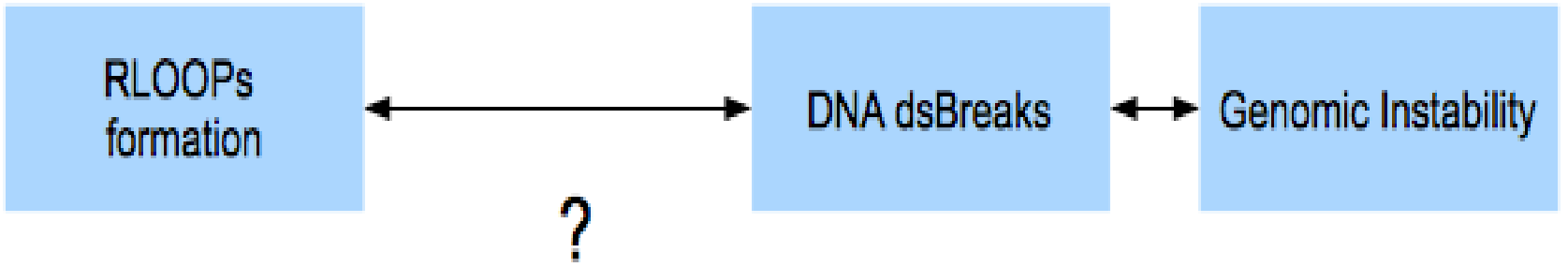
An example of how RLoops induced DNA damages



Molecular Cell
Short Article

CellPress

Transcription-Coupled Nucleotide Excision Repair Factors Promote R-Loop-Induced Genome Instability

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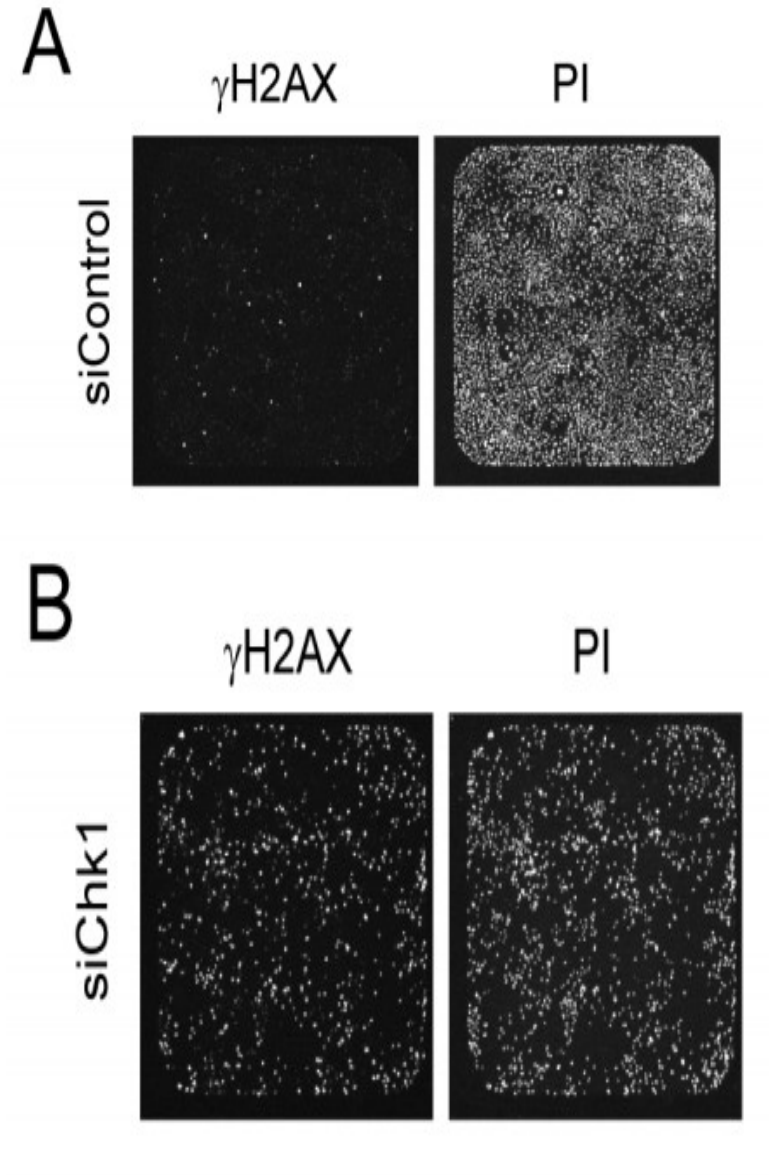
How to choose the cellular model

- Search in data derives from a genomic-wide SiRNA screening performed in HeLa cells.

- The measure of H2AX phosphorilation was the readout to obtain « candidate gene » with a key role in genomic stability.

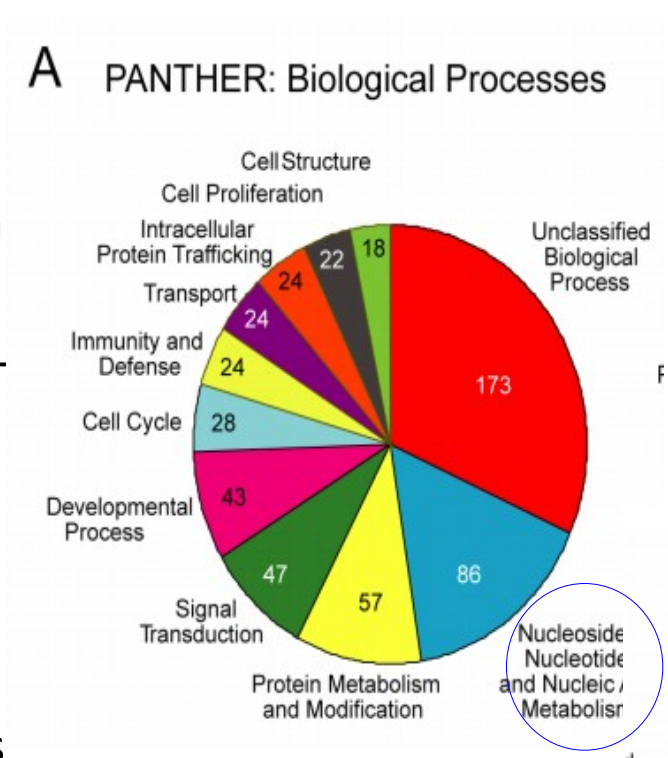
The cellular model should show genomic instability-related characteristics (DNA damages)

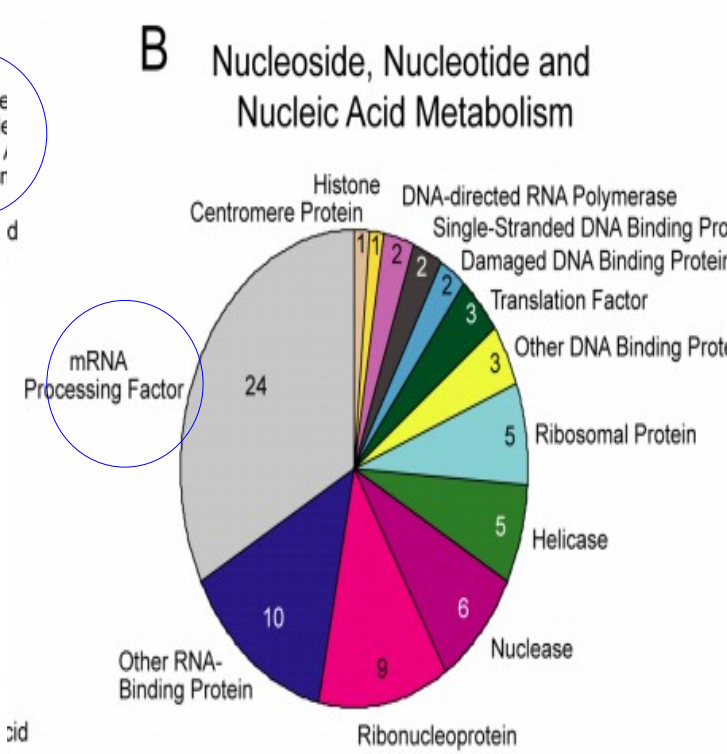


How to choose the cellular model

- Search in data derives from a genomic-wide SiRNA screening performed in HeL cells.

- Bioinformatics analysis reveals the spectrum of biological function of genes involved in genomic instability → choose mRNA processing





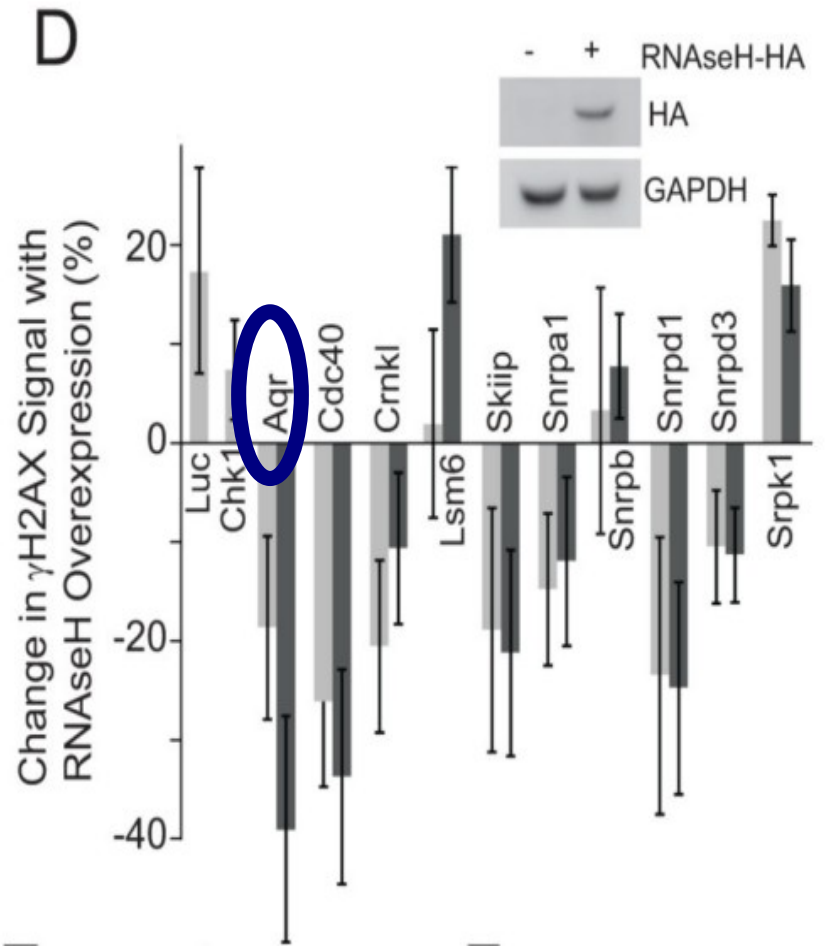
The cellular model should show Genomic instability-related characteristics (DNA damages) in a RNA processing context

How to choose the cellular model

- Test if DNA damage caused by mRNA processing genes may involve the formation of Rloops.

↓

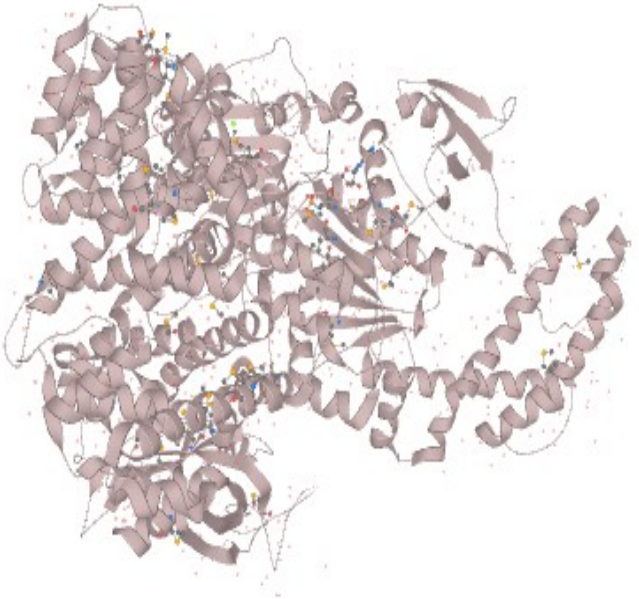
- Analyse phosphorilated H2AX signal before and after the over expression of RNaseH, a preventing Rloops factor



The cellular model should show a correlation between Rloops and DNA damages

AQUARIUS (AQR)

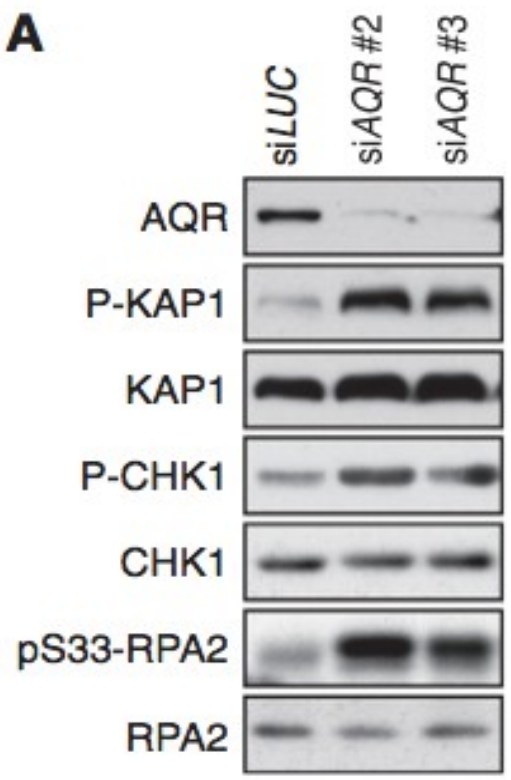
Aquarius is a spliceosome associated factor that binds precursor-mRNA introns at a defined position.

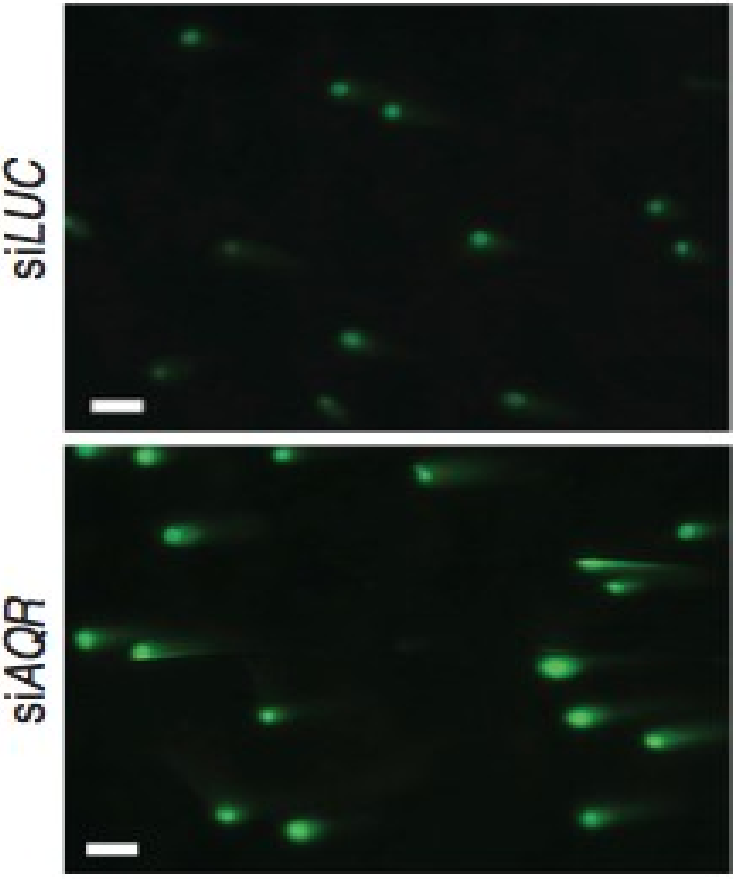


belonge to a subfamily of protein possessing a DEAxQ domain that is a putative **RNA/DNA helicase** domain.

The cellular model should show a correlation between Rloops and DNA damages

Aquarius knockdown (AQR⁻) in HeLa cells

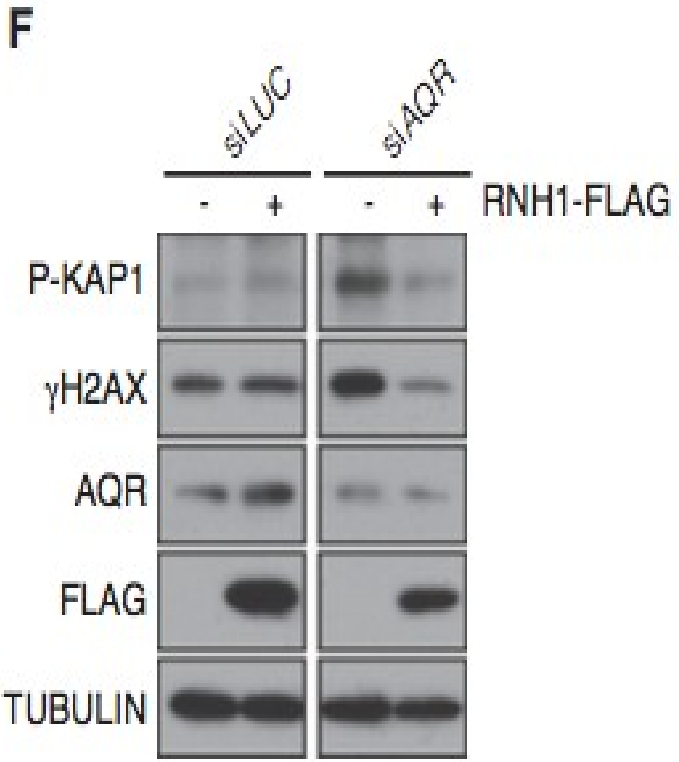


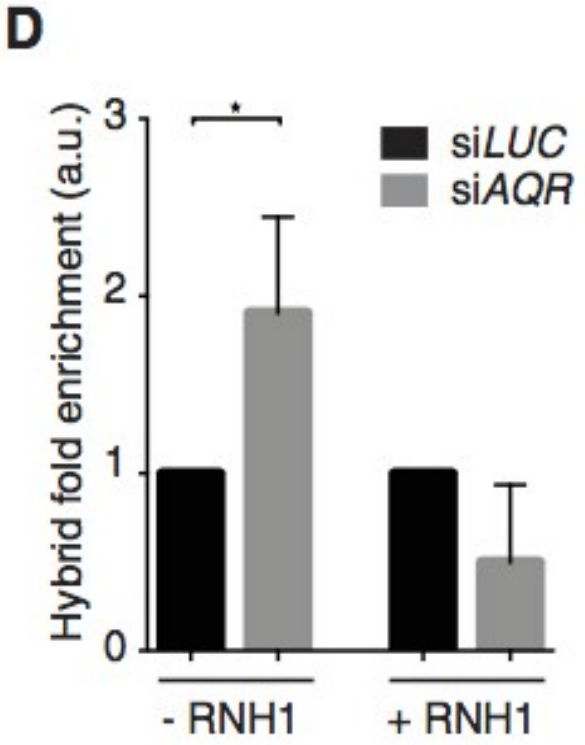


- Knockdown of AQR induced the DNA damage response (DDR) by phosphorilation of its actors
- Knockdown of AQR induce DDR by dsBreaks production (comet assay)

AQR depleted cells are a model for genomic instability

RNase H1 overexpression in AQR- cells





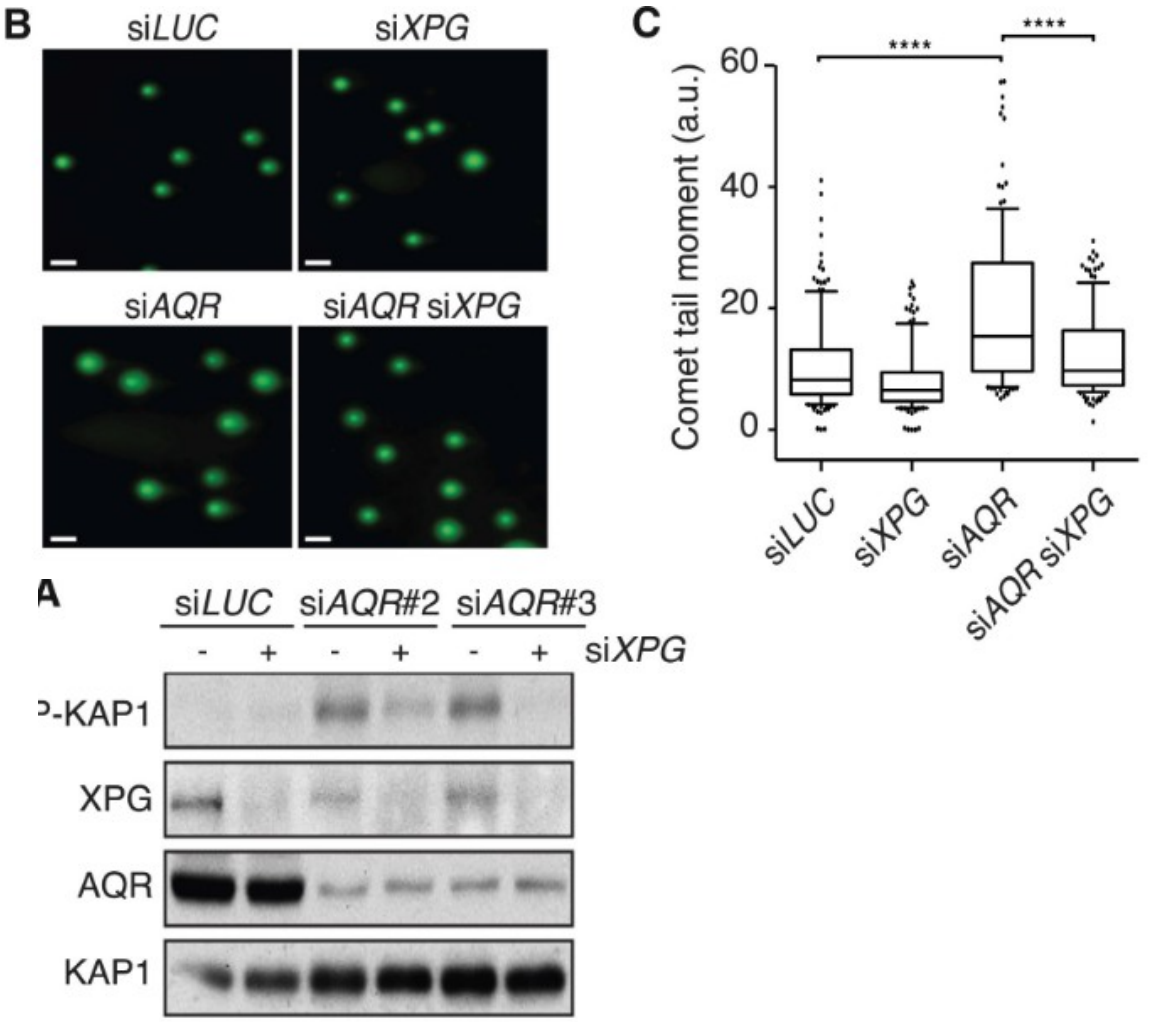
AQR- depleted cells show a correlation between Rloops and DNA damages

- Inducible system HeLa Tet-ON cells transfected with siLUC siAQR, expressing TET-tight inducible Flag tagged RNasi show reduction of H2AX phosphorilation levels
- Quantification of RNA:DNA hybrids with antibody s.9.6 in genomic DNA extractions reveals enrichment of hybrids in AQR depleted cells, abolished by RNasi pretratment

How RLoops induced DNA damages?

HP: similar nuclear structures could be recognized and processed by similar nuclear factors.

- Open DNA structures, such as Rloop, could be processed by Nucleotide Excision Repair factors (NER), ando so by the endonucleases XPF and XPG.



Double knockdown AQR- XPG-show a reduction of DDR reponse and dsB formation

Effects of XPG in AQR depleted cells

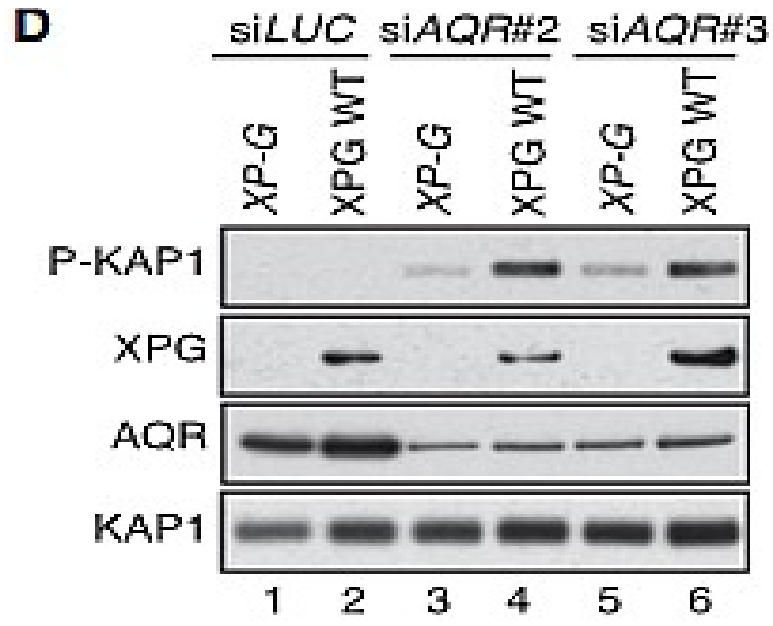
Cellular model: immortalized XPG- fibroblast from xeroderma pigmentosum patient, isogenic cell line complemented with XPG wild-type.

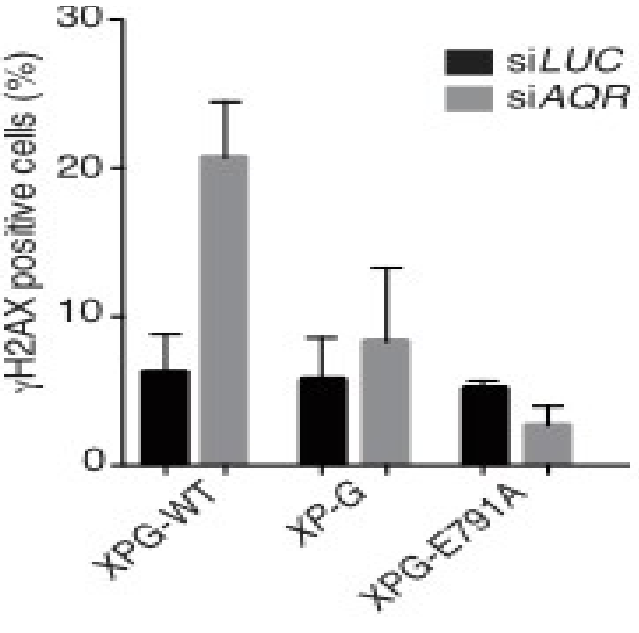
- AQR- in XPG complemented cells show induced phosphorilation of PKAP1. In XPG deficient cells this phosphorilation is reduced.

  XPG plays only a structural role?

- **Cellular model:** XPG- complemented with a nuclease-dead form.

- H2AX phosphorilation signal is lower in XPG-dead form AQR- cells.





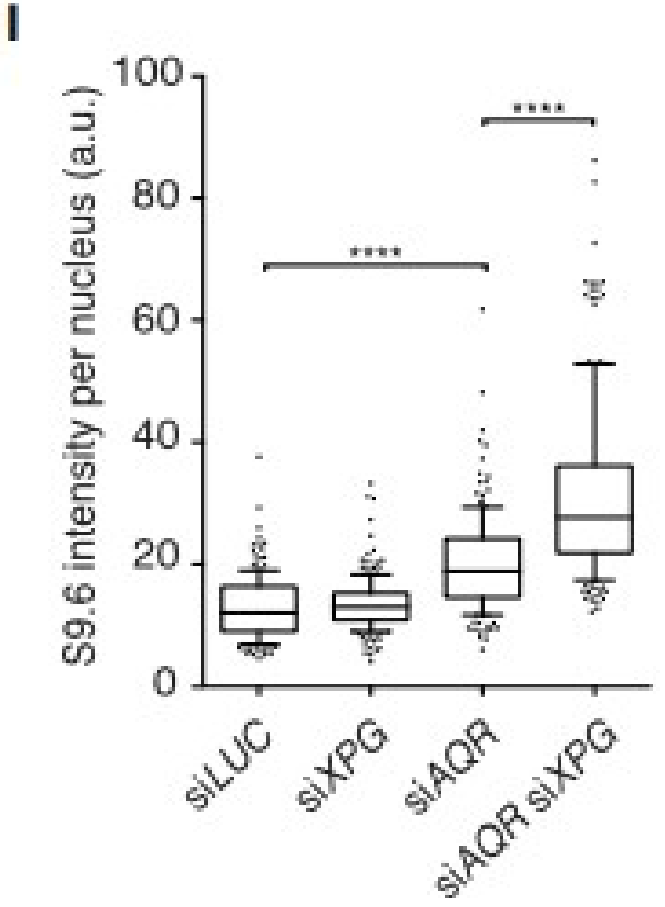
XPG increase DDR reponse, Its nuclease activity is required to generate DSBs from AQR knockdown

XPG knockdown is directly associated with Rloop processing?

Two model:

1) XPG defeciency cause a decrease in general transcriptional processes,and Rloops levels →reduction in DNA damages

2) XPG deficiency cause lack of processing Rloops in DSBs → reduction in DNA damages but increase in Rloops level



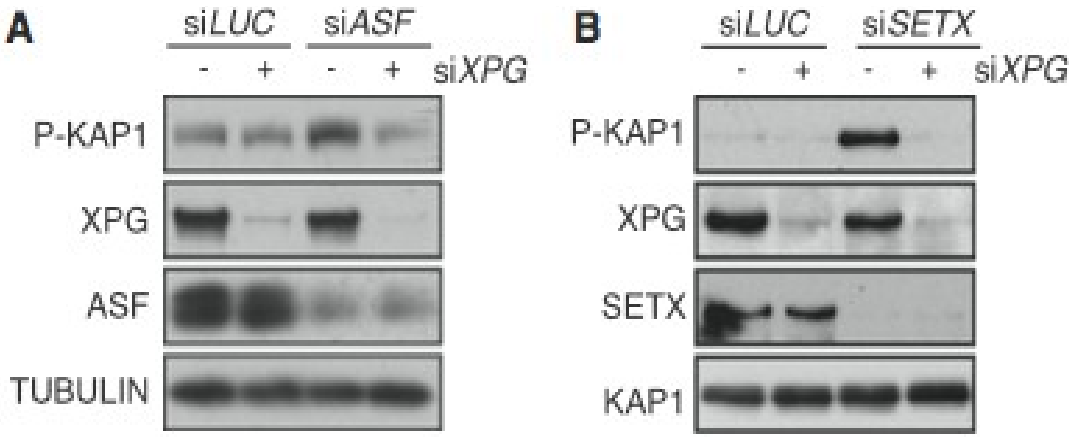
RNA:DNA hybrids presence is increased when both AQR and XPG are knocked down XPG induce dsB by processing Rloops

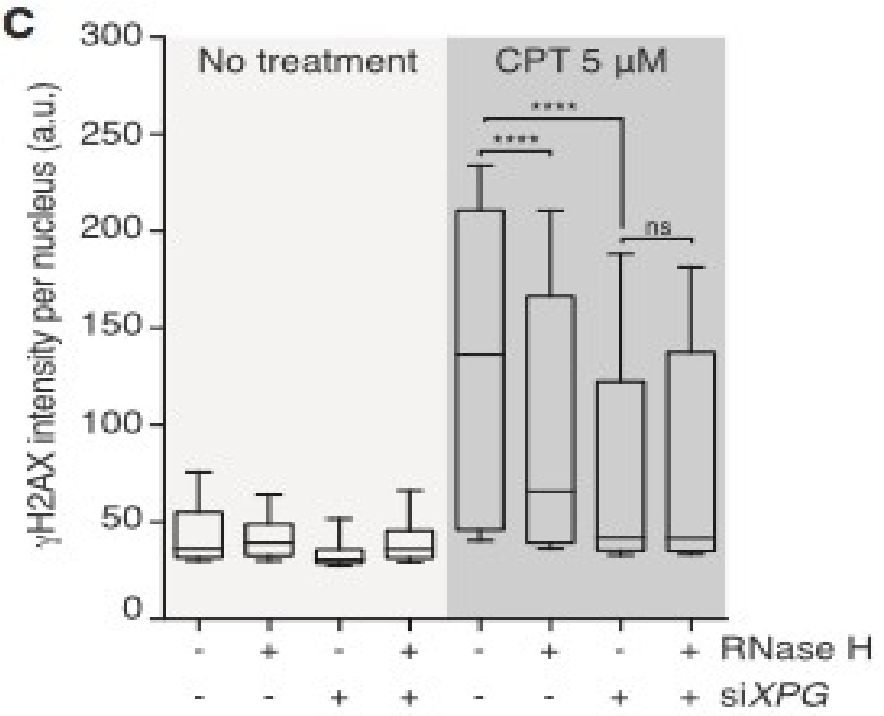
XPG role is conserved between induced-Rloops factors

- XPG knockdown in splicing factor ASF/SF2 depleted cells and helicase SETX depleted cell abrogates DDR response

- XPG knockdown in cells treated with a topoisomerasi I inhibitor (CPT) reduce H2AX phosphorilation

XPG has a general role in processing Rloops into DSBs



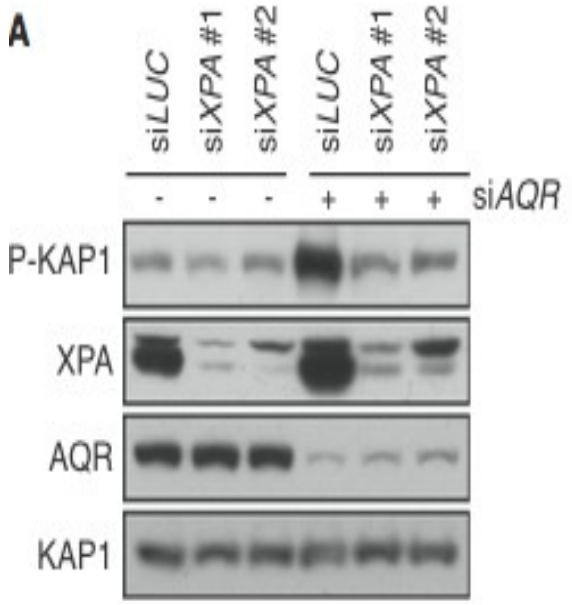


XPG act throught a NER like pathway

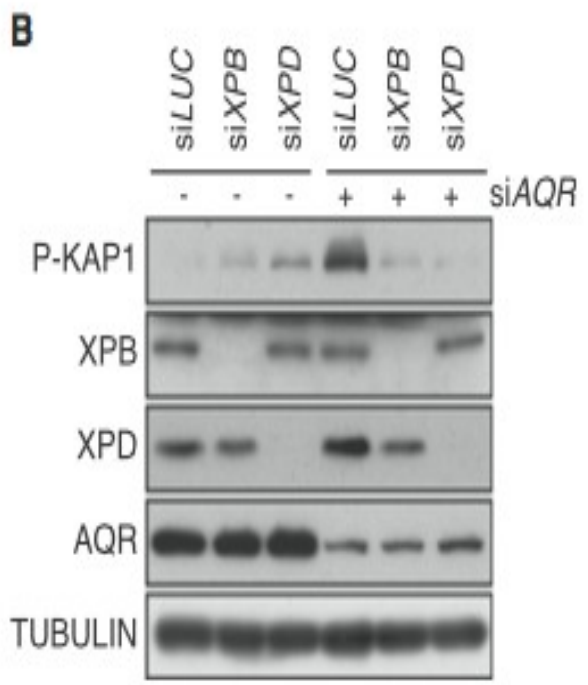
A canonical NER pathway is constitued by XPA that control the positioning of NER factors, such as XPG and XPF nucleases,TFIIH complex, formed by XPB XPD, an ATPasi and Helicase.

•XPA,XPB,XPD single depletion in AQR knockdown cells suppress DDR response

XPA



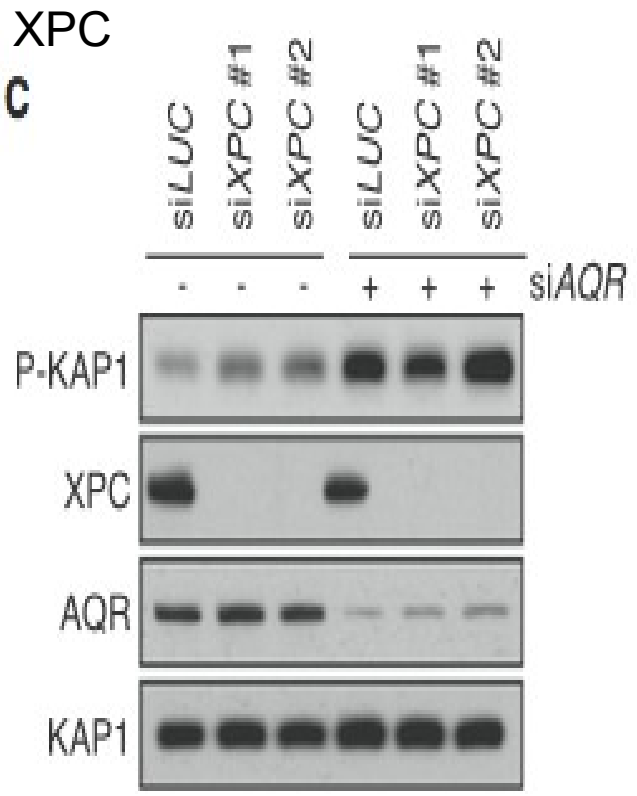
XPB,XPD

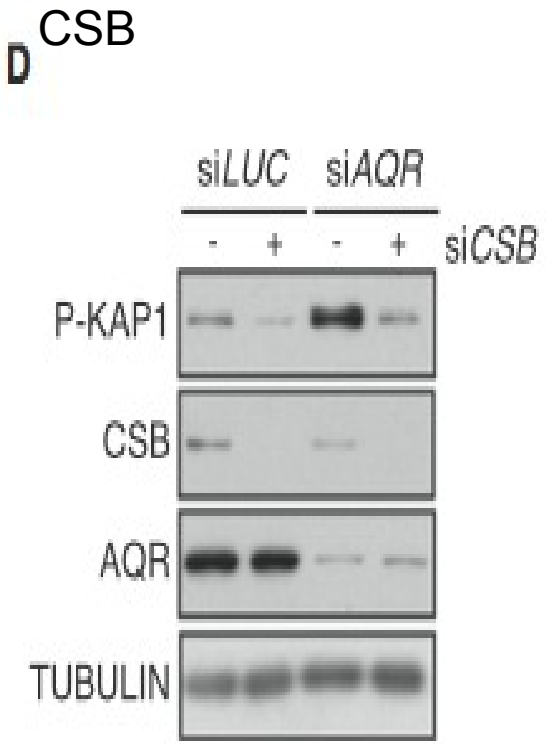


Rloops processing during transcription requires the NER factors to create DNA damages

XPG act throught a NER like pathway

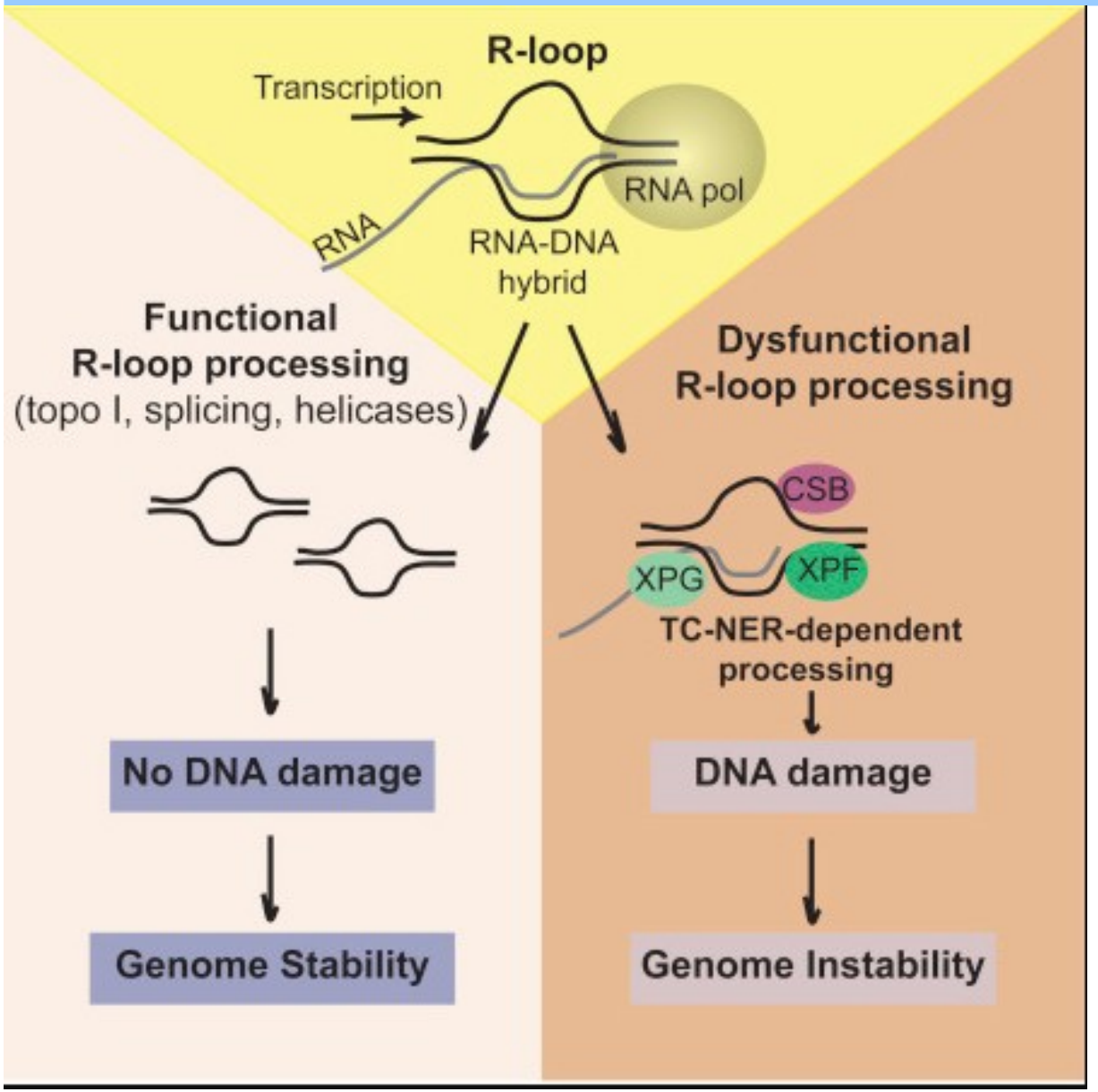
- CSB single depletion in AQR knockdown cells suppress DDR response
- But XPC depletion in AQR- cells did not affect DDR response





Rloops processing during transcription requires the TC-NER factors to create DNA damages

Conclusions



- The RNA-DNA helicase AQR prevents R loop-induced DSB formation
- R loop-dependent DSBs are formed by the endonucleases XPF and XPG
- The processing of R loops is a TC-NER-like event