Beyond reversal: ubiquitin and ubiquitin-like proteases and the orchestration of the DNA double strand break repair response

Alexander J. Garvin

Institute of Cancer and Genomic Sciences, University of Birmingham, Edgbaston, Birmingham, U.K.

Correspondence: Alexander J. Garvin (a.j.garvin@bham.ac.uk)

The cellular response to genotoxic DNA double strand breaks (DSBs) uses a multitude of post-translational modifications to localise, modulate and ultimately clear DNA repair factors in a timely and accurate manner. Ubiquitination is well established as vital to the DSB response, with a carefully co-ordinated pathway of histone ubiquitination events being a central component of DSB signalling. Other ubiquitin-like modifiers (Ubl) including SUMO and NEDD8 have since been identified as playing important roles in DSB repair. In the last five years ~20 additional Ub/Ubl proteases have been implicated in the DSB response. The number of proteases identified highlights the complexity of the Ub/Ubl signal present at DSBs. Ub/Ubl proteases regulate turnover, activity and protein–protein interactions of DSB repair factors both catalytically and non-catalytically. This not only ensures efficient repair of breaks but has a role in channelling repair into the correct DSB repair sub-pathways. Ultimately Ub/Ubl proteases have essential roles in maintaining genomic stability. Given that deficiencies in many Ub/Ubl proteases promote sensitivity to DNA damaging chemotherapies, they could be attractive targets for cancer treatment.

Introduction

DNA double stranded breaks are highly toxic lesions that occur when both strands of DNA are broken. The breaks can be formed from endogenous processes such as DNA replication, or exogenous sources such ionising radiation. Two major pathways repair double strand breaks (DSBs) (Figure 1). Non-Homologous End Joining (NHEJ) occurs throughout the cell cycle and uses small regions of homology present in DNA overhangs followed by direct re-ligation for repair. If the overhangs are not compatible loss of nucleotides can lead to mutations. The second pathway Homologous Recombination (HR) is restricted to late S/G2 phases of the cell cycle as it relies on homologous DNA sequences, provided by sister chromatids, for homology directed repair. HR repair is generally error free [1]. Ub plays essential roles in orchestrating both repair pathways. A central function of Ub in DSB repair is maintaining the balance between NHEJ and HR in S/G2 cells. RNF168 mono-ubiquitination on K15 of H2A or H2AX promotes the recruitment of the pro-NHEJ factor 53BP1, while BRCA1–BARD1 mediated mono-ubiquitination of H2A K125/K127 or K129 promotes displacement of 53BP1 and subsequent DNA end-resection, the essential precursor step for HR [2]. Maintaining the correct balance is critical, as too much NHEJ can be mutagenic, but un-restrained DNA end-resection is also toxic. To co-ordinate DSB repair Ub and the Ubl SUMO and NEDD8 play multiple roles, they promote the recruitment and retention of repair factors at damaged chromatin, alter protein–protein interactions and enable clearance and termination of repair signalling [3,4]. As all of these processes require exquisite temporal and spatial tuning, a plethora of Ub/Ubl proteases are deployed by DNA damage signalling to ensure efficient and accurate DSB repair [5]. The profound consequences that result from the mis-regulation of these proteases offers fascinating insight into the intricacies of DSB repair.
Ku70/80 and the MRN complex

DSBs are rapidly detected by two complexes, the Ku70/Ku80 heterodimer which promotes NHEJ and MRN (MRE11A/RAD50/NBS1) which, with the exonuclease CtIP promotes HR [6,7]. Multiple Ub-E3 ligases ubiquitinate Ku heterodimers which ultimately results in extraction by the VCP/p97 complex [8,9]. The importance of this regulation is highlighted by the toxic effects of trapped Ku heterodimers which not only prevent NHEJ repair termination but also block the early steps in HR repair [9]. Ubiquitination of Ku heterodimers is countered by at least two DUBs (De-ubiquitinating proteases). UCHL3 de-ubiquitinates and stabilises Ku80 which is required for Ku80 retention at DSBs [10]. OTUD5 also stabilises Ku80, loss of OTUD5 reduces NHEJ repair efficiency [11] (Figure 2).

The MRN complex performs several activities, including DNA unwinding, promotion of DNA end resection and recruitment of the master DSB kinase ATM to the DSB [6]. ATM phosphorylates multiple DSB repair proteins, including histone H2AX at Ser139 (γH2AX) [12]. Pellino generates K63-Ub chains on NBS1 promoting ATM activation and HR repair, suggesting that Ub signalling also regulates MRN activity [13]. Indeed, USP4 is recruited to DSBs through interaction with NBS1 where it also regulates CtIP ubiquitination [14].

MDC1 (Mediator of Damage Checkpoint 1)

MDC1 interacts with γH2AX via its BRCT repeats [15], and has multiple roles in DSB signalling, it aids in the retention of ATM which further propagates the γH2AX signal along damaged chromatin and it recruits RNF8.

Figure 1. NHEJ occurs at all stages of the cell cycle.
Ku70/80 heterodimers bind and stabilise the ends of the DSB. Other factors are also recruited by the Ku dimer. DNA end processing chemically modifies the bases to allow re-ligation. Ligase 4 (LIG4), XLF and other repair factors ligate the broken DNA ends. HR is restricted to S/G2, initial DNA 3’ end resection is initiated by the MRN complex and CtIP. Further resection promoted by the BRCA1–BARD1 heterodimer, EXO1 and DNA2 promote long range resection. The ssDNA generated by end resection is coated in the RPA heterotrimeric complex, which is then displaced by RAD51 nucleofilaments with the aid of the BRCA1–PALB2–BRCA2 complex. After RAD51 filament formation, homology search followed by invasion of the sister chromatid and synthesis of new DNA occurs. As the biology of these later processes in the context of Ub/Ubl modifications is poorly understood they are not illustrated here. As NHEJ can also occur in S/G2 pathway choice between HR and NHEJ occurs. The pro-NHEJ factor 53BP1 is recruited by K15 Ub modified H2A/H2AX while modification of K125/K127 or K129 by mono-Ub aids in nucleosome remodelling and displacement of 53BP1, this favours DNA end-resection and HR. Thus maintaining the balance of Ub signals dictates repair pathway choice.
Figure 2. The DNA bound Ku70/80 heterodimer is ubiquinated and SUMOylated by multiple E3 ligases. Part 1 of 2
Ultimately this signals for Ku70/80 dimer removal from chromatin by VCP/p97. MDC1 is constitutively protected from excessive SUMOylation through its interaction with SENP2. This interaction is disrupted by ATM signalling which allows the DSB located MDC1 to be hyperSUMOylated by PIAS4 which also localises to DSBs. The SUMOylated MDC1 is then recognised by the SUMO directed Ub ligase RNF4 which ubiquitinates MDC1 and promotes extraction from chromatin by VCP. The RNF4 step can be countered by ATXN3, slowing MDC1 turnover at DSBs. RNF8 is recruited by MDC1 where it forms K63-Ub linkages on...
and K63-Ub signalling [24]. The RNF4 dependent clearance of MDC1 is antagonised by ATXN3, a DUB that undergoes premature PIAS4-RNF4-VCP dependent clearance from DSBs resulting in ablated RNF8 accrual shortly after DSB induction. This allows unrestrained SUMOylation of MDC1 by PIAS4 on DSBs which ultimately promotes RAP80-BRCA1 accrual. USP13 trims Ub from the RAP80 UIM domain to allow efficient Ub recognition. BRCA1–BARD1 also recruit to DSBs independently of K63-Ub signalling. Ubiquitination inhibits the BARD1–HP1γ interaction needed for efficient accumulation of BRCA1–BARD1 at DSBs. This disruptive ubiquitination event is countered by USP15. The ubiquitination and turnover of BRCA1 is countered by USP9X. BRCA1–BARD1 also generates mono-Ub on the far C terminus of H2A (K125, K127 or K129). This signal promotes DNA end-resection and aids in the displacement of 53BP1 from DSBs. USP48 restricts the spread of H2A K125/127/129-Ub preventing excessive DNA end-resection. The MRN complex and CtIP, both of which are essential for the early steps in DNA end-resection are ubiquitinated, USP4 antagonises these modifications. SUMOylation promotes degradation of the DNA exonuclease EXO1, to control DNA end-resection which is countered by SENP6. The loading of the RPA hetero-trimer onto single stranded DNA (generated through DNA end-resection) and its exchange with RAD51 are essential steps in HR. The RPA70 subunit is SUMOylated and degraded by RNF4, this is countered by SENP6. The loading of RAD51 onto ssDNA is promoted by BRCA2 in the BRCA1–PALB2–BRCA2 complex. This complex can only form due to the activity of USP11 which removes an inhibitory Ub modification that is disruptive to BRCA1–PALB2 interactions. USP11 levels are cell cycle regulated by KEAP1. USP1–UAF1 non-catalytically aids in the loading of RAD51, UCHL3 de-ubiquitinates RAD51 promoting its ability to load onto DNA, while USP21 protects the unstable BRCA2 protein from degradation.

[16,17]. While MDC1 recruitment is phosphorylation dependent, turnover at DSBs in G1 requires SUMOylation and ubiquitination. Following recruitment to DSBs MDC1 is SUMOylated by PIAS4 [18–22]. MDC1 SUMOylation promotes recognition by RNF4, an Ub-E3 that interacts with SUMOylated substrates [23]. This in turn promotes VCP/p97 dependent extraction of MDC1 from DSBs [20–22,24]. The basal SUMOylation state of MDC1 is maintained by the SUMO protease SENP2 which dissociates from MDC1 shortly after DSB induction. This allows un-restrained SUMOylation of MDC1 by PIAS4 on DSBs which ultimately triggers MDC1 removal by VCP. In the absence of SENP2 the constitutively hyperSUMOylated MDC1 undergoes premature PIAS4-RNF4-VCP dependent clearance from DSBs resulting in ablated RNF8 accrual and K63-Ub signalling [24]. The RNF4 dependent clearance of MDC1 is antagonised by ATXN3, a DUB that is rapidly recruited to DSBs by SUMO [25]. USP7 also interacts with and stabilises the MDC1–MRN complex [26].

**RNF8**

RNF8 is recruited to DSBs via its FHA domain that recognises phosphorylated MDC1 [27], where along with its partner E2 (UBE2N) K63-Ub chains are conjugated on histone H1 [28]. HUWE1 primes the ubiquitination of H1 prior to extension by RNF8 [29]. Several DUB dependent modes of RNF8 regulation have been identified. OTUB1 blocks the generation of K63-Ub through a non-enzymatic activity that involves disruption of the RNF8–UBE2N catalytic cycle [30,31]. The RNF8–UBE2N complex is also regulated by HERC2 which
stimulates the generation of K63-Ub at DSBs by stabilising or promoting the interaction of RNF8 with UBE2N [32]. This activity is promoted by SUMOylation of HERC2 through PIAS4 [33]. HERC2 interacts with USP20, a DUB that is recruited to DSBs and is involved in checkpoint signalling in response to replication stress and HR repair [34–37]. HERC2 also interacts with and stabilises USP16 which is involved in H2A de-ubiquitination in DSB repair [38]. RNF8 auto-ubiquitination is countered by ATXN3, which is required to balance VCP/p97 dependent extraction from damaged chromatin [39].

**RNF168**

H1 K63-Ub is recognised by a MIU domain of RNF168, which conjugates mono-ubiquitin to H2A or H2AX at K15 [28,40]. This serves as a docking site for the resection antagonist/pro-NHEJ factor 53BP1 [41,42]. A second MIU domain in RNF168 also recognises its own Ub product, allowing spreading and amplification of H2A/H2AX-UbK15 [28]. RNF168 is de-stabilised following DSB induction and at least two DUBs, USP34 and USP7 are involved in countering RNF168 turnover. Loss of either DUBs results in reduced RNF168 and less K63-Ub signalling at DSBs [43,44]. RNF168 accumulation at damaged chromatin is also regulated by DUBs. A20 interacts with RNF168 upon DSB induction and disrupts recruitment to H1K63-Ub non-catalytically [45]. USP14 also recruits to DSBs where it interacts with RNF168 via its MIU1 domain and impairs RNF168 Ub signalling [46].

USP3, USP51 and USP16 de-ubiquitinate H2A/H2AXK15-Ub with varying degrees of specificity [47–49]. Loss of these DUBs causes excessive spreading of H2A/H2AXK15-Ub and its downstream reader 53BP1. In addition to K63-Ub, RNF168 generates K27-Ub conjugates, which are involved for the recruitment of 53BP1 and RAP80–BRCA1 [50]. Although the biology of K27-Ub in DSB repair is not well understood, it has since been shown that RNF168 generated K27-Ub conjugates can be antagonised by UCHL3 [51].

H2A/H2AXK15-Ub is also recognised by the RNF168 paralog RNF169 which competes with 53BP1. RNF169 therefore limits the recruitment of the pro-NHEJ/anti-resection factor 53BP1 and promotes HR repair [41,52–55]. Like RNF168, RNF169 is also stabilised by USP7 [56].

Several DUBs were characterised for their roles in DSB signalling prior to the identification of H2A/H2AXK15-Ub generated by RNF168 and therefore it is not clear if they only target this modification, other sites on H2A/H2AX or non-histone K63-Ub modified proteins. USP44, USP17L2/DUB3, USP11, ZUFSP and POH1 loss promotes the excessive spreading of Ub at DSBs and concomitant excessive accumulation of 53BP1 [57–65].

**H2a-K118/K119**

H2A is predominantly mono-ubiquitinated at K118/K119 by BMI1:RING1B E3 ligase. H2AK118/K119-Ub has important, but not fully understood the role in DSB repair [66]. BAP1 is the dominant H2AK118/K119-Ub DUB [67] and is rapidly and transiently recruited to DSBs [37,68,69]. BAP1 loss specifically reduces HR but not NHEJ [68,69].

**53BP1**

53BP1 is a major adapter protein that recruits to DSBs via histone phosphorylation [70], methylation [71] and ubiquitination [42]. While 53BP1 has no enzymatic activity it does recruit a number of other proteins that antagonise DNA end-resection and subsequent HR pathways mediated by BRCA1. The major effectors of 53BP1 include PTIP, RIF1 and the Rev7-Shieldin-CST complexes [72].

The Tudor domain of 53BP1 recognises H4K20me2, which is also recognised with higher avidity by L3MBTL1 and JMJD2A [73,74]. To allow maximal 53BP1 recruitment these factors need to be displaced from chromatin. L3MBTL1 is K48 ubiquitinated by RNF8 and extracted by VCP/p97 from chromatin [73]. This ubiquitination is antagonised by OTUB2, a DUB that is recruited to DSBs and slows L3MBTL1 Ub dependent removal from chromatin. This limits excessive 53BP1 spreading [37,75]. SUMOylation at DSBs is also important for 53BP1 recruitment, and 53BP1 is itself SUMOylated by PIAS4 in G1 [18,76]. SUMOylation of 53BP1 is antagonised by SENP1, as displacement of SENP1 from nuclear pores reduces 53BP1 SUMOylation and disrupts NHEJ repair [77]. The 53BP1 interacting partner RIF1 is also PIAS4 SUMOylated with similar kinetics to 53BP1 [76].

**RAP80 and the BRCA1-A complex**

RAP80 is a reader of K63-Ub polymers at DSBs and serves as a docking module for the BRCA1-A complex [78–81]. In addition to K63-Ub, RAP80 interacts with SUMO chains, likely through its recognition of mixed
K63-Ub-SUMO linkages for which it has an eighty times higher binding preference over K63-Ub chains. RNF4 is the likely source of these linkages due to its localisation at DSBs and ability to generate mixed K63-Ub-SUMO conjugates [82,83].

BRCC36 (BRCA1/2 Containing Complex 36) was the first DUB characterised in DSB repair [84] and resides within the BRCA1-A complex. BRCC36 de-ubiquinatises H2A/H2AX and RAP80. Loss of BRCC36 results in extensive spreading of K63-Ub at DSBs, suggesting its role is to limit the amplitude of K63-Ub at DSBs [85,86]. BRCA1-A components, including BRCC36, are antagonists of HR repair. The BRCA1-A complex has been proposed to divert BRCA1–BARD1 Ub ligase activity to dampen DNA end-resection. Loss of BRCA1-A components causes a hyper-resection phenotype [87,88]. USP26 and USP37 are both recruited to DSBs and act on conjugates generated by RNF168. However, these DUBs act on the RAP80 pathway rather than the 53BP1 pathway. USP26/USP37 depletion increases size but not number of BRCA1 foci. This excessive spreading of BRCA1 can be countered by depletion of RAP80, suggesting the BRCA1 is being recruited into enlarged BRCA1-A complexes. In cells depleted of USP26/USP37, BRCA1 interacts less efficiently with PALB2, which bridges interaction with BRCA2. As BRCA2 is required for RAD51 loading this is a likely cause of failed HR in these cells [57,58]. The K63-Ub dependent interaction by RAP80 is disrupted by ubiquitination of lysines in close proximity to its UIM domain. USP13 counters this modification to enable recruitment of RAP80 and the BRCA1-A complex to DSBs [89]. BRCA1 recruitment in S/G2 is also regulated by USP1–UAFl1, which antagonises K63-Ub chains that serve as recruiters for BRCA1. Unlike several other DUBs that antagonise K63-Ub, over-expression of USP1 only affected BRCA1 recruitment and not 53BP1. USP1 is locally inactivated by K11-Ub modification generated by APC\textsubscript{Cdh1}/Ub\textsubscript{2S} which is also localised to DSBs. The APC\textsubscript{Cdh1} dependent inactivation of USP1 thus removes the restraint on K63-Ub generation in a cell cycle dependent manner [90].

**BRCA1–BARD1**

BARD1 aids in the recruitment of its heterodimeric partner BRCA1 to DSBs through a number of different interacting partners, including HP1\textgamma [91]. The BARD1 BRCT repeats are modified by K63-Ub which disrupts interaction with HP1\textgamma and therefore localisation of BRCA1–BARD1 to DSBs. This inhibitory ubiquitination is removed by USP15, a cytoplasmic DUB that accumulates at DSBs through interaction with BARD1 [37,92]. The constitutive turnover of BRCA1 is countered by USP9X, loss of which causes reduction in BRCA1 protein levels and reduced HR repair [93]. The BRCA1–BARD1 heterodimer constitutes an additional source of H2A ubiquitination in the DSB repair response, being responsible for mono-ubiquitination of the extreme C terminus of H2A at K125/127/129 [94]. This ubiquitination promotes chromatin alterations which facilitate long range DNA end resection [95]. This modification is regulated by USP48 which specifically cleaves mono-ubiquitinated H2AK125/127/129Ub. USP48 controls the extent of H2AK125/127/129 ubiquitination to prevent hyper-resection. In the absence of USP48, DNA is over-resected and the cells utilise the single strand annealing (SSA) sub-pathway in addition to HR. SSA is mutagenic due to the use of homologous repeats for bridging DSB ends, resulting in sections of DNA being deleted [48].

**CtIP**

CtIP activity is essential for the early commitment to HR and is extensively regulated by both Ub and SUMO E3 ligases [96,97]. Ubiquitination and SUMOylation are important for regulating CtIP steady state, promoting recruitment to DSBs and limiting excessive resection. USP4 recruits to DSBs and interacts with both the MRN complex and CtIP [14,98]. In its inactive ubiquitinated form USP4 cannot interact with these factors but due to auto-de-ubiquitination these modifications are removed, enabling interaction with MRN-CtIP. It is not fully understood how USP4 regulates CtIP activity [14,98].

**EXO1**

EXO1 is degraded after DSB induction as a means to limit hyper-resection [99]. EXO1 is SUMOylated by PIAS4 in response to DSB induction, which promotes its degradation independently of RNF4. SENP6 antagonises EXO1 SUMOylation promoting stabilisation [100]. UCHL5 is also required for BLM/EXO1 dependent end resection through de-ubiquitinating the INO80 subunit NFRKB, although the function of this subunit in regulating end resection is not currently understood [37].
RPA complex

USP1 both directly and indirectly — through its partner UAF1 affects HR repair [101–103]. UAF1 is critical to the HR response through its association with RAD51AP1–RAD51–DNA complex which is required for efficient RAD51 loading and unloading [101–103]. The RAD51AP1 interaction is mediated through a SUMO-like domain, interestingly SUMO has previously been shown to be important for RAD51 foci formation [104]. The RPA70 subunit is SUMOylated in response to DSB induction due to the release of the constitutive interaction with SENP6. The SUMOylation of RPA70 improves interaction with RAD51 which is required for the exchange of RPA subunits for RAD51 during HR [105].

BRCA2 and RAD51

Assembly of the BRCA1–PALB2–BRCA2 complex is restricted to S/G2 phases of the cell cycle. The Cullin3 adapter KEAP1 is responsible for preventing the formation of this complex outside of S/G2 by conjugating Ub on the interface between BRCA1 and PALB2. This disruptive ubiquitination is countered by USP11 [106,107]. USP11 turnover is regulated by KEAP1 in a cell cycle dependent manner, with USP11 protein levels at their lowest in G1. Thus cell cycle regulated turnover of USP11 allows a Ub dependent modification event to disrupt the formation of the BRCA1–PALB2–BRCA2 complex. This prevents RAD51 loading from occurring outside of S/G2 phases. Cells deficient of USP11 are defective in RAD51 loading due to failure in the formation of this complex, resulting in loss of HR [107,108]. USP21 interacts with, and stabilises the BRCA2–RAD51 complex by antagonising degradative BRCA2 ubiquitination. Down-regulation of USP21 results in failure of RAD51 loading and HR repair [109]. UCHL3 interacts with RAD51 and de-ubiquitinates residues that disrupt the RAD51–BRCA2 interaction. In the absence of UCHL3, RAD51 interaction with BRCA2 is disrupted, leading to reduced RAD51 foci formation and HR repair efficiency [110].

Chromatin relaxation

USP8 forms a complex with the early DDR factor BRIT1. Under basal conditions BRIT1 is K63-Ub modified, which is antagonised by USP8. Low levels of BRIT1 K63-Ub modification are needed for recruitment to γH2AX which promotes the further recruitment of chromatin remodellers. The subsequent relaxation of chromatin then allows accumulation of downstream repair factors [111]. SENP7 maintains steady state SUMOylation levels of the chromatin component KAP1. ATM dependent phosphorylation of KAP1 is required to disrupt the SUMO dependent interaction with the CHD3 subunit of the NuRD complex. This disruption allows dispersion of the NuRD CHD3 complex from DSBs and subsequent chromatin relaxation [112]. In the absence of SENP7 the hyperSUMOylated KAP1 retains interaction with CHD3 resulting in failure of chromatin relaxation and HR repair [113]. The NuRD complex also recruits USP11 to DSBs promoting both histone de-ubiquitination and de-acetylation which enforces proper chromatin remodelling post DSB induction [65].

Maintaining the free pool of Ub/Ubl for effective DSB response

Disruption of proteasome function results in attenuation of Ub driven DSB repair which can be overcome by suppling excess free Ub [62,114]. In addition to the proteasome, Ub/Ubl proteases are required for maintaining modifier availability, either by processing immature precursors or by recycling modifiers from substrates. Only six SUMO proteases are responsible for the bulk of SUMO maturation and recycling. Loss of SENP6 provokes a HR defect due to failure in RPA70 deSUMOylation and RAD51 filament formation [105], however, SENP6 is the major polySUMO2/3 protease, depletion of which causes substantial alterations in SUMO2/3 homeostasis in cells. These pleiotropic effects contribute to the HR defect, as re-supply of SUMO2 restores HR in SENP6 depleted cells [113]. Depletion of SENP2 also causes SUMO starvation resulting in HR failure that can be rescued with additional free SUMO [24].

NEDDylation and DSB repair

NEDD8 conjugates are enriched at DSBs [115]. NEDD8 likely plays multiple roles in DSB resolution, but a role in H4 NEDDylation by RNF111, required for RNF168 accrual has been proposed [115]. To add further complexity RNF168 has also been proposed to NEDDylate H2A acting as a competitor for Ub signalling [116]. Additionally RNF111 NEDDylates CtIP and thus regulates DNA end-resection [117]. NEDDylation is reversed
by just two known active proteases — the JAMM type DUB CSN5 within the COP9 signalosome and SENP8 [118]. Given the role of NEDDylation in the DSB repair response it is unsurprising that the COP9 signalosome and its DUB component CSN5 are recruited to DSBs in a NEDD8 dependent fashion. Disruption of this complex reduces HR [37,119].

### Ub/Ubl proteases and cancer

Imbalances in DSB associated Ub/Ubl protease activity can be caused by mutations or expression alterations. For example, inactivating mutations in BAP1 are common in mesothelioma, renal clear cell carcinomas and uveal melanomas. Cell lines carrying these mutations are particularly sensitive to ionising radiation and PARP inhibitors due to the important roles BAP1 plays in HR repair [68,69,120]. The amplification of the long arm of chromosome 3q is common in epithelial cancers, including lung squamous cell carcinoma, oesophageal, cervical and ovarian cancer. The 3q amplification encompasses the RNF168, USP13 and SENP2 genes. Over-expression of each of these individually promotes resistance to irradiation through altered Ub/SUMO signalling and DSB repair kinetics [24,89,121]. Several other DUBs are amplified in cancers including UCHL3 in breast cancer [110] and USP21 in hepatocellular carcinoma [109]. Dependency on Ub/Ubl proteases for cancer survival could make these useful targets in patient stratification. Indeed, inhibition of specific DUBs is currently being investigated as a means to enhance sensitivity to chemo/radiotherapies [122]. USP1 inhibitors have been proven effective in BRCA1 mutant tumours as USP1 is required for replication fork stability in the absence of functional BRCA1 [123]. The USP13/USP10 inhibitor Spautin-1 improves the anti-cancer activity of PARP inhibitors in an ovarian cancer model in mice [89]. USP7 inhibitors are also effective at sensitising therapy resistant CLL cells to HR directed therapies [124].

| Function | Ub/Ubl Protease |
|----------|----------------|
| Ku dimer retention | UCHL3, OTUD5 |
| MDC1 retention | SENP2, ATXN3, USP7 |
| RNF8 stabilisation | ATXN3 |
| RNF8–UBE2N catalysis antagonist | OTUB1 |
| RNF168 stabilisation | USP34, USP7 |
| RNF168 accumulation antagonist | A20, USP14 |
| H2A/H2AX/K13Ub spread antagonist | USP3, USP51, USP16 |
| H2A-K118/119Ub antagonist | BAP1 |
| K63-Ub/53BP1 spread antagonist | USP44, DUB3, USP11, ZUFSP, POH1, BRCC36, USP26, USP37 |
| 53BP1 spread (methyl dependent) antagonist | OTUB2 |
| RAP80–BRCA1-A complex regulators | BRCC36, USP26, USP37, USP13, USP1-UAF1 |
| BRCA1–BARD1 accumulation | USP15 |
| BRCA1 stabilisation | USP9X |
| H2A-K125/K127/K129 antagonist | USP48 |
| CtIP–MRN regulators | USP4 |
| EXO1 stabilisation | SENP6, UCHL5 |
| RPA–RAD51 interaction | USP1-UAF1, SENP6 |
| BRCA2 stabilisation | USP21 |
| RAD51 loading | USP11, UCHL3 |
| Chromatin remodelers | USP8, SENP7, USP11 |
| Free SUMO pool regulators | SENP2, SENP6 |

Note that many proteases play multiple roles in DSB signalling e.g. USP11.
Perspectives

- The sheer number and lack of redundancy of DSB associated Ub/Ubl proteases highlights the complexity of Ub/Ubl signalling in genomic stability (summarised in Table 1). In many cases, the reduction or inactivation of a single Ub/Ubl protease is sufficient to entirely block DSB repair.

- The RNF8–RNF168–K63-Ub signalling node generates easily detectable DSB associated foci that can be visualised by a number ofUb specific antibodies such as FK2 and K63-Ub. As these modifications are read by 53BP1 which also forms readily detectable foci much of the initial research in the field focused on DUBs that regulate this step, indeed ∼8 DUBs have so far been identified that regulate 53BP1 dependent foci spreading [2]. However in more recent years Ub/Ubl proteases that regulate the earliest steps of DSB repair, the ordered clearance of repair factors and the later steps of RAD51 loading have been identified, suggesting Ub/Ubl modifiers are involved in multiple steps of DSB repair. Even greater nuance in Ub/Ubl modifier roles in DSB repair has been highlighted by a number of DUBs that remove disruptive Ub conjugates that impair protein–protein interactions needed for DSB repair.

- Further layers of complexity arise from the multiple Ub chain types now implicated in DSB repair. Additionally SUMOylation is unlikely to act separately from ubiquitination as co-modification and mixed chains are important signalling elements of the DSB response [125]. Therefore the diversity of chains types present at DSBs is likely many times greater than currently appreciated. SUMOylation is essential for the recruitment, activity and clearance of several DSB repair factors but we know relatively little concerning the activity of deSUMOylases in the DSB response, indeed there appears to be little redundancy between SENP enzymes as depletion of each causes specific DSB repair defects [24,105,113]. Finally, in both NHEJ and HR repair pathways there are multiple steps that are regulated by Ub/Ubls but the roles for their respective proteases await discovery.

Abbreviations
DSBs, double strand breaks; HR, homologous recombination; NHEJ, non-homologous end joining; SSA, single strand annealing.

Competing Interests
The Author declares that there are no competing interests associated with this manuscript.

References
1 Scully, R., Panday, A., Elango, R. and Willis, N.A. (2019) DNA double-strand break repair-pathway choice in somatic mammalian cells. Nat. Rev. Mol. Cell Biol. 20, 698–714. https://doi.org/10.1038/s41580-019-0152-0
2 Uckelmann, M. and Soma, T.K. (2017) Histone ubiquitination in the DNA damage response. DNA Repair 56, 92–101. https://doi.org/10.1016/j.dnarep.2017.06.011
3 Garvin, A.J. and Morris, J.R. (2017) SUMO, a small, but powerful, regulator of double-strand break repair. Philos. Trans. R. Soc. Lond. B Biol. Sci. 372, 20160281. https://doi.org/10.1098/rstb.2016.0281
4 Brown, J.S., Lukashchuk, N., Szcaniecka-Cliff, M., Britton, S., le Sage, C., Calsou, P. et al. (2015) Neddylation promotes ubiquitylation and release of Ku from DNA-damage sites. Cell Rep. 11, 704–714. https://doi.org/10.1016/j.celrep.2015.03.058
5 Nishi, R. (2017) Balancing act: to be, or not to be ubiquitylated. Mutat. Res. 803, 43–50. https://doi.org/10.1016/j.mrfmmm.2017.07.006
6 Syed, A. and Tainer, J.A. (2018) The MRE11-RAD50-NBS1 complex conducts the orchestration of damage signaling and outcomes to stress in DNA replication and repair. Annu. Rev. Biochem. 87, 263–294. https://doi.org/10.1146/annurev-biochem-062917-012415
7 Shibata, A., Jeggo, P. and Lobrich, M. (2018) The pendulum of the Ku-Ku clock. DNA Repair 71, 164–171. https://doi.org/10.1016/j.dnarep.2018.08.020
8 Kragelund, B.B., Weterings, E., Hartmann-Petersen, R. and Keijzers, G. (2016) The Ku70/80 ring in non-homologous end-joining: easy to slip on, hard to remove. Front. Biosci. 21, 514–527. https://doi.org/10.2741/4406
101 Cukras, S., Lee, E., Palumbo, E., Benavidez, P., Moldovan, G.L. and Kee, Y. (2016) The USP1-UAF1 complex interacts with RAD51AP1 to promote homologous recombination repair. Cell Cycle 15, 2636–2646. https://doi.org/10.1080/15384101.2016.1209613

102 Liang, F.S., Longerich, S., Miller, A.S., Tang, C., Buzovetsky, O., Xiong, Y. et al. (2016) Promotion of RAD51-mediated homologous DNA pairing by the RAD51AP1-UAF1 complex. Cell Rep. 15, 2118–2126. https://doi.org/10.1016/j.celrep.2016.05.007

103 Murai, J., Yang, K.L., Desjuphong, D., Hirota, K., Takeda, S. and D’Andrea, A.D. (2011) The USP1-UAF1 complex promotes double-strand break repair through homologous recombination. Mol. Cell 31, 2462–2469. https://doi.org/10.1016/MCB.05058-11

104 Shima, H., Suzuki, H., Sun, J.Y., Kono, K., Shi, L., Kinomura, A., et al. (2013) Activation of the SUMO modification system is required for the accumulation of RAD51 at sites of DNA damage. J. Cell Sci. 126, 5284–5292. https://doi.org/10.1242/jcs.133744

105 Dou, H., Huang, C., Singh, M., Carpenter, P.B. and Yeh, E.T.H. (2013) Regulation of DNA repair through deSUMOylation and SUMOylation of replication protein A complex. Mol. Cell 39, 333–345. https://doi.org/10.1016/j.molcel.2010.07.021

106 Schoenfeld, A.R., Apgar, S., Doloe, G., Wang, R. and Aaronsan, S.A. (2004) BRCA2 is ubiquitinated in vivo and interacts with USP11, a deubiquitinating enzyme that exhibits prosurvival cellular function in the DNA damage response. Mol. Cell Biol. 24, 7444–7455. https://doi.org/10.1128/MCB.24.17.7444-7455.2004

107 Orthwein, A., Noordermeer, S.M., Wilson, M.D., Landry, S., Enchev, R.I., Sherker, A., et al. (2015) A mechanism for the suppression of homologous recombination in G1 cells. Nature 528, 422–426. https://doi.org/10.1038/nature16142

108 Wiltshire, T.D., Lovejoy, C.A., Wang, T., Xia, F., O'Donnell, L., Williams, T.A. et al. (2015) USP8 and BRUCE regulate DNA double-strand break repair and tumor growth. EMBO Rep. 16, 1495–1504. https://doi.org/10.15252/embr.201543029

109 Li, J.P., Kruwicki, A., Dang, H., Tran, A.D., Kwon, S.M., Wang, X.W. et al. (2017) Ubiquitin-specific protease 21 stabilizes BRCA2 to control DNA repair and tumor growth. Nat. Commun. 8, 1377. https://doi.org/10.1038/s41467-017-00206-2

110 Luo, K.T., Li, L., Li, Y.H., Wu, C.M., Yin, Y.J., Chen, Y.P. et al. (2016) A phosphorylation-deubiquitination cascade regulates the BRCA2-RAD51 axis in homologous recombination. Genes Dev. 30, 2581–2595. https://doi.org/10.1101/gad.289439.116

111 Ge, C.M., Che, L.X., Ren, J.Y., Pandita, R.K., Lu, J., Li, K.Y. et al. (2015) BRUCE regulates DNA double-strand break response by promoting USP8 deubiquitination of BRIT1. Proc. Natl Acad. Sci. U.S.A. 112, E1210–E1219. https://doi.org/10.1073/pnas.1418335112

112 Goodarzi, A.A., Kurka, T. and Jeggo, P.A. (2011) KAP-1 phosphorylation regulates CHD3 nucleosome remodeling during the DNA double-strand break response. Nat. Struct. Mol. Biol. 18, 831–U112. https://doi.org/10.1038/nsmb.2077

113 Garvin, A.J., Densham, R., Blair-Reid, S.A., Pratt, K.M., Stone, H.R., Weekes, D. et al. (2013) The deSUMOylase SENP7 promotes chromatin relaxation during homologous recombination DNA repair. EMBO Rep. 14, 975–983. https://doi.org/10.1038/embr.2013.141

114 Dantuma, N.P., Groothuis, T.M., Saloms, F.A. and Neelefs, J. (2006) A dynamic ubiquitin equilibrium couples prososomal activity to chromatin remodeling. J. Cell Biol. 173, 19–26. https://doi.org/10.1083/jcb.200501001

115 Ma, T., Chen, Y.B., Zhang, F., Yang, C.Y., Wang, S.M. and Yu, X.C. (2013) RNF111-Dependent neddylation activates DNA damage-induced ubiquitination. Mol. Cell 49, 897–907. https://doi.org/10.1016/j.molcel.2013.01.006

116 Li, T.T., Guan, J.H., Huang, Z.J., Hu, X. and Zheng, X.F. (2014) RNF168-mediated h2A neddylation antagonizes ubiquitilation of h2A and regulates DNA damage repair. J. Cell Sci. 127, 2238–2248. https://doi.org/10.1242/jcs.138891

117 Jimeno, S., Fernandez-Avilia, M.J., Cruz-Garcia, A., Copedoa-Garcia, C., Gomez-Cabello, D. and Huertas, P. (2015) Neddylation inhibits CSN-mediated resection and regulates DNA double strand break repair pathway choice. Nucleic Acids Res. 43, 987–999. https://doi.org/10.1093/nar/gku1384

118 Brown, J.S. and Jackson, S.P. (2015) Ubiquitilation, neddylation and the DNA damage response. Open Biol. 5, 150018. https://doi.org/10.1098/rsob.150018

119 Meir, M., Galanty, Y., Kashani, L., Blank, M., Khoosrov, R., Fernandez-Avilia, M.J., et al. (2015) The COP9 signalosome is vital for timely repair of DNA double-strand breaks. Nucleic Acids Res. 43, 4517–4530. https://doi.org/10.1093/nar/gkv270

120 Pena-Lopez, S., Vega-Rubin-De-Celis, S., Liao, A., Leng, N., Pavia-Jimenez, A., Wang, S., et al. (2012) BAP1 loss defines a new class of renal cell carcinoma. Nat. Genet. 44, 751–759. https://doi.org/10.1038/ng.2233

121 Chroma, K., Mistrik, M., Moudry, P., Gursky, J., Liptay, M., Strauss, R., et al. (2017) Tumors overexpressing RNF168 show altered DNA repair and responses to genotoxic treatments, genomic instability and resistance to proteotoxic stress. Oncogene 36, 2405–2422. https://doi.org/10.1038/onc.2016.392

122 Harrigan, J.A., Jacq, X., Martin, N.M. and Jackson, S.P. (2018) Deubiquitilating enzymes and drug discovery: emerging opportunities. Nat. Rev. Drug Discov. 17, 57–77. https://doi.org/10.1038/nrd.2017.152

123 Lim, K.S., Li, H., Roberts, E.A., Gaudiano, E.F., Clairmont, C., Sambol, L.A., et al. (2018) USP1 is required for replication fork protection in BRC1-deficient tumors. Mol. Cell 72, 925–941.e4. https://doi.org/10.1016/j.molcel.2018.10.045

124 Agathanggelou, A., Smith, E., Davies, N.J., Kwok, M., Zlatanou, A., Oldreive, C.E., et al. (2017) USP7 inhibition alters homologous recombination repair and targets CLL cells independently of ATM/p53 functional status. Blood 130, 156–166. https://doi.org/10.1182/blood-2016-12-758219

125 Morris, J.R. and Garvin, A.J. (2017) SUMO in the DNA double-stranded break response: similarities, differences, and cooperation with ubiquitin. J. Mol. Biol. 429, 3376–3387. https://doi.org/10.1016/j.jmb.2017.05.012