Sumoylation of CtIP controls DNA end resection

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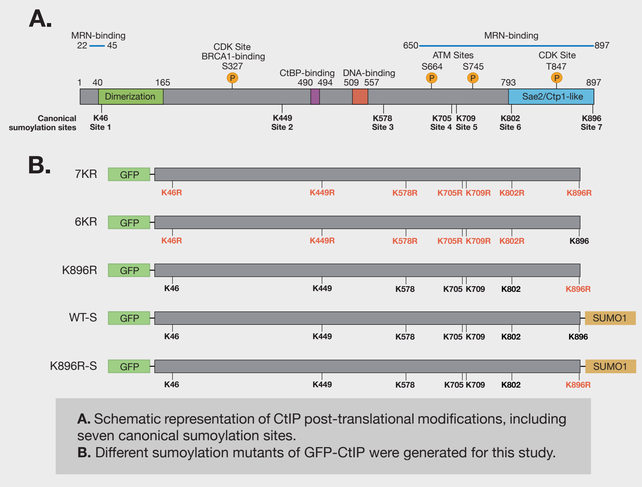
Double strand breaks (DSBs) can be repaired by two major mechanisms. Both ends can be simple re-joined with little or no processing, a mechanism known as non-homologous end-joining. On the other hand, DSBs can be processed and engaged in a more complex repair pathway called homologous recombination (HR). Repair by HR requires a first step called DNA end resection, consisting in the 5' to 3' nucleolitic degradation of one DNA strand at both DNA ends. Resection leads to the appearance of a 3' ssDNA overhang that is immediately coated by the RPA protein complex for protection. The RPA-coated ssDNA is an essential intermediate of all HR subpathways. CtIP is a key protein controlling DNA end resection, but is also involved in other nuclear processes, such as transcription and checkpoint activation. The functional regulation of this protein depends on the interaction with several proteins but also on post-translational modifications.

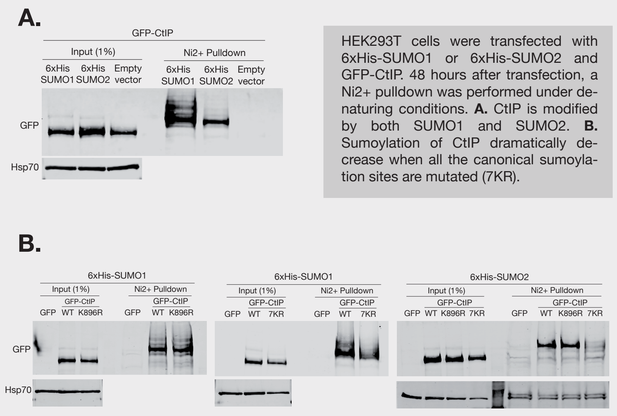
We have analyzed the role of sumoylation in the regulation of CtIP-mediated DNA end resection. In summary, we have found that a small fraction of CtIP is constitutively sumoylated by both SUMO1 and SUMO2 in human cells. Although sumoylation happens at several sites, we have identified a single lysine which sumoylation is absolutely essential for CtIP-mediated resection and repair. Using single point mutations that block sumoylation at this residue in combination with mutants that are constitutively sumoylated we have studied the role of this post-translational modification in the repair of DSBs. Strikingly, we have noticed that constitutive sumoylation at this specific residue trigger a second post-translational modification of CtIP. Overexpression of the ubiquitin-editing enzyme TNFAIP3 reduces this secondary modification, suggesting that CtIP suffers a SUMO-dependent ubiquitylation.

CtIP variants used in this study



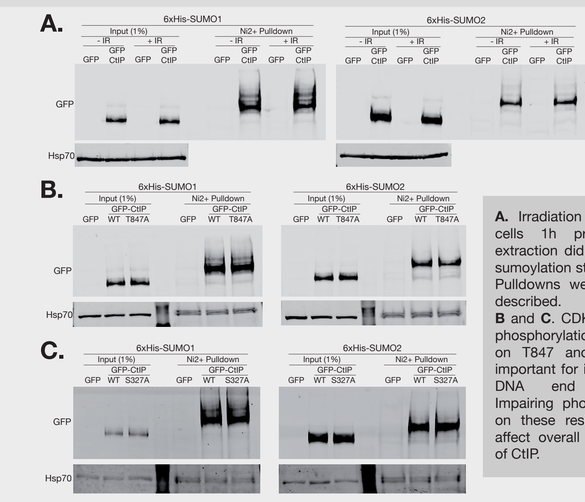
A. Schematic representation of CtIP post-translational modifications, including seven canonical sumoylation sites.
B. Different sumoylation mutants of GFP-CtIP were generated for this study.

CtIP is modified by SUMO1 and SUMO2



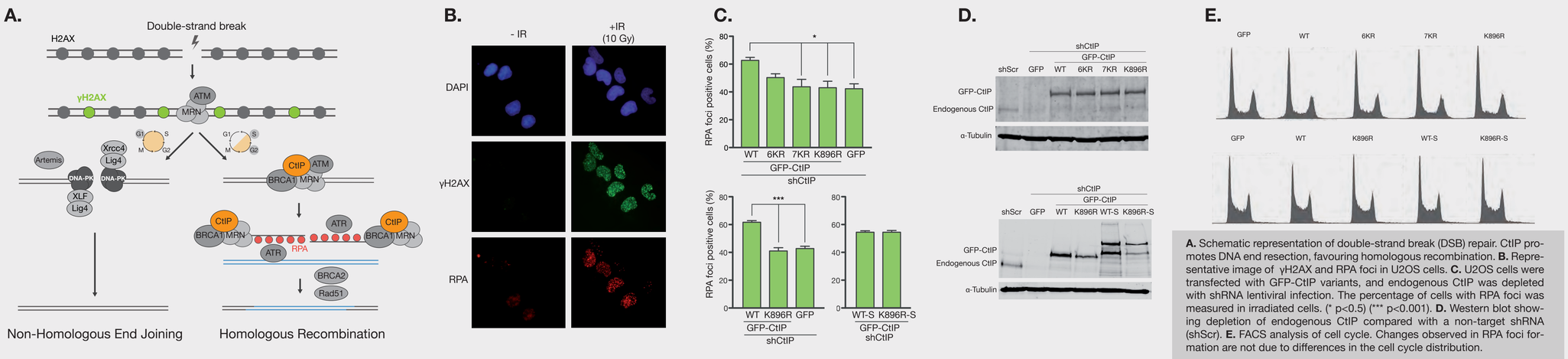
HEK293T cells were transfected with 6xHis-SUMO1 or 6xHis-SUMO2 and GFP-CtIP. 48 hours after transfection, a Ni2+ pulldown was performed under denaturing conditions. **A.** CtIP is modified by both SUMO1 and SUMO2. **B.** Sumoylation of CtIP dramatically decrease when all the canonical sumoylation sites are mutated (7KR).

Sumoylation of CtIP is independent of DNA damage and CDK-mediated phosphorylation



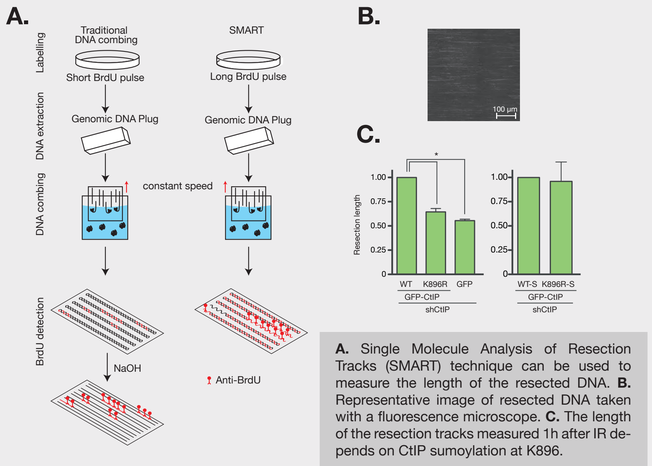
A. Irradiation (10 Gy) of cells 1h prior protein extraction did not change sumoylation status of CtIP. Pulldowns were done as described.
B and **C**. CDK-dependent phosphorylations of CtIP on T847 and S327 are important for its activity in DNA end resection. Impairing phosphorylation on these residues didn't affect overall sumoylation of CtIP.

Sumoylation of CtIP is required for RPA foci formation upon DNA damage



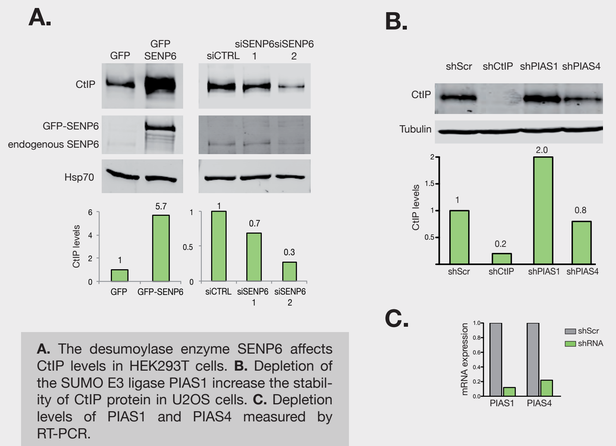
A. Schematic representation of double-strand break (DSB) repair. CtIP promotes DNA end resection, favouring homologous recombination. **B.** Representative image of γH2AX and RPA foci in U2OS cells. **C.** U2OS cells were transfected with GFP-CtIP variants, and endogenous CtIP was depleted with shRNA lentiviral infection. The percentage of cells with RPA foci was measured in irradiated cells. (* $p<0.5$) (*** $p<0.001$). **D.** Western blot showing depletion of endogenous CtIP compared with a non-target shRNA (shScr). **E.** FACS analysis of cell cycle. Changes observed in RPA foci formation are not due to differences in the cell cycle distribution.

Effect of CtIP sumoylation mutants in the length of the resection tracks



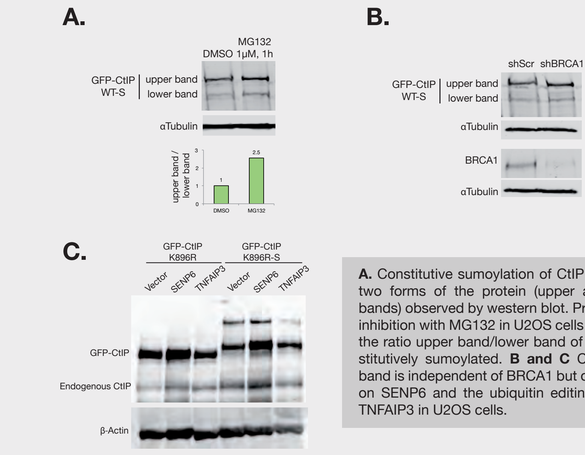
A. Single Molecule Analysis of Resection Tracks (SMARAT) technique can be used to measure the length of the resected DNA. **B.** Representative image of resected DNA taken with a fluorescence microscope. **C.** The length of the resection tracks measured 1h after IR depends on CtIP sumoylation at K896.

CtIP stability is affected by sumoylation



A. The desumoylase enzyme SENP6 affects CtIP levels in HEK293T cells. **B.** Depletion of the SUMO E3 ligase PIAS1 increase the stability of CtIP protein in U2OS cells. **C.** Depletion levels of PIAS1 and PIAS4 measured by RT-PCR.

Constitutive sumoylation triggers a second post-translational modification of CtIP



A. Constitutive sumoylation of CtIP produces two forms of the protein (upper and lower bands) observed by western blot. Proteasome inhibition with MG132 in U2OS cells increased the ratio upper band/lower band of CtIP constitutively sumoylated. **B and C** CtIP upper band is independent of BRCA1 but dependent on SENP6 and the ubiquitin editing enzyme TNFAIP3 in U2OS cells.

Conclusions

> CtIP sumoylation at K896 is required for proper DNA end resection.

> Sumoylation of CtIP affects its stability.

> Sumoylation at K896 triggers a second post-translational modification of CtIP, probably ubiquitylation.

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