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53BP1-mediated DNA double strand break repair: Insert bad pun here

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ABSTRACT

53BP1 is an established player in the cellular response to DNA damage and is a canonical component of ionizing-radiation induced foci – that cadre of proteins which assemble at DNA double strand breaks following radiation exposure and which are readily visualized by immunofluorescence microscopy. While its roles in p53 regulation and cell cycle checkpoint activation have been studied for some time, the impact of 53BP1 on DNA double strand break rejoining has only come to light in the past few years. Convincing evidence now exists for 53BP1 significantly affecting the outcome of DNA double strand break repair in several contexts, many of which hint to an important role in modulating chromatin structure surrounding the break site. Here, we highlight the known and emerging roles of 53BP1 in DNA double strand break repair, including the repair of lesions induced within heterochromatin, following telomere uncapping, in long-range V(D)J recombination, during immunoglobulin class switch recombination and its much debated role in regulating resection during homologous recombination.

1. 53BP1, DNA breakage and radiation-induced foci formation

53BP1 (p53 Binding Protein 1) was originally identified in a yeast two-hybrid screen looking for proteins that interact with the p53 tumor suppressor [1]. Subsequent studies established 53BP1 as a substrate for Ataxia Telangiectasia (A-T) Mutated (ATM) signaling and that it re-localized to discrete foci overlapping with the phosphorylated form of histone H2AX (γH2AX), demarcating sites of DNA double strand breaks (DSBs) following radiation exposure [2–4]. Following a decade of research, we now know that 53BP1 functions downstream of an γH2AX-dependent hierarchy of proteins that collectively establish ionizing radiation (IR)-induced foci (IRIF) at DSB sites; this hierarchy includes (but is not necessarily limited to) the Mre11/Rad50/NBS1 (MRN) complex, ATM, MDC1, RNF8, RNF168 and HERC2 [5–8]. With the exception of ATM, whose function to generate γH2AX may be partially compensated by the activity of the DNA-dependent kinase (DNA-PK), all of these proteins are physically and functionally required to recruit 53BP1 to the DSB site. In brief, this involves DSB recognition by MRN, ATM activation, γH2AX-formation, MDC1-recruitment, MRN-retention (leading to further ATM-activation and γH2AX spreading) and RNF8/RNF168/HERC2-mediated histone H2A and H2AX mono and poly-ubiquitination. DSB-induced histone ubiquitination is presently thought to engender chromatin alterations that enable the re-localization of 53BP1 to exposed methyl-groups within the chromatin surrounding a DSB.

The ability of 53BP1 to form IRIF is attributable to its two tandem tudor domains, oligomerization activity and a Glycine/Arginine-Rich (GAR) motif, which are collectively referred to as the kinetochore binding domain (KBD) [9–11]. The region of 53BP1 necessary for oligomerization maps to residues 1231–1270 and its function is DNA damage independent but necessary for 53BP1 recruitment to IRIF [11]. Early studies focusing on the DNA binding ability of the 53BP1's tudor domains revealed a dependence upon di-methylated histone H3 for foci formation [12]. However, more recent work found that the first of the two 53BP1 tudor domains bound di-methylated K20 of Histone H4 (H4K20^{me2}) with a much higher affinity, forming a molecular pocket-like structure able to specifically bind H4K20^{me2}, which is largely inaccessible in undamaged chromatin [13]. Although unequivocal proof has yet to emerge, a significant body of work supports a model whereby the ubiquitination of histones H2A and H2AX facilitates local chromatin opening that renders histone methyl groups more amenable to 53BP1 binding in the area surrounding a DSB [6–8,14,15]. Perhaps the most direct evidence for ubiquitination 'exposing' otherwise buried histone moieties is the finding that RNF8 and CHFR activity synergistically regulate histone ubiquitination and that this is prerequisite for acetylating K16 of histone H4 [16]. An alternative model is suggested by work demonstrating that MDC1-dependent MMSET histone methyltransferase activity is increased at sites of DNA damage, enabling 53BP1 IRIF formation via de novo histone H4 K20 methylation [17]. While it is conceivable that both pathways may equally contribute to 53BP1 recruitment, it should be noted that 98% of new histone H4 is di-methylated at K20 within 2–3 cell cycles following deposition in S-phase, with little to no detectable turnover of this modification in vivo [18]. Under these circumstances, it is likely that DNA damage-inducible de novo H4 methylation is most relevant to 53BP1 IRIF formation in newly synthesized chromatin (particularly towards the end of S-phase or the beginning of G2) and that this is less relevant in other cell cycle phases and even less so in non-dividing cells. By contrast, the visible

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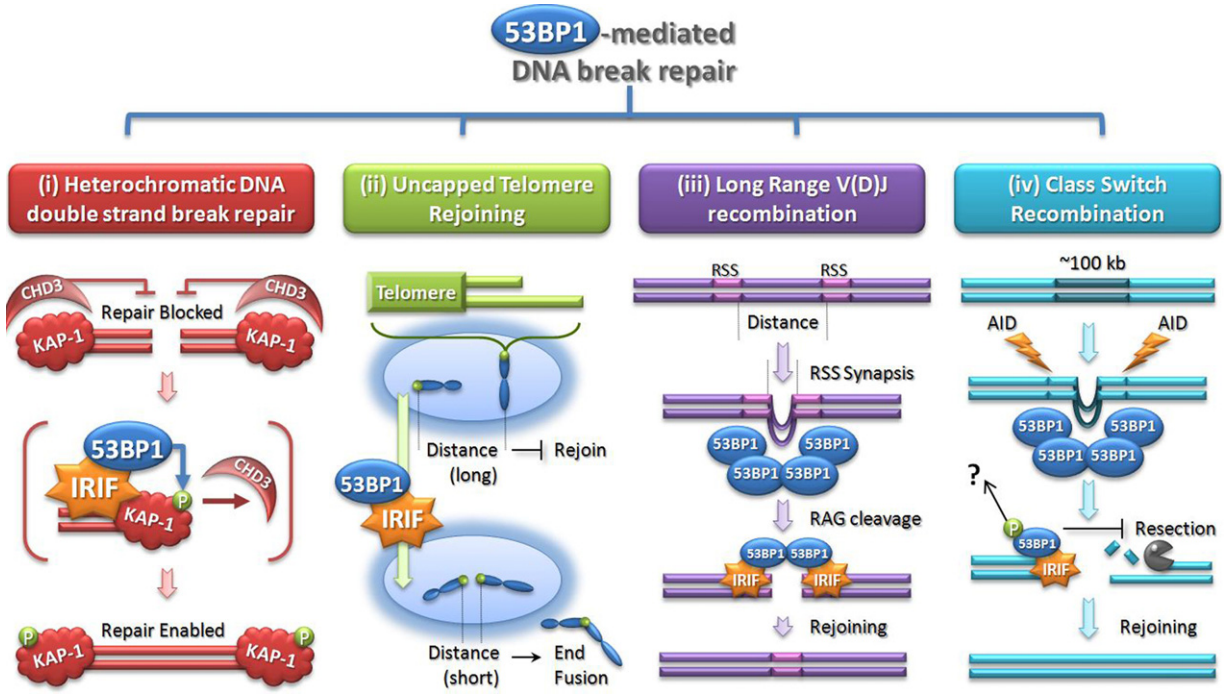

Fig. 1. Known pathways of 53BP1-dependent DNA double strand break repair. (i) Heterochromatic DNA double strand break repair fails in the presence of CHD3 ATP-dependent chromatin remodeling activity recruited by KAP-1. When recruited to IRIF, 53BP1 promotes robust KAP-1 phosphorylation by concentrating active ATM at the break site, which disperses CHD3 activity and, hence, enables repair. (ii) When telomeres are uncapped, 53BP1 enables chromosomal end-to-end fusion by increasing the distance across which the DNA end may traverse. (iii) During V(D)J recombination of distally separated V, D or J segments, recombination signal sequences (RSS) are brought together via the action of 53BP1 oligomers; following RAG endonuclease cleavage, 53BP1 at IRIF further assists the synapsis of RSS until rejoining can take place. (iv) AID cleavage sites are generally separated by ~100 kb and are brought into proximity by 53BP1 oligomers. Following DNA breakage, 53BP1 at IRIF attenuates DNA end resection, preventing excessive sequence loss before rejoining. The phosphorylation of 53BP1's N-terminus is required for rejoining, although the mechanism by which this occurs is unclear.

accumulation of 53BP1 at IRIF requires the presence and action of localized histone ubiquitination within all cell cycle phases examined [6–8,14].

Cells deficient in 53BP1 or its localization to IRIF display mild ATM-signaling defects and impaired ATM-dependent DSB repair [19,20]. 53BP1's role in ATM-dependent cell cycle checkpoint activation and maintenance is subtle and dependent upon the dose of DNA damage present at any given time. At higher DSB levels, 53BP1 is dispensable for maintaining ATM activity and both ATM substrate phosphorylation and cell cycle checkpoint arrest are effectively normal [20]. As such, it has been proposed that 53BP1 serves more to amplify ATM signaling than initiate it [21]. Indeed, 53BP1 is known to 'build upon' the prior action of MDC1, which drives early MRN-ATM recruitment to DSB sites, promoting progressive MRN-ATM concentration at IRIF via interactions between 53BP1's C-terminal BRCT domain and the Rad50 component of the MRN complex [19,22]. Thus, 53BP1 serves to maintain a certain level of ATM signaling per DSB that, when DSBs are scarce, is required for checkpoint activation. The subtle role of 53BP1 in cell cycle checkpoint activation contrasts with its role in DSB repair, where loss causes dose-independent defects equating, in effect, with a complete failure to repair lesions induced within regions of heterochromatin [19,23]. 53BP1 is additionally known to promote DSB repair during long-range V(D)J recombination in developing B and T lymphocytes [24], the rejoining of un-protected telomeres in cells depleted for the shelterin complex [25] and the repair of DSBs during immunoglobulin class switch recombination [26] (Fig. 1). The molecular mechanism(s) by which 53BP1 mediates DSB repair in each of these cases is only starting to become clear and numerous, potentially over-lapping explanations have been proposed, including: promoting chromatin relaxation, increasing DSB mobility within the nucleus or facilitating DSB synapsis. This is an emerging 'hot topic' within the DNA repair field that will be reviewed in detail here.

2. 53BP1-dependent DSB repair: heterochromatin

Non-dividing cells lacking or depleted for 53BP1 show a DSB repair defect following IR or X-ray treatment that is comparable to and epistatic with ATM loss, accounting for approximately 10–25% of induced DSBs [23]. Cells derived from RIDDLE syndrome patients, which are unable to form 53BP1 foci due to RNF168 mutation, also fail to repair 10–25% of DSBs and represent the first human case of IRIF failure (with otherwise normally initiated ATM signaling) leading to serious disease [6,27]. Further human cases of RNF168 mutation leading to disease and an inability to recruit 53BP1 to IRIF are now confirmed [28]. In 2008, within the laboratory of Penny Jeggo (University of Sussex, UK), we demonstrated that the ATM-dependent DSB repair fraction corresponds to lesions overlapping with heterochromatin enriched for the transcriptional co-repressor KAP-1 (KRAB-Associated Protein 1) and that phosphorylation of KAP-1 at S824 (pKAP-1), by ATM, was essential for DSB repair in these regions [29]. Mutation of KAP-1 S824 to alanine generated a constitutive DSB repair defect epistatic with ATM loss, while mutation of S824 to a phosphomimetic amino acid (aspartate) bypassed the need for ATM signaling in DSB repair altogether. We later demonstrated that 53BP1 depletion or deletion also produced a heterochromatic DSB repair defect that could be bypassed by expression of the KAP-1 S824D mutant [19]. However, quizzically, pKAP-1 levels by immunoblot were essentially normal in 53BP1 deficient cells – in stark contrast to ATM loss which completely ablated pKAP-1 formation. 53BP1, it turned out, was specifically required to support localized pKAP-1 at late-repairing, heterochromatic DSBs while pKAP-1 occurring away from DSBs (as a result