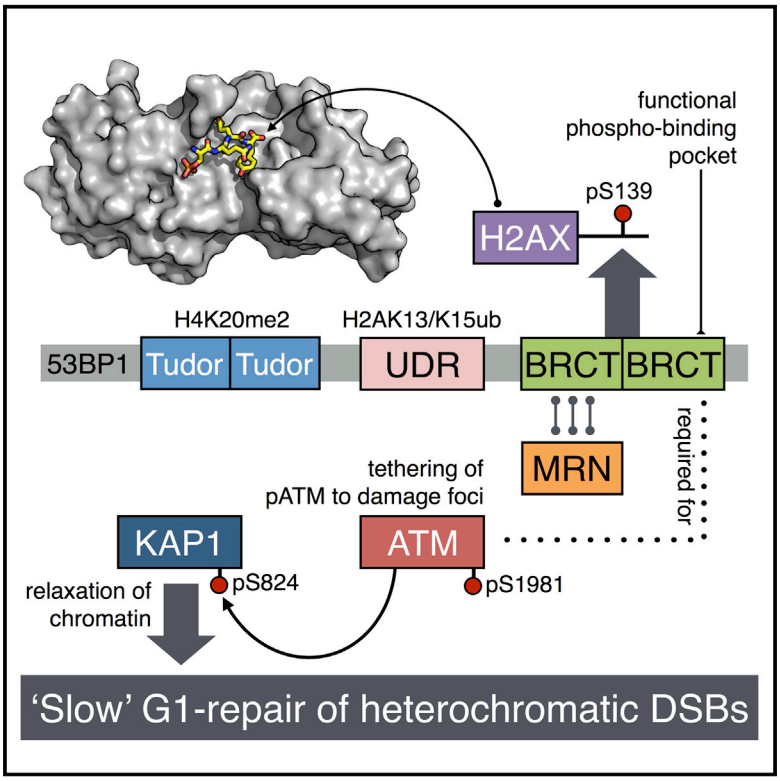
Cell Reports

Report

ATM Localization and Heterochromatin Repair Depend on Direct Interaction of the 53BP1-BRCT$_2$ Domain with γH2AX

Graphical Abstract



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In Brief

Baldock et al. find that the BRCT$_2$ domain of 53BP1 specifically recognizes γH2AX, the primary chromatin mark at DNA double-strand breaks. Mutational disruption of this recognition in cells affects pATM recruitment into foci in G1 and results in a defect in repair of DNA damage in heterochromatin.

Highlights

- The BRCT$_2$ domain of 53BP1 binds the DNA damage chromatin mark γH2AX
- Crystal structure of γH2AX bound to 53BP1-BRCT$_2$ reveals the basis of specificity
- 53BP1-BRCT$_2$ responds to γH2AX formation by DNA damage in cells
- Disruption of γH2AX binding disrupts pATM foci and DSB repair in heterochromatin

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ATM Localization and Heterochromatin Repair Depend on Direct Interaction of the 53BP1-BRCT$_2$ Domain with γH2AX

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SUMMARY

53BP1 plays multiple roles in mammalian DNA damage repair, mediating pathway choice and facilitating DNA double-strand break repair in heterochromatin. Although it possesses a C-terminal BRCT$_2$ domain, commonly involved in phospho-peptide binding in other proteins, initial recruitment of 53BP1 to sites of DNA damage depends on interaction with histone post-translational modifications—H4K20me2 and H2AK13/K15ub—downstream of the early γH2AX phosphorylation mark of DNA damage. We now show that, contrary to current models, the 53BP1-BRCT$_2$ domain binds γH2AX directly, providing a third post-translational mark regulating 53BP1 function. We find that the interaction of 53BP1 with γH2AX is required for sustaining the 53BP1-dependent focal concentration of activated ATM that facilitates repair of DNA double-strand breaks in heterochromatin in G1.

INTRODUCTION

TP53 binding protein 1 (53BP1) is a large multi-domain protein with multiple roles in the DNA damage response (Panier and Boulton, 2014; Zimmermann and de Lange, 2014). Following DNA damage and activation of the DNA-damage-responsive protein kinase ATM, 53BP1 is recruited rapidly to nuclear foci (Schultz et al., 2000) containing the primary mark of DNA damage—phosphorylation of Ser139 close to the C terminus of the histone H2A variant—H2AX (Rogakou et al., 1998), generally known as γH2AX. Although 53BP1 has a C-terminal tandem BRCT domain (BRCT$_2$), which in its orthologs, *Saccharomyces cerevisiae* Rad9p and *Schizosaccharomyces pombe* Crb2, mediates binding to the equivalents of γH2AX (Hammet et al., 2007; Kilkenny et al., 2008), the role of the 53BP1-BRCT$_2$ domain remains controversial. Although some studies indicated an interaction with γH2AX (Stewart et al., 2003; Ward et al., 2003), others have contradicted this (Stucki et al., 2005; Ward et al., 2006), and a significant role for this domain in the DNA damage response has been largely discounted (Bothmer et al., 2011; Callen et al., 2013).

Current models suggest that 53BP1 recruitment to ionizing radiation induced nuclear foci (IRIF) depends only indirectly on γH2AX and is instead mediated by two other post-translational modifications: (1) H2AK13/15-anchored ubiquitin chains (Fradet-Turcotte et al., 2013) generated by the E3 ubiquitin ligases RNF8 and RNF168, which are themselves recruited by MDC1, whose BRCT$_2$ domain interaction with γH2AX is required for its own recruitment (Bekker-Jensen and Mailand, 2010; Pinder et al., 2013); and (2) direct interaction of the tandem Tudor domains of 53BP1 with dimethylated H4K20 (Botuyan et al., 2006) exposed by release of JMJD2A and L3MBTL1 following their ubiquitylation by RNF8 and RNF168 (Acs et al., 2011; Mallette et al., 2012).

We have re-examined the role of the 53BP1-BRCT$_2$ domain and show unambiguously that it is a competent binding module for phosphorylated peptides with a clear specificity for the DNA-damage marker γH2AX, and in isolation from other parts of 53BP1 is sufficient for localization to sites of DNA damage in cells associated with γH2AX.

Structure-based mutational disruption of γH2AX binding by 53BP1 interferes with the 53BP1-dependent localization of pATM required for repair of DNA damage in regions of heterochromatin and results in a defect in the slow phase of DNA break repair in G1. These data add a third histone post-translational mark to the ligand repertoire of 53BP1, and a clear functional role for phosphopeptide binding by its BRCT$_2$ domain.

RESULTS AND DISCUSSION

53BP1-BRCT$_2$ Binds γH2AX In Vitro

Comparison of the tandem BRCT domains of 53BP1 with those of MDC1 (Rodriguez et al., 2003; Stucki et al., 2005) and Crb2 (Kilkenny et al., 2008) shows strong conservation of residues