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Phosphorylation of Exo1 modulates homologous recombination repair of DNA double-strand breaks

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ABSTRACT

DNA double-strand break (DSB) repair via the homologous recombination pathway is a multi-stage process, which results in repair of the DSB without loss of genetic information or fidelity. One essential step in this process is the generation of extended single-stranded DNA (ssDNA) regions at the break site. This ssDNA serves to induce cell cycle checkpoints and is required for Rad51 mediated strand invasion of the sister chromatid. Here, we show that human Exonuclease 1 (Exo1) is required for the normal repair of DSBs by HR. Cells depleted of Exo1 show chromosomal instability and hypersensitivity to ionising radiation (IR) exposure. We find that Exo1 accumulates rapidly at DSBs and is required for the recruitment of RPA and Rad51 to sites of DSBs, suggesting a role for Exo1 in ssDNA generation. Interestingly, the phosphorylation of Exo1 by ATM appears to regulate the activity of Exo1 following resection, allowing optimal Rad51 loading and the completion of HR repair. These data establish a role for Exo1 in resection of DSBs in human cells, highlighting the critical requirement of Exo1 for DSB repair via HR and thus the maintenance of genomic stability.

INTRODUCTION

DNA double-strand breaks (DSBs) can be induced by a variety of factors such as chemotherapeutic agents, ionising radiation (IR) and by the products of cellular metabolism, including replication fork collapse. In order to maintain genomic stability, cells possess a complex network of signalling pathways involved in the detection, signalling and repair of DNA damage. Defects in these DNA repair pathways can lead to human genomic instability syndromes, with increased cancer susceptibility, neurological syndromes and immunodeficiency. The resection of DSBs to produce 3′ single-stranded DNA (ssDNA) tracts is a critical step in the repair of DSBs by homologous recombination (1). The ssDNA at the break site is essential for activation of the ATR signalling cascade which re-enforces ATM-induced cell cycle checkpoints (2). The MRN (MRE11, Rad50 and NBS1) complex, in association with CtIP, has been previously reported to be important for DSB resection (3). However, recent reports clearly indicate that yeast MRX (Mre11, Rad50 and XRS1) is involved only in limited resection at the break sites while extensive resection requires additional, redundant nucleases such as Exonuclease 1 (Exo1) and/or DNA2 (4).

Exo1 was first identified in *Schizosaccharomyces pombe* as a nuclease that is induced during meiosis (5). Exo1 belongs to the RAD2 family of nucleases and possesses 5′–3′ nuclease activity and 5′-flap endonuclease activity (6,7). Alternate splicing leads to two isoforms of Exo1 (a and b). The isoforms differ at the C-terminus, with Exo1b having an additional 48 amino acids. Exo1 is known to interact with several other proteins involved in replication and DNA repair including PCNA and mismatch repair (MMR) proteins (8). Exo1 is implicated in several DNA repair pathways including MMR, post-replication repair, meiotic and mitotic recombination (9–11). The involvement of Exo1 in DNA repair pathways including MMR suggests it may also be a target for mutation in tumourigenesis. Consistent with this, a cancer-prone phenotype can be observed in Exo1-deficient mice including increased susceptibility to lymphoma development (12). In addition, patients with atypical human non-polyposis colon cancer and other forms of colorectal cancer have been found to have

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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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