Human exonuclease 1 role in response to UV irradiation

Dipartimento di Scienze Biomolecolari e Biotecnologie

Sertic S.[1], Giannattasio M.[1], Puddu F.[1], Marini F. [1],Rotondo G. [1], Muzi Falconi M. [1], Plevani P. [1]

1University of Milan

DNA damage checkpoints are surveillance mechanisms that monitor the integrity of the genome. Nucleotide excision repair (NER) is a DNA repair mechanism that cells use to remove UV-induced DNA lesions. Previous publication from our laboratory demonstrated that recognition and processing of UV-induced damage by NER is required for proper activation of checkpoint through interactions between NER proteins and checkpoint factors in yeast and human primary fibroblasts. From a two hybrid screening in yeast exonuclease 1 (Exo1) was identified as a 9-1-1 complex interactor. Exo1 is a 5'-3' exonuclease and 5'-flap-endonuclease with many different roles in DNA metabolism such as meiotic and mitotic recombination, mismatch repair and telomere processing. Characterization of an *exo1* yeast deleted strain has shown that this protein is involved in the early steps of UV-induced DNA damage checkpoint.
In human cells EXO1 is present as two isoforms named hEXO1a and hEXO1b genetarated by alternative splicing. We are analyzing the role of EXO1 in checkpoint activation in response to UV-C damage in human cells: using siRNA against both a and b isoform of hEXO1 in G1 cells we were able to observe a defect in Chk1 and p53 phosphorylation induced by UV-C irradiation.

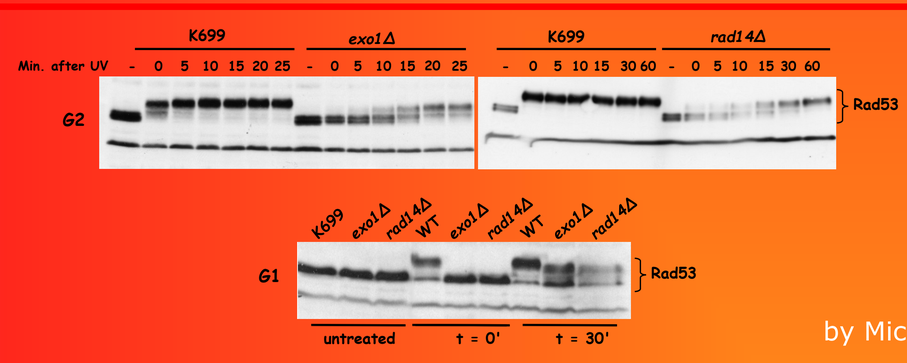

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Fig.1: Rapid Rad53 activation after UV irradiation is dependent upon Exo1 and Rad14.
WT, *rad14Δ* and *exo1Δ* cells were arrested in G2/M with nocodazole or G1/S with alfa-factor and UV irradiated with 75 J/m². Cells were then kept arrested for the indicated times before preparing TCA extracts. Activation of checkpoint was monitored by looking at Rad53 phosphorylation. While WT cells immediately phosphorylates Rad53 after irradiation, the NER and Exo1 deficient strains show a delayed and incomplete Rad53 phosphorylation.

IN YEAST

Saccharomyces cerevisiae

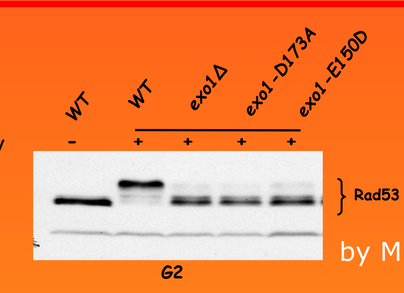

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Fig.2: Exo1 nuclease activity is required for the rapid Rad53 activation after UV treatment.
WT, *exo1Δ* and *exo1* indicated mutants were arrested in G2/M and UV irradiated with 75 J/m². Protein extracts were prepared immediately after UV treatment.
exo1-D173A and *exo1-E150D* point mutants, which are defective in the 5'-3' Exo1 associated exonuclease activity, do not activate Rad53 immedialtely after UV treatment.

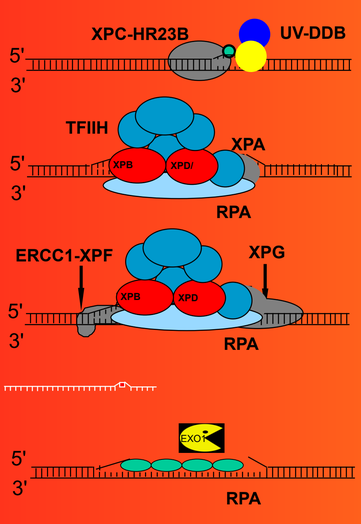


Fig.3: Working model for checkpoint activation after UV treatment.

Immediately after the UV treatment NER recognize the damage and promote the formation of open structure.

Subsequent recruitment of two specific endonucleases cut at the 5' and 3' of the lesion so the damage strand can be removed.

Some gaps created by the NER activities could be stabilized and Exo1 dependent 5'-3' directed resection can take place generating ssDNA.
ssDNA covered by RPA is produced and checkpoint factors can be recruited and activated.

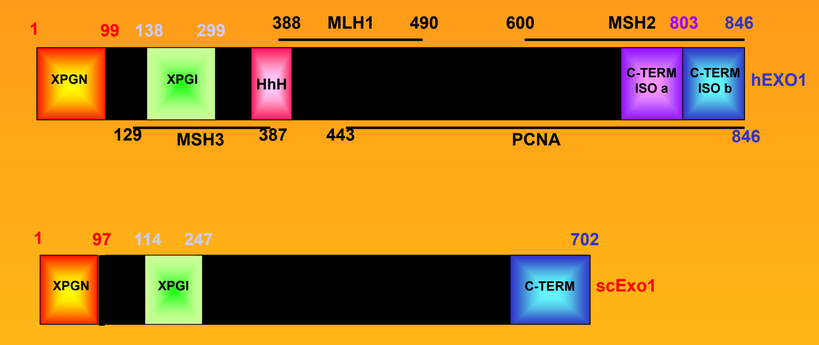
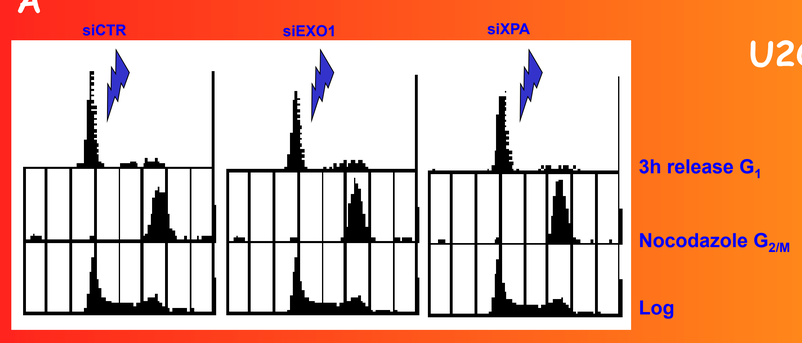
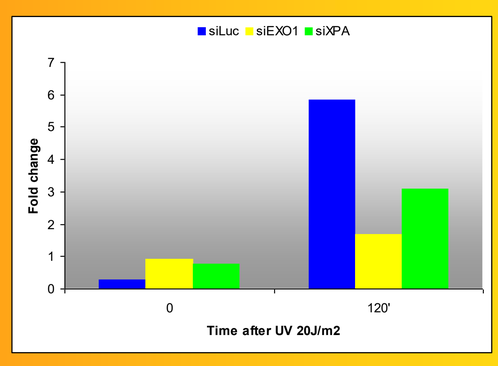
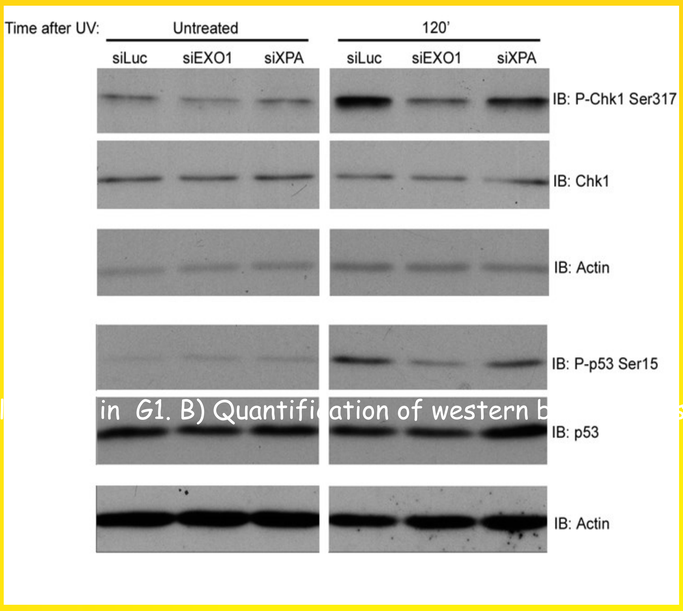


Fig 4. Schematic representation of the structure of *Saccharomyces* cerevisiae and human exonuclease 1. the N-terminal part of the protein is conserved, but in human cells two isoforms are present generated by alternative splicing.

IN HUMAN CELLS







s of log cells, nocodazole blocked cells and nocodazole released cells. UV irradiation was performed 3 hours after release from nocodazole when cel in G1. B) Quantifi ation of western b s showing p

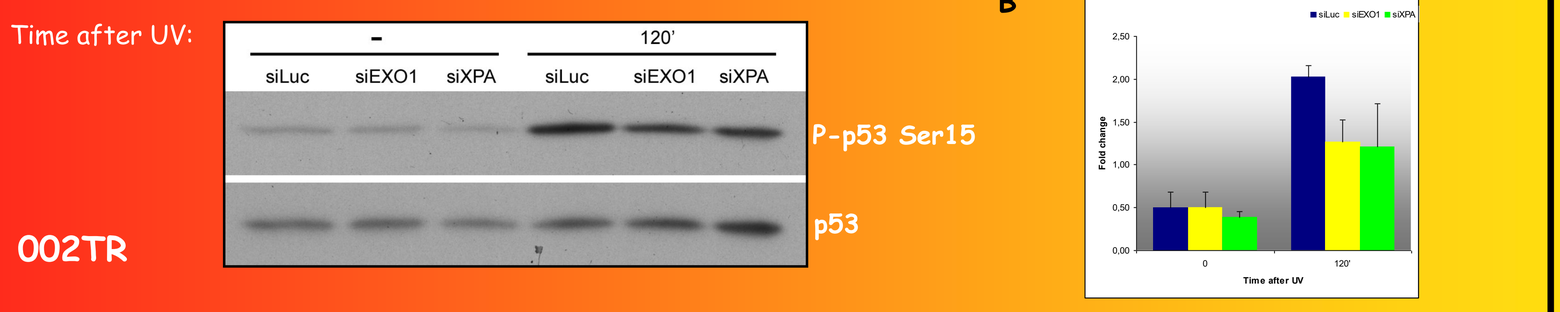


Fig. 6. Serum starved primary fibroblasts depleted for Exo1 are defective in p53 phoshorylation after UV irradiation.
A) Western blot analysis of starved primary cells silenced for Exo1 and XPA, monitoring p53 phoshorylation on serine 15. Cells were irradiated with 20J/m² B) Quantification of independent experiments showing p53 phosphorylation defect in siEXO1 and siXPA quiescent cells after UV irradiation.

C) western blot analysis monitoring Chk1 and p53 phosphorylation status after UV irradiation in G1 cells depleted for EXO1 or XPA

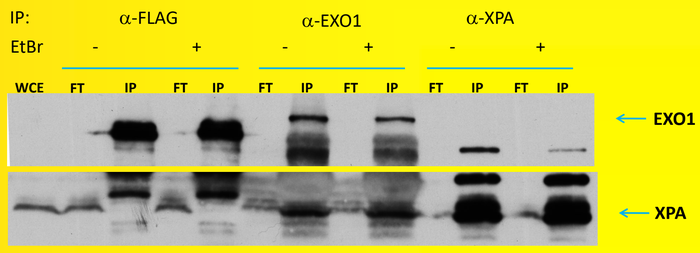


Fig. 7. Exo1 interacts with XPA in absence of DNA damage. Protein extracts from MRC5VA cells was incubated either with anti-EXO1 Ab or anti-XPA Ab, co-IP experiments were separated on an SDS-PAGE and interactor presence was monitored by western blot analysis. EXO1 is able to immunoprecipitate XPA, but not vice versa.

CONCLUSIONS:

- scExo1 is required for the rapid Rad53 activation and this depends upon it's nuclease activity
- hEXO1 is required for Chk1 and p53 phosphorylation in G1 U2OS cells after UV irradiation
- hEXO1 is required for p53 phoshopylation in quiescent primary fybroblasts after UV irradiation
- hEXO1 phisically interact with NER protein XPA even in absence of DNA damage