

The histone H2AX does not inhibit resection of DNA double strand breaks induced by heavy ions

N. B. Averbeck¹, O. Ringel¹, B. Jakob¹, A. L. Leifke¹, G. Becker¹, M. Durante^{1,2},
and G. Taucher-Scholz^{1,2}

¹GSI Helmholtzzentrum für Schwerionenforschung GmbH, Darmstadt, Germany;

²Technical University Darmstadt, Germany

The histone H2A variant H2AX plays an important role in the repair of DNA double strand breaks (DSBs) within eukaryotes. Very rapidly upon DSB induction this H2A variant becomes phosphorylated at Ser139 [1]; H2AX phosphorylated at this site is named γ H2AX. It serves as a platform for several DSB repair factors [reviewed in 2] and further is thought to stabilize the loose ends of a DSB [3]. In addition, γ H2AX controls the end processing of recombination activating gene (RAG) endonuclease induced DSBs within V(D)J recombination, which takes place in the G1 cell cycle phase of lymphocytes. Absence of γ H2AX within this process allows CtIP-mediated DSB resection [4]. Hence, γ H2AX prevents resection within the V(D)J recombination and it is conceivable that resection of genotoxic caused DSBs is as well under the control of γ H2AX [4], especially in the cell cycle phase G1 when DSB resection would lead to genomic instability.

Studies here at GSI clearly demonstrated that CtIP-driven resection of heavy ion induced DSBs occurs not only in the late S and G2 cell cycle phase but in G1 as well [5] sustaining the notion that resection of ion induced DSBs in G1 is not inhibited by γ H2AX. This is further supported by the observation that in ion irradiated confluent human fibroblasts (AG1522D) (figure 1), where 98 % of cells were in the G1/G0 cell cycle phase, most DSBs are resected and the resection marker pRPA always colocalizes with γ H2AX at ion induced DSBs. There was not one cell where RPA was recruited to a break site where H2AX was not phosphorylated. This was observed in three independent experiments ($2 \times {}^{238}\text{U}$ irradiation, LET: 15 000 keV/ μm , fluence: 3×10^6 p./cm²; $1 \times {}^{207}\text{Pb}$ irradiation, LET 13500 keV/ μm , fluence 3×10^6 p./cm²). Thus, γ H2AX seems not to inhibit DSB resection of heavy ion induced lesions.

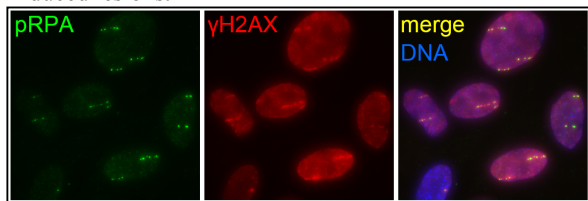


Figure 1: Resection of ion induced DSBs is never observed independently of γ H2AX. Confluent human fibroblasts were irradiated with ${}^{207}\text{Pb}$ ions (LET: 13500 keV/ μm ; fluence: 3×10^6 p./cm²) and fixed 1 h after irradiation. Cells were immuno-stained for γ H2AX (DSB marker) and pRPA (resection marker). DNA was visualized by DAPI staining.

In order to further characterize whether or not DSB resection in G1 is controlled by H2AX and in particular by γ H2AX we studied DSB resection in H2AX deficient cells. Murine H2AX $-/-$ and wild-type cells were ${}^{12}\text{C}$ ion irradiated (LET: 170 keV/ μm ; fluence: 3×10^6 p./cm²) and immuno-stained against RPA (resection marker) and 53BP1 (DSB marker) 1 h after irradiation. If γ H2AX were inhibiting ion induced DSB resection in the G1 cell cycle phase one would expect that the H2AX deficient cells show an increase in resected DSBs. However, H2AX deficient and wild type cells both show about 20 % of resected DSBs. A different cell cycle distribution cannot be responsible for the obtained result as approximately 50 % of both populations were in the G1 cell cycle phase (determined by flow cytometry). As H2AX deficiency did not influence the fraction of resected ion induced DSBs in G1 we conclude that γ H2AX indeed does not inhibit resection of heavy ion induced DSBs in cells within the G1 cell cycle phase.

Taken together, from these observations we conclude that H2AX most likely has no function in controlling end resection of genotoxic-induced DSBs. Its influence on DSB resection might, thus, be unique to V(D)J recombination.

References

- [1] E.P. Rogakou *et al.*, DNA double-stranded breaks induce histone H2AX phosphorylation on serine 139, *J Biol Chem*, 273 (1998) 5858-5868.
- [2] J. Yuan *et al.*, Focus on histone variant H2AX: to be or not to be, *FEBS Lett*, 584 (2010) 3717-3724.
- [3] B. Yin *et al.*, Histone H2AX stabilizes broken DNA strands to suppress chromosome breaks and translocations during V(D)J recombination, *J Exp Med*, 206 (2009) 2625-2639.
- [4] B.A. Helmink *et al.*, H2AX prevents CtIP-mediated DNA end resection and aberrant repair in G1-phase lymphocytes, *Nature*, 469 (2011) 245-249.
- [5] N. Averbeck *et al.*, DNA double strand break resection after heavy ion irradiation, in: K. Große (Ed.) GSI Scientific Report 2011, GSI Helmholtzzentrum für Schwerionenforschung GmbH, Darmstadt, 2012.

Acknowledgements

This work is partially funded by the BMBF grant 02NUK001A.