We have shown here that following DSB-induction Exo1 is initially phosphorylated by an ATM-dependent mechanism, however, at later time points Exo1 phosphorylation is independent of ATM suggesting that it is phosphorylated redundantly, possibly by ATR or DNA-PK. We show that while RPA34 recruitment to foci was normal in cells expressing the non-phosphorylatable mutant of Exo1 (S714A), Rad51 recruitment to foci was defective. This suggests that the phosphorylation event at S714 is not required for the generation of the ssDNA tracks but is possibly required to allow Rad51 loading. Interestingly we observed that cells expressing a phospho-mimic Exo1 (S714E) are deficient in generation of ssDNA/DNA resection as measured by the recruitment of RPA34 and Rad51 to foci. This phospho-mimic mutant also has impaired HR activity. At present we can only speculate as to the specific function of the phosphorylation event, however a recent paper examining four phosphorylation sites in yeast Exo1 suggested that phosphorylation probably inhibits the activity of Exo1 (35).

It is tempting to speculate that S714 phosphorylation is not required for the initiation of Exo1-mediated DNA resection, but rather that the phosphorylation is required to attenuate Exo1 activity and perhaps prevent the generation of substrates unsuitable for HR. Inactivation of a nuclease by phosphorylation after DNA damage is not without precedent. A recent paper has also shown that phosphorylation of Mre11 on SQ/TQ residues decreases its affinity for DNA, causes disassembly from chromatin and thereby results in its inactivation, it is possible that phosphorylation of Exo1 may function in a similar way (40). Our interpretations do not exclude additional functions of phosphorylated Exo1 in recombinational DNA repair. It is also possible that phosphorylation of Exo1 on S714 changes its binding partners. We have observed here that phosphorylation of Exo1 by ATM was dispensable for resection as revealed by normal RPA foci formation in non-phosphorylatable mutant (S714A) expressing cells; however these cells are likely to be defective in recombinational repair due to defective loading of Rad51. Indeed we also found that cells expressing the non-phosphorylatable mutant displayed defective *I-SceI*-induced HR. Taken together, our data suggests that the DNA substrates generated by action of non-phosphorylatable Exo1 cannot be used efficiently by Rad51 to form nucleoprotein filaments. Further studies will be required to address whether this is due to the over-resection by unregulated Exo1.

A recent study has shown that HR of IR-induced DSBs that arise during G2 is reduced in ATM-deficient cells (41). Since we have shown that ATM is required for the timely phosphorylation of Exo1 and that this phosphorylation event is required for the regulation of HR, this may further confirm a role for ATM in controlling recombinational repair of DSBs. However, the role of ATM in Exo1 phosphorylation appears to be redundant suggesting that other proteins such as ATR or DNA-PK can compensate albeit at a later time-point.

DNA DSBs induce a vast array of signaling networks, which can greatly influence DNA repair, cell cycle checkpoint activation and cell viability and therefore it is understandable that multiple levels of regulation may exist to inactivate destructive proteins such as nucleases. Exo1 activity may be attenuated by ATM-dependent phosphorylation following its role in resection to prevent the destruction of substrates required for HR. Given the importance of accurate recombination in preserving the genome, our findings clearly illustrate the role of Exo1 in maintaining genomic stability.

SUPPLEMENTARY DATA

Supplementary Data are available at NAR Online.

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REFERENCES

1. Mimitou,E.P. and Symington,L.S. (2009) Nucleases and helicases take center stage in homologous recombination. *Trends Biochem. Sci.*, **34**, 264–272.
2. Shiotani,B. and Zou,L. (2009) Single-stranded DNA orchestrates an ATM-to-ATR switch at DNA breaks. *Mol. Cell*, **33**, 547–558.
3. Sartori,A.A., Lukas,C., Coates,J., Mistrik,M., Fu,S., Bartek,J., Baer,R., Lukas,J. and Jackson,S.P. (2007) Human CtIP promotes DNA end resection. *Nature*, **450**, 509–514.
4. Bernstein,K.A. and Rothstein,R. (2009) At loose ends: resecting a double-strand break. *Cell*, **137**, 807–810.
5. Szankasi,P. and Smith,G.R. (1992) A DNA exonuclease induced during meiosis of Schizosaccharomyces pombe. *J. Biol. Chem.*, **267**, 3014–3023.
6. Lee Bi,B.I., Nguyen,L.H., Barsky,D., Fernandes,M. and Wilson,D.M. III (2002) Molecular interactions of human Exo1 with DNA. *Nucleic Acids Res.*, **30**, 942–949.
7. Qiu,J., Qian,Y., Chen,V., Guan,M.X. and Shen,B. (1999) Human exonuclease 1 functionally complements its yeast homologues in DNA recombination, RNA primer removal, and mutation avoidance. *J. Biol. Chem.*, **274**, 17893–17900.
8. Lee,S.D. and Alani,E. (2006) Analysis of interactions between mismatch repair initiation factors and the replication processivity factor PCNA. *J. Mol. Biol.*, **355**, 175–184.
9. Fiorentini,P., Huang,K.N., Tishkoff,D.X., Kolodner,R.D. and Symington,L.S. (1997) Exonuclease I of Saccharomyces cerevisiae functions in mitotic recombination in vivo and in vitro. *Mol. Cell Biol.*, **17**, 2764–2773.
10. Kirkpatrick,D.T., Ferguson,J.R., Petes,T.D. and Symington,L.S. (2000) Decreased meiotic intergenic recombination and increased meiosis I nondisjunction in exo1 mutants of Saccharomyces cerevisiae. *Genetics*, **156**, 1549–1557.
11. Tsubouchi,H. and Ogawa,H. (2000) Exo1 roles for repair of DNA double-strand breaks and meiotic crossing over in Saccharomyces cerevisiae. *Mol. Biol. Cell*, **11**, 2221–2233.
12. Wei,K., Clark,A.B., Wong,E., Kane,M.F., Mazur,D.J., Parris,T., Kolas,N.K., Russell,R., Hou,H. Jr, Kneitz,B. *et al.* (2003) Inactivation of Exonuclease 1 in mice results in DNA mismatch repair defects, increased cancer susceptibility, and male and female sterility. *Genes Dev.*, **17**, 603–614.
13. Sun,X., Zheng,L. and Shen,B. (2002) Functional alterations of human exonuclease 1 mutants identified in atypical hereditary