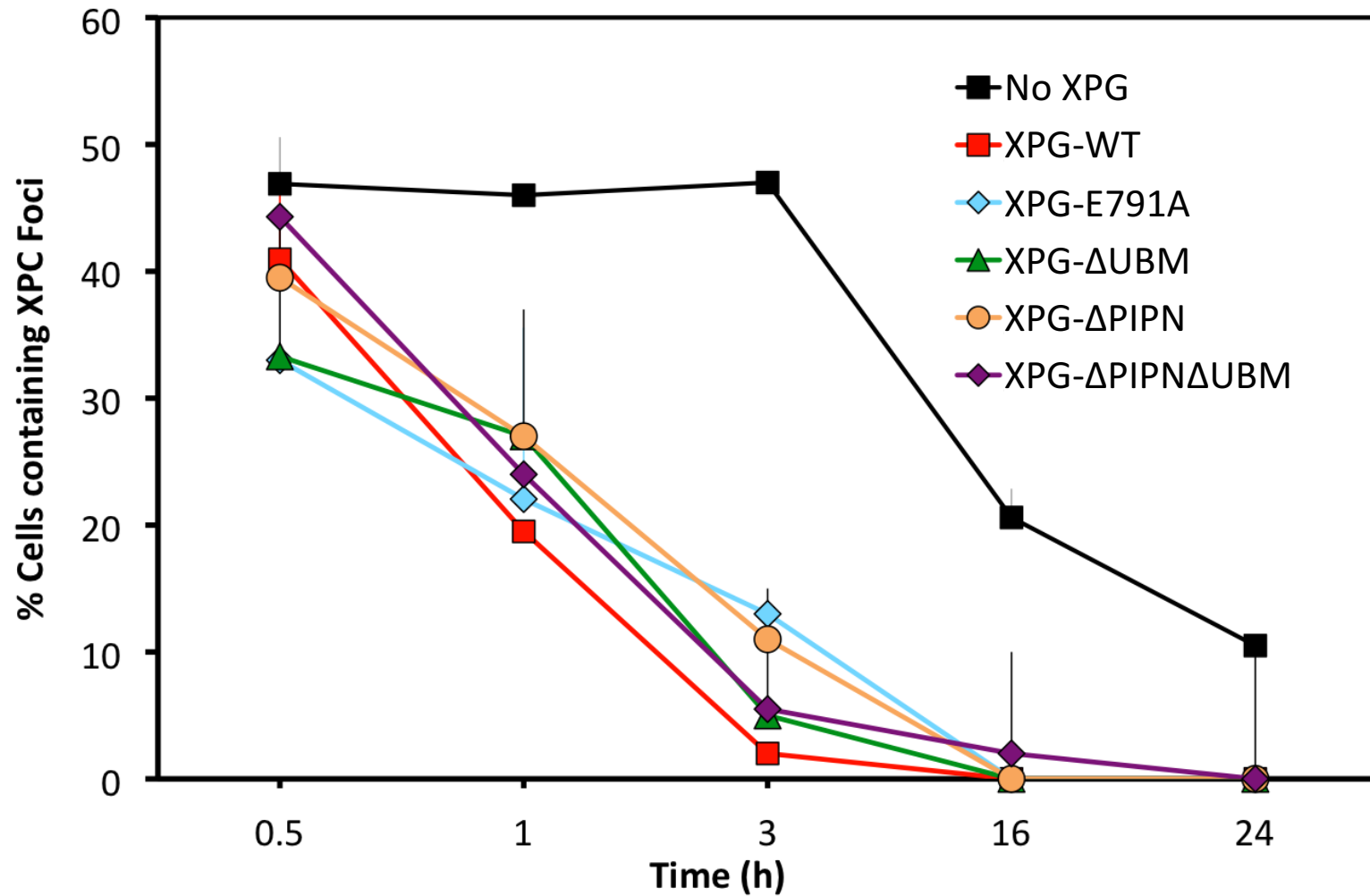
XPG

↓

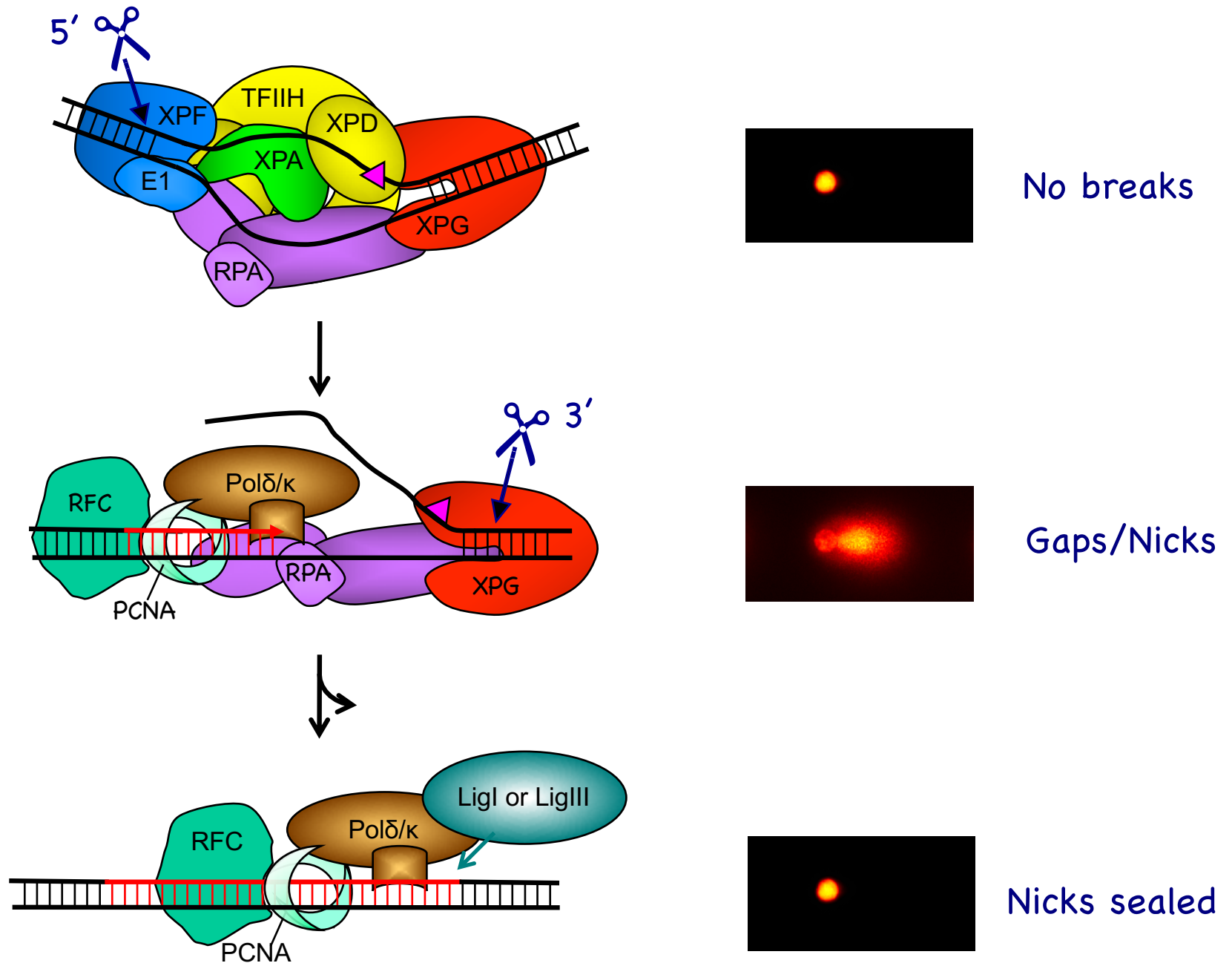
XPC-
RAD23B



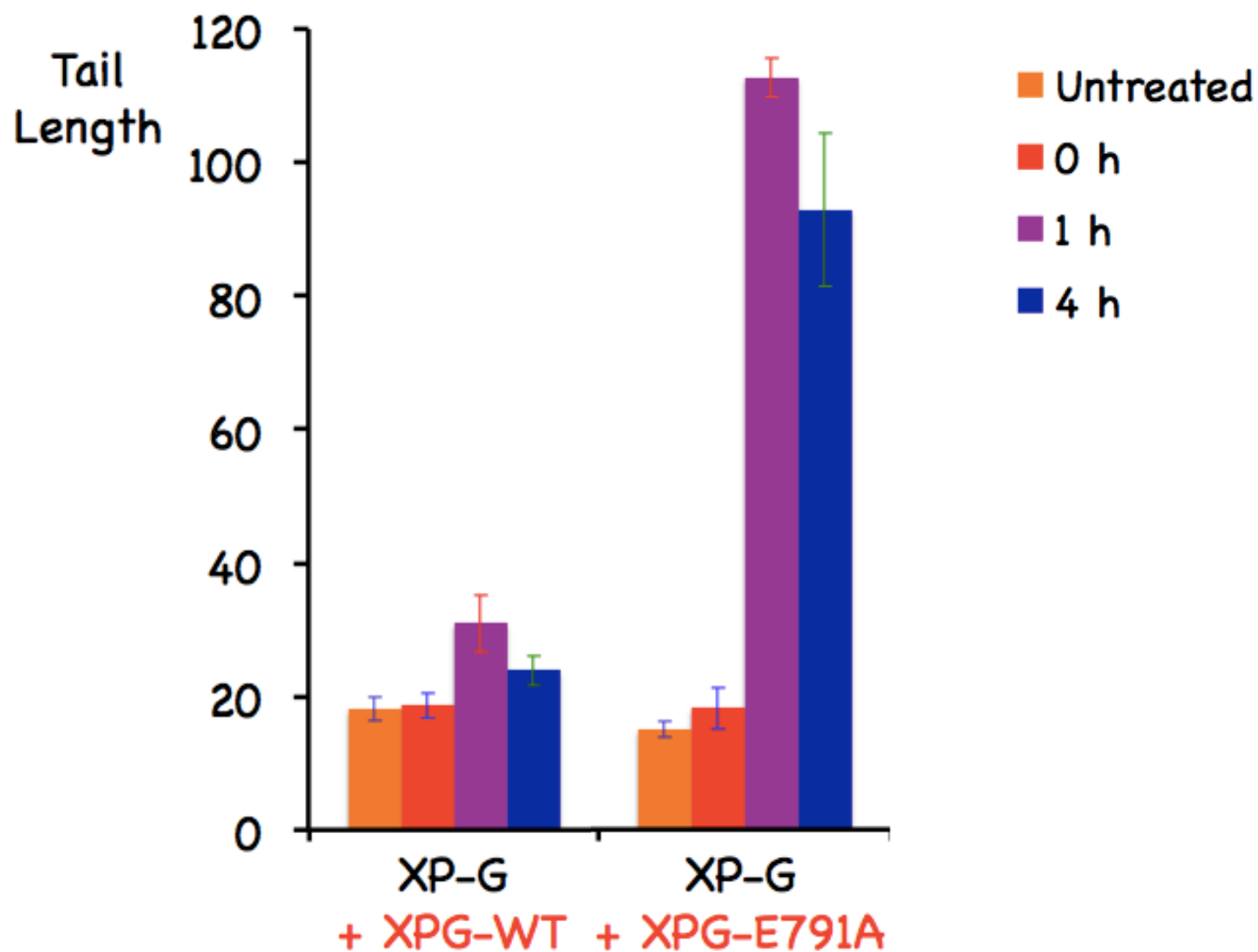
Δ UBM & Δ PIP HAVE XPC DYNAMICS LIKE WT-XPG



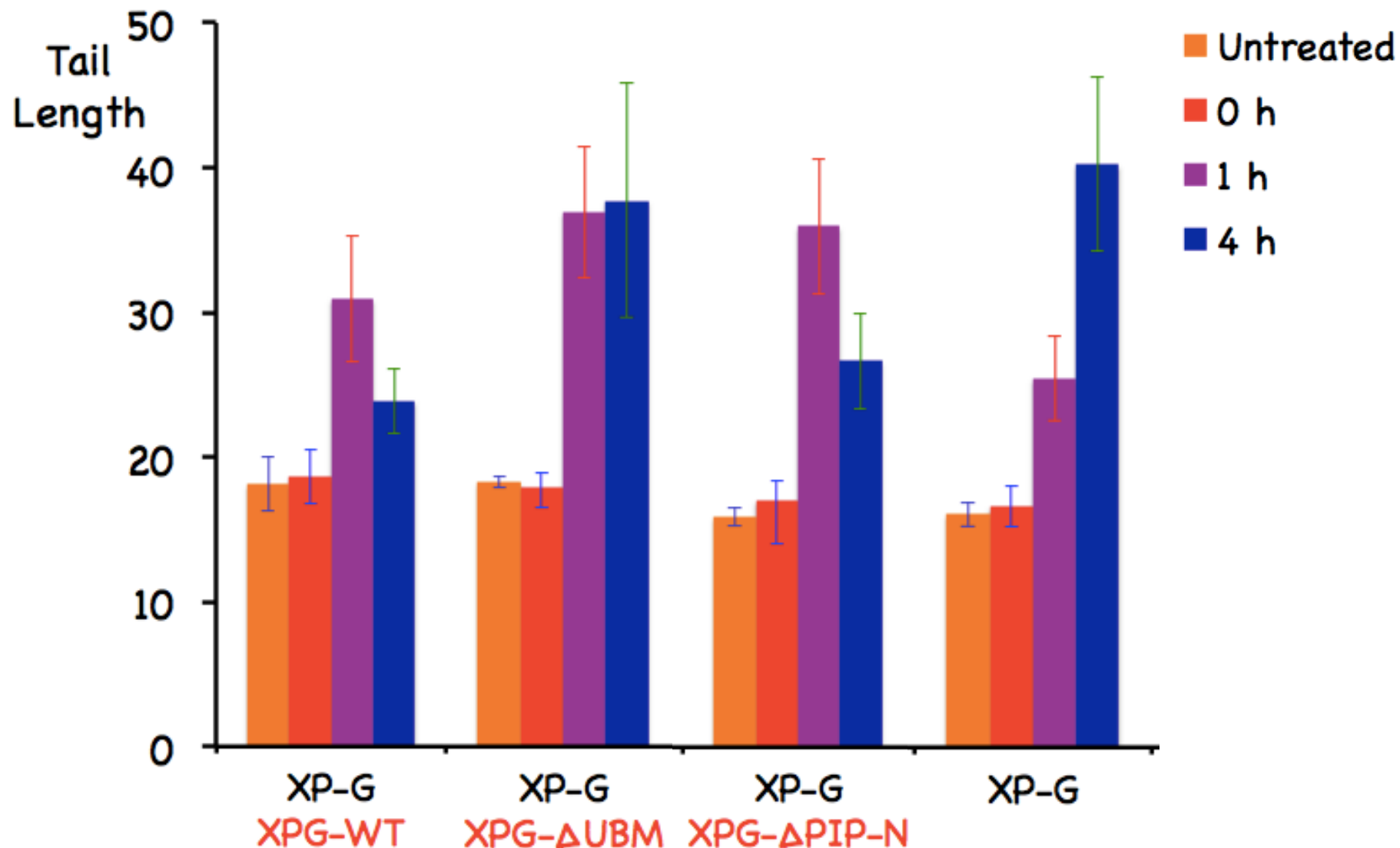
USING COMET CHIP TO FOLLOW NER



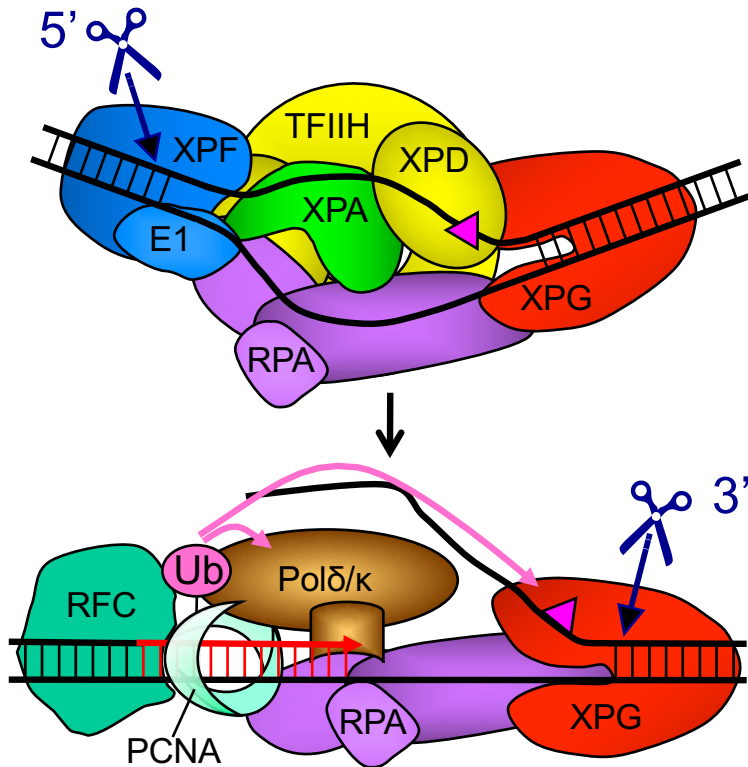
NICKS PERSIST IN XPG-E791A CELLS AFTER UV



COMET TAILS PERSIST IN XPG- Δ UBM CELLS



SUMMARY



- Nuclease activities in NER are tightly regulated to avoid inappropriate incisions and persistence of toxic intermediates.
- XPG is a latent endonuclease and is activated only after 5' incision and partial repair synthesis.
- XPG incision activity is regulated by interaction of XPG with PCNA and ubiquitin.
- Regulation of XPG incision may be the key switch between NER and DNA damage signaling

THANK YOU!

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Burak Derkunt

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Tom Ellenberger (WashU)

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Laura Niedernhofer (Scripps)

Nikhil Bhagwat

Advaitha Maddireddy



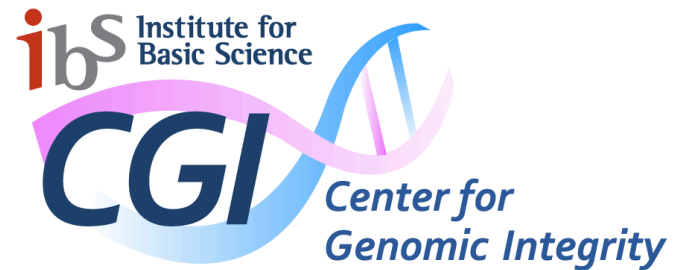
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