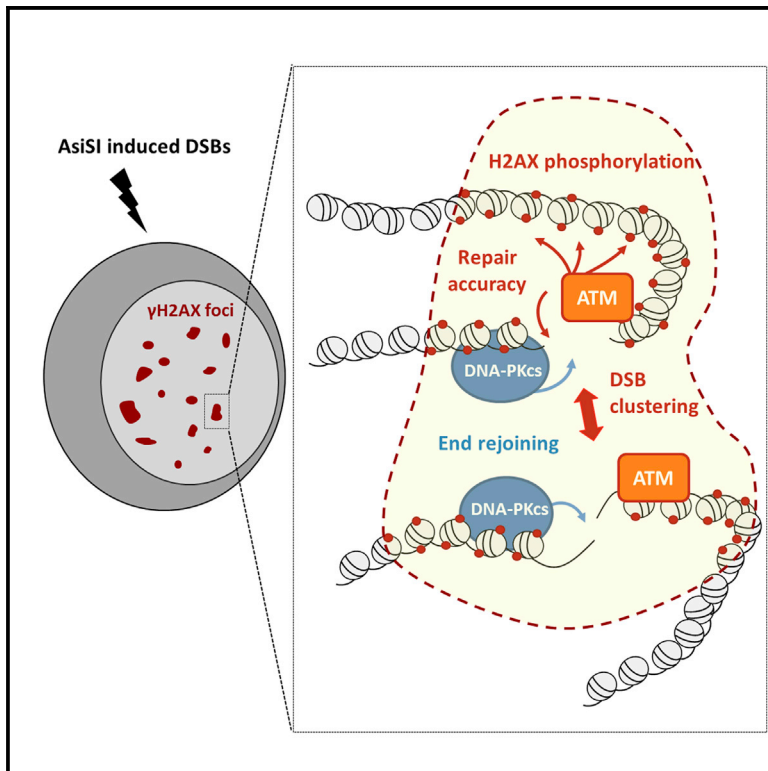# Cell Reports

Non-redundant Functions of ATM and DNA-PKcs in Response to DNA Double-Strand Breaks

Graphical Abstract

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In Brief

By inducing multiple annotated DNA double-strand breaks in the human genome, Caron et al. show that two DNA damage response kinases, ATM and DNA-PKcs, are co-recruited at DSBs but exhibit non-redundant functions in promoting end joining, repair accuracy, H2AX phosphorylation, and DSB clustering.

Highlights

- Both ATM and DNA-PKcs are recruited at AsiSI-induced DSBs

- Once recruited, both kinases exhibit complementary and non-redundant functions

- DNA-PKcs activity is required for end joining at all AsiSI-induced DSBs

- ATM activity promotes repair accuracy, H2AX phosphorylation, and DSB clustering

CellPress

Non-redundant Functions of ATM and DNA-PKcs in Response to DNA Double-Strand Breaks

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http://dx.doi.org/10.1016/j.celrep.2015.10.024

SUMMARY

DNA double-strand breaks (DSBs) elicit the so-called DNA damage response (DDR), largely relying on ataxia telangiectasia mutated (ATM) and DNA-dependent protein kinase (DNA-PKcs), two members of the PI3K-like kinase family, whose respective functions during the sequential steps of the DDR remains controversial. Using the DIvA system (DSB inducible via *AsiSI*) combined with high-resolution mapping and advanced microscopy, we uncovered that both ATM and DNA-PKcs spread in *cis* on a confined region surrounding DSBs, independently of the pathway used for repair. However, once recruited, these kinases exhibit non-overlapping functions on end joining and γH2AX domain establishment. More specifically, we found that ATM is required to ensure the association of multiple DSBs within "repair foci." Our results suggest that ATM acts not only on chromatin marks but also on higher-order chromatin organization to ensure repair accuracy and survival.

INTRODUCTION

Among the various DNA damage types, DNA double-strand breaks (DSBs) are the most deleterious since they can lead to various mutations and chromosomal rearrangements linked to tumor initiation and progression. DSBs can both arise during development as part of scheduled processes, such as V(D)J and immunoglobulin class-switch recombination, and be generated by environmental stresses, such as pollutants and irradiation. DSBs are mainly repaired by two distinct pathways: homologous recombination (HR), involving extensive resection and utilizing an intact copy of the damaged locus, and non-homologous end joining (NHEJ), in which the two broken ends are able to be joined with no or minimal homology (reviewed in Deriano and Roth, 2013; Jasin and Rothstein, 2013). Defects in either repair pathway results in genome instability and can be lethal at very early developmental stages.

DSB detection rapidly elicits the so-called DNA damage response (DDR), which largely relies on the activity of the phosphatidylinositol 3-kinase (PI3K)-related kinases DNA-PKcs, ATM, and ATR (reviewed in Sirbu and Cortez, 2013). All three of these kinases have been found to be mutated in human disorders associated with genome instability: severe combined immunodeficiency (DNA-PKcs), ataxia telangiectasia (ATM), and Seckel syndrome (ATR). While DNA-PKcs and ATM have a function restricted to the DSB response, ATR is activated following a wider range of damage types, especially those occurring during DNA replication. These kinases are rapidly recruited and activated at DSBs through direct interactions with the Ku heterodimer (DNA-PKcs), the MRN complex (ATM), and ATRIP (ATR) (Falck et al., 2005). Once recruited, they have been proposed to participate in repair on three different levels (Sirbu and Cortez, 2013).

First of all, both ATM and DNA-PKcs play a direct role in repair at the break site in a manner that largely depends on their kinase activity. DNA-PKcs is a core component of the NHEJ machinery that allows both synapsis of DNA ends and the stable recruitment of the XRCC4/DNA Ligase 4 complex, required for end joining (Calsou et al., 2003). Consequently, its impairment leads to ends rejoining defects as measured by pulse field gel electrophoresis (PGFE) (Beamish et al., 2000). In contrast, ATM is dispensable for repair of most DSBs arising after irradiation, but required for efficient repair of DSBs induced in heterochromatin (Beucher et al., 2009; Goodarzi et al., 2008) or with blocked DNA ends (Álvarez-Quilón et al., 2014).

Second, upon activation, PI3K-like kinases elicit checkpoint activation by phosphorylating a large number of substrates that either remain at the break site and thus play a direct role in signal amplification or diffuse from the break and mediate signal transduction that eventually leads to cell cycle arrest (Sirbu and Cortez, 2013).

Finally, these DSB-activated kinases trigger a profound remodeling of the chromatin structure at the vicinity of the break. One of their main substrates is the H2AX histone variant, incorporated in roughly one-tenth of nucleosomes (although its