Published in final edited form as:

DNA Repair (Amst). 2009 September 2; 8(9): 1068–1076. doi:10.1016/j.dnarep.2009.04.007.

Choreography of Recombination Proteins during the DNA Damage Response

Michael Lisby¹ and Rodney Rothstein²

¹Department of Biology, University of Copenhagen, Ole Maaloees Vej 5, DK-2200 Copenhagen N, Denmark

²Department of Genetics & Development, Columbia University Medical Center, 701 West 168th Street, New York, NY 10032-2704

Abstract

Genome integrity is frequently challenged by DNA lesions from both endogenous and exogenous sources. A single DNA double-strand break (DSB) is lethal if unrepaired and may lead to loss of heterozygosity, mutations, deletions, genomic rearrangements and chromosome loss if repaired improperly. Such genetic alterations are the main causes of cancer and other genetic diseases. Consequently, DNA double-strand break repair (DSBR) is an important process in all living organisms. DSBR is also the driving mechanism in most strategies of gene targeting, which has applications in both genetic and clinical research. Here we review the cell biological response to DSBs in mitotically growing cells with an emphasis on homologous recombination pathways in yeast *Saccharomyces cerevisiae* and in mammalian cells.

Keywords

homologous recombination; foci; DNA double-strand break repair; *Saccharomyces cerevisiae*; mammalian

1. Introduction

Advances in automated live cell fluorescence microscopy, digital image analysis, and engineering of spectral variants of green and red fluorescent proteins over the last decade has greatly contributed to our understanding of the cellular response to DNA damage [1]. The ability to induce a single DSB in a site-specific manner by mega-nucleases or a subnuclear cluster of DSBs by laser micro-irradiation and other approaches has further facilitated the study of DSB repair. Many proteins are recruited to a DSB in multiple copies, resulting in a high local concentration of the protein, which is visualized cytologically as a focus using either GFP-tagged (green fluorescent protein) fusion proteins or immunohistochemistry (Fig. 1). The recruitment of checkpoint and repair proteins to foci is being used to study the choreography of DSB repair inside cells.

© 2009 Elsevier B.V. All rights reserved.

Correspondence to Michael Lisby: mlisby@bio.ku.dk and Rodney Rothstein: rothstein@cancercenter.columbia.edu.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

2. Pathways of DSB repair

Two major pathways exist for repairing DSBs: non-homologous end-joining (NHEJ) and homologous recombination (HR) (Fig. 2). In general, NHEJ is the preferred pathway in the G1 phase of the cell cycle, while HR is favored in S and G2 phases [5,6]. Moreover, NHEJ makes a greater contribution to DSB repair in higher eukaryotes with large intergenic regions and many repetitive sequences, whereas HR is preferred in yeast and other eukaryotes with small compact genomes of short intergenic regions and low redundancy [7,8]. Biochemically, NHEJ consists of a religation of the two ends of a DSB. Since ligation is often preceded by nucleolytic processing of ends, NHEJ is prone to causing deletions [9]. By contrast, DSBs that are repaired by HR do so without loss of genetic information, because the break is resealed by copying genetic information from an intact donor sequence.

The monitoring of DNA damage-induced foci is a useful tool for studying HR in living cells. Foci are dynamic giga-Dalton structures typically containing hundreds to thousands of copies of a given repair protein. The high local concentration of proteins at the DNA lesion is likely to facilitate the efficiency of individual biochemical steps during recombination. For example, budding yeast Rad52 is present at approximately 50-fold higher concentration within foci than elsewhere in the nucleus. At the same time, the low abundance of repair proteins at undamaged DNA likely constitutes a barrier to untimely recombination or potentially deleterious interactions with intact DNA. For each specific DNA repair protein in question, its accumulation at a DNA lesion results from a combination of novel binding sites being generated and a change in the residence time of the bound protein, as elegantly demonstrated by fluorescence recovery after photobleaching (FRAP) and fluorescence loss in photobleaching (FLIP) analyses in mammalian cells [10]. Finally, it is important to keep in mind that although HR foci are likely to reflect an attempt to perform recombination, they are not evidence of successful completion of the process. Moreover, it is likely that some HR events do not form cytologically detectable foci, because the reaction is fast and therefore completed before the accumulation of a detectable concentration of repair proteins at the DNA lesion.

3. Cellular choreography of DNA damage response

3.1 Choreography of DSB repair in *Saccharomyces cerevisiae*

In budding yeast, DNA double-strand breaks are initially sensed independently by the Ku (Yku70-Yku80) and MRX (Mre11-Rad50-Xrs2) complexes [11-13], both of which bind directly to DNA ends [14-16]. The relationship between these complexes is a question under active investigation, but it is reported that the Ku complex delays the onset of 5' end resection likely creating a window of opportunity for NHEJ [17], which itself is stimulated 3-fold by Ku *in vitro* [16]. In contrast, MRX promotes the onset of resection using its intrinsic 3'-exo- and endonuclease activity [18-24]. Likely, the competition between Ku and MRX for binding to DSBs is important for determining the choice between NHEJ and HR during DSB repair.

The two ends of a DSB are held together by a mechanism that is partially dependent on MRX and Sae2 [16,25-27]. For this reason, the two ends of a DSB form a single Mre11 focus. Further, the MRX complex interacts with the Tel1 kinase and is required for its recruitment to foci [28]. The Tel1 kinase phosphorylates histone H2A, which is a chromatin mark specific for damaged DNA in most eukaryotes [29-31] (Fig. 3). The recognition of DSBs by MRX occurs independently of cell cycle phase and other factors, consistent with the notion that the complex binds directly to free DNA ends [13,16]. Importantly, the modification of chromatin by H2A phosphorylation facilitates binding of Rad9 to histone H3 methylated at lysine 79 (H3-K79^{Me}) and subsequent recruitment and activation of Rad53 [13,32].

Unless a DSB is repaired by NHEJ, it is eventually processed by 5' to 3' nucleolytic resection, which commits the cell to HR. In yeast, resection is a two-step process catalyzed by numerous partially redundant nucleases including Mre11, Sae2, Dna2 and Exo1 [18-25,33]. First, resection is initiated by Sae2 and the MRX complex. Sae2 and MRX are particularly important for removal of hairpins, bulky adducts and other irregular end structures [23,34-36]. Second, a more processive resection is catalyzed by Exo1 and/or Dna2 in collaboration with the Sgs1 helicase [18,19]. The efficiency of resection depends both on cell cycle phase and the type of DSB. While endonuclease induced DSBs are resected only in S and G2 phase, DSB ends induced by ionizing radiation are resected throughout the cell cycle [37,38]. Resection is accompanied by the dissociation of MRX, Sae2 and Tel1 from the DSB and binding of replication protein A (RPA) to the 3' single-stranded overhangs (Fig. 4A) [13,37]. RPA is necessary for recruiting a number of checkpoint and HR proteins including the Mec1-Ddc2, Rad24-RFC and 9-1-1 (Ddc1-Mec3-Rad17) complexes. Notably, Tel1 and Mec1 have many of the same phosphorylation targets including histone H2A. As a consequence, Tel1-dependent checkpoint signaling is likely replaced by Mec1-dependent signaling upon resection of DSB ends. Interestingly, recent experiments have shown that tethering of Ddc1 and Ddc2 to DNA is sufficient for checkpoint activation [39]. Notably, efficient Rad53 phosphorylation is observed only if 80 or more copies of Ddc1 and Ddc2 are tethered to DNA, which is analogous to the accumulation of these proteins to foci during the DNA damage response [39]. Similar activation of the DNA damage response is observed in mammalian cells upon tethering of NBS1, MRE11, ATM or MDC1 to chromatin, but not after tethering the downstream effector kinases Chk1 and Chk2, which underscores the functional significance of this observation [40]. Consistent with a functional crosstalk between the Ddc2-Mec1 and 9-1-1 complexes during checkpoint signaling, there is a reported requirement for the 9-1-1 complex to stabilize Ddc2 foci in irradiated G1 cells [37]. Further, in S and G2 phase the 9-1-1 complex and the Cdc28 kinase both contribute to the stabilization of DNA damage-induced Ddc2 foci [37], demonstrating how checkpoint signaling is coordinated with cell cycle phase.

In S and G2 phase, RPA facilitates the recruitment of Rad52 to DSBs likely via a direct physical interaction [13,41]. Rad52 interacts with the Rad51 recombinase and Rad59 to recruit these proteins to foci [13,42,43]. In addition, Rad59 also requires Rad52 for its nuclear localization [13]. Rdh54 is a DNA-dependent ATPase belonging to the SWI/SNF family of chromatin remodeling complexes [44]. Interestingly, Rdh54 is recruited both to DSBs and to the kinetochore, although the functional significance of this dual localization is unknown. The recruitment of Rdh54 to DSBs is Rad52- and Rad51-dependent, while its localization to the kinetochore is independent of the recombination machinery. Rdh54 is a homologue of Rad54, which itself is recruited to DSBs but not to the kinetochore in wild-type cells. Interestingly, Rad54 does localize to the kinetochore in a *rdh54Δ* mutant [13], likely explaining some of the functional redundancy between these two proteins [45]. While Rdh54 appears to be recruited to DSBs immediately downstream of Rad51, Rad54 also requires the Rad51 paralogues, Rad55-Rad57, suggesting that Rad54 focus formation requires Rad51 nucleoprotein filament formation [13]. These requirements for focus formation indicate that Rdh54 acts upstream of Rad54 during homologous recombination. Together with Rad52, Rad55-Rad57 mediate the formation of Rad51 filaments and themselves require Rad51 for focus formation [13,46]. Importantly, Rad55 foci are transient suggesting that Rad55-Rad57 is not incorporated into the Rad51 filament (unpublished data) (Fig. 4A). At present, it remains to be determined which proteins are localized to DSBs during the late stages of homologous recombination, when recombination intermediates are resolved, and how chromatin is restored to its initial state.

3.2 Choreography of DSB repair in mammalian cells

Many of the proteins involved in the DNA damage response are evolutionarily conserved between yeast and mammals (Fig. 3). Accordingly, the cellular response to DSBs in

mammalian cells shows similarities to yeast, but also exhibits some significant differences. Similar to yeast, the MRE11-RAD50-NBS1 (MRN) and KU70-KU80 (Ku) complexes are recruited to DSB ends independently [47-50]. Ku recruits the DNA-PK_{cs} kinase to form the DNA-PK holoenzyme, which facilitates the juxtaposition of ends prior to recruitment of additional end-joining factors [51,52]. A notable difference between yeast and higher eukaryotes in DSB recognition is the absence of a DNA-PK_{cs} homologue in yeast and the requirement for the yeast MRX in both NHEJ and HR, while human MRN is required only for HR [53-55]. Perhaps DNA-PK is responsible for the higher efficiency of NHEJ in mammalian cells compared to yeast.

During HR, MRN recruits the ATM kinase, which phosphorylates histone H2AX at serine 139 to generate a chromatin mark (γ -H2AX) similar to that made by Tel1 in budding yeast [56-58]. Mice lacking H2AX are radiation sensitive, immune deficient, and males are infertile [59]. In mammalian cells, γ -H2AX is bound by the master regulator of recombinational DNA repair, MDC1 [60-63], which itself interacts with ATM via its FHA domain and with NBS1 [61,64-66]. The interactions between γ -H2AX, MDC1, ATM and NBS1 have been proposed to generate a positive feedback loop to amplify the γ -H2AX signal and promote additional recruitment of MDC1, ATM and NBS1 [61] (Fig. 4B). MDC1 itself is phosphorylated in an ATM-dependent manner [67,68], and this modification stimulates binding by the E3 ubiquitin ligase, RNF8, which in turn specifies the ubiquitylation of histone H2A and H2AX [69-71]. At the cell biological level, the recruitment of MRN and ATM, phosphorylation of H2AX, and recruitment of MDC1 and RNF8 occur very quickly and with similar kinetics, suggesting that each of these events are part of an early, coordinated initiation of the response to DSBs [69].

Ubiquitylated histones H2A and H2AX are bound by RAP80 in a second wave of recruitment of proteins to a DSB [69,72,73]. During this wave, RAP80 interacts with Abraxas (CCDC98) [74], which associates with the deubiquitylating enzyme BRCC36 and with the BRCT domain of BRCA1 [72,75-77]. The complex also contains BARD1 (BRCA1-associated RING domain protein 1), which interacts with BRCA1 through their respective RING domain-containing N-termini to form an active E3 ubiquitin ligase [78,79]. In addition to the Abraxas interaction, BRCA1 can also interact with the BACH1 helicase and the CtIP nuclease [80,81]. Interactions of Abraxas, BACH1 and CtIP with the BRCT domain of BRCA1 are mutually exclusive, suggesting that the three proteins compete for the same binding site to form distinct BRCA1-containing complexes, of which only the Abraxas-BRCC36-BRCA1-BARD1 complex is recruited to sites of DNA damage by RAP80 [76]. In support of this notion, BACH1 knockdown does not disrupt BRCA1 focus formation [72]. Moreover, sedimentation velocity analysis of IR-induced BRCA1-BARD1 complexes identified distinct complexes containing either MRN and CtIP or BACH1 and TopBP1 [82]. Further, IR-induced Abraxas and RAP80 foci are BRCA1 independent [76], indicating that BRCA1 is acting at DSBs downstream of RAP80. Similarly, BACH1 and CtIP foci require BRCA1 [80,82,83], suggesting that BACH1 and CtIP are downstream of BRCA1. At the moment, it is unclear how the multiple interactions of the BRCT domain of BRCA1 with these proteins are coordinated *in vivo* (reviewed in [84]).

Ubiquitylation of histones H2A and H2AX also facilitates binding of 53BP1 to H4-K20^{Me} [69,85,86]. The alternative binding of 53BP1 to H3-K79^{Me} is apparently three orders of magnitude lower although the literature is not in full agreement on this point [85,87]. The similarities of these observations to the recruitment of the 53BP1 homologues to foci in *S. cerevisiae* and *S. pombe* (Rad9 and Crb2, respectively) makes it tempting to speculate that ubiquitylation plays a hitherto uncharacterized role in recruitment of these proteins to sites of DNA damage in yeast. However, deletion of the two putative RNF8 homologues in *S. cerevisiae*, *DMA1* and *DMA2*, does not significantly affect Rad9 focus formation in response to DNA damage (unpublished results). The two BRCT domains of 53BP1 interact with the DNA-binding surface of the p53 tumor suppressor [88], which possibly allows 53BP1 to recruit

p53 to activated Chk2, where p53 is phosphorylated at serine 20 in response to DNA damage [89]. Phosphorylation of p53 leads to its stabilization and activation as a transcription factor [90,91]. Both 53BP1 and BRCA1 foci are dependent on MDC1 and RNF8, but appear to be mutually independent [69,92], although some studies report an interdependency of 53BP1 and BRCA1 [93]. However, the independent recruitment of 53BP1 and BRCA1 is consistent with the notion that these proteins represent distinct protein complexes being recruited to chromatin by H4-K20^{Me} and RAP80, respectively.

A prerequisite for recombinational repair of a DSB is the 5' to 3' resection of the two ends to yield 3' single-stranded overhangs. In mammalian cells, a number of nucleases contribute to the resection of ends including MRE11, EXO1 and CtIP [94-96]. MRE11 and CtIP form a complex, but the two proteins form foci independently of each other and independently of BRCA1 [97]. In budding yeast, Mre11 and Sae2 are also recruited to foci independently [13]. In contrast, the fission yeast CtIP is recruited to DSBs in an Mre11-dependent manner [24]. End resection is accompanied by a third wave of recruitment of proteins to the DNA lesion and a simultaneous loss of binding of NHEJ factors [50]. However, in contrast to yeast, MRN is retained at DSBs after resection by an interaction of NBS1 to γ -H2AX and/or MDC1 [64, 98]. As in all organisms studied, RPA binds directly to single-stranded DNA formed by resection of DSBs [99] and the resulting RPA foci are remarkably alike across species (Fig. 1). RPA recruits the ATR-ATRIP checkpoint kinase [69,100,101]. In addition, RPA also recruits the alternative DNA clamp loader RAD17-RFC, which loads the 9-1-1 complex (RAD9-HUS1-RAD1) onto DNA [102-105]. Both ATR-ATRIP and ATM contribute to the phosphorylation of H2AX and CHK2 to activate the DNA damage checkpoint [106]. Similar to the yeast homologs, phosphorylated CHK2 exhibits very weak retention at sites of DNA damage and its dispersal throughout the nucleoplasm is crucial for checkpoint signaling [107].

Initiation of recombination requires the recruitment of the Rad51 recombinase. In contrast to yeast wherein Rad52 recruits Rad51 to foci [13,108], the RAD51 recombinase in mammalian cells is recruited to IR-induced DSBs by BRCA2 [109]. This difference could explain the reduced requirement for RAD52 during HR in mammalian cells compared to yeast. Nevertheless, RAD52 is recruited to RAD51 foci in IR-treated cells [110]. BRCA2 (FancD1) is part of the Fanconi anemia (FA) pathway (reviewed in [111]) and it remains to be fully established how BRCA2 is recruited to foci. However, at least three observations seem to be connected to this question. First, BRCA2 binds directly to single-stranded DNA as suggested by the reported *in vitro* complex of BRCA2 and DSS1 with single-stranded DNA [112]. Second, RPA directly interacts with the acidic N-terminus of BRCA2, which may contribute to recruiting BRCA2 to RPA-coated single-stranded DNA [113]. Third, BRCA2-PALB2 foci colocalize with FancD2 and BRCA1 foci [114-116] and their interdependencies suggest that BRCA2-PALB2 recruitment is initiated by the binding of BRCA1 to chromatin in the vicinity of a DSB. BRCA1 facilitates the recruitment of FancD2 to foci [117,118], which also requires FancD2 monoubiquitylation at lysine 561 by the FA core complex [118]. Yeast two-hybrid analysis indicates that FancD2 interacts directly with BRCA2 [119], raising the possibility that FancD2 directly contributes to recruiting BRCA2 to foci. Fittingly, FancD2 foci are independent of BRCA2, supporting the notion that FancD2 is upstream of BRCA2 [119]. Taken together, the data suggest that BRCA2 is recruited to foci by multiple protein and DNA interactions likely depending on the context of the DNA lesion.

In addition to BRCA2, IR-induced RAD51 focus formation also requires RAD54, and the five RAD51 paralogues RAD51B, RAD51C, RAD51D, XRCC2 and XRCC3 [109,120-126]. The RAD51 paralogues form two distinct complexes: a RAD51B-RAD51C-RAD51D-XRCC2 complex and a RAD51C-XRCC3 complex [127] with RAD51 interacting directly with XRCC3 in the smaller complex [128,129]. Unlike in mammalian cells, the Rad51 paralogues in *S.*

cerevisiae, Rad55 and Rad57, are not required for Rad51 focus formation in yeast [13]. The formation of XRCC3 foci is early and independent of RAD51 foci [130]. During S phase, RAD51 can form BRCA2-independent foci [109], which are likely to represent replication-coupled HR, where RAD51 is recruited by a direct interaction with RPA [131,132]. Human cells have two homologues of yeast Rad54, RAD54 and RAD54B, both of which are recruited to DNA damage-induced RAD51 foci [133]. These foci also contain the WRN helicase, which interacts with both RAD51 and RAD54B [134]. IR-induced WRN focus formation is defective in Nijmegen breakage syndrome (NBS) cells, whereas Werner syndrome (WS) cells retain the ability to form NBS1 foci consistent with the notion that WRN acts downstream of NBS1 in HR [135]. In addition to its RAD51 and RAD54B interactions, WRN physically and functionally interacts with RAD52 *in vitro* and *in vivo* at foci after mitomycin C treatment [136]. Interestingly, simultaneous deletion of RAD52 and XRCC3 in chicken DT40 cells is lethal, suggesting that RAD52 and the XRCC3-RAD51C complex have redundant functions [137]. Consistent with this idea, RAD52 foci in mammalian cells form independently of BRCA2 and the RAD51 paralogues XRCC2, XRCC3 and RAD51C [126]. Most likely RAD52 is recruited to foci by a direct physical interaction with RPA, although this remains to be formally demonstrated [138].

4. Spatial organization of DSB repair

DNA double-strand break repair by homologous recombination is organized into foci, which can be viewed as repair factories, where a high local concentration of the proteins facilitates efficient repair. However, the function of repair foci likely extends beyond this simplistic biochemical point of view. In fact, most DSBs consist of two ends, which likely require tethering to ensure correct rejoining and perhaps prevent telomere addition [139]. In budding yeast, the tethering of broken ends requires the MRX complex, Sae2 and the Tel1 kinase [26, 27,139], but it is likely that other factors are also needed. There is *in vitro* evidence to indicate that MRN and ATM play a similar role in metazoans [47,140].

Yeast cells that experience multiple DSBs have the ability to recruit these lesions to the same repair focus [141]. In contrast, DSBs in mammalian cells induced by γ -irradiation or the I-SceI endonuclease are largely immobile [142,143] with the interesting exception of DSBs induced by α -irradiation [144], and deprotected telomeres, which are mobilized in a 53BP1-dependent manner [145]. Possibly, the aggregation of multiple DSBs results from the attempt to tether DNA ends in general. In mammalian KU70^{-/-} cells, translocation between DSBs on different chromosomes are increased [146]. Thus, the Ku complex is important for proper tethering of ends, allowing the cell “to know” which ends belong together in order to avoid translocations.

Although HR is important for maintaining genome integrity, the process must be tightly regulated to avoid untimely and toxic recombination. An example of such regulation at the cell biological level is the exclusion of most HR proteins from the nucleolar compartment in budding yeast despite the fact that these same proteins are required to maintain rDNA homeostasis [147]. Interestingly, DSBs in the rDNA must relocate to a position outside the nucleolus before association with a Rad52 focus. Sumoylation of Rad52, MRX and the Smc5-Smc6 complex jointly contribute to this process and a failure to suppress the formation of Rad52 foci inside the nucleolus results in rDNA hyperrecombination and formation of extra-chromosomal rDNA circles [147].

Recently, it was demonstrated that persistent DSBs relocate to the nuclear pore complex (NPC) in an Slx5-Slx8- and Mec1/Tel1-dependent manner to stimulate gene conversion [148]. Interestingly, “old” NPCs and associated DNA circles are retained in the mother cell during cell division by a lateral diffusion barrier at the septum [149]. Thus, these findings leads to the intriguing possibility that irreparable DSBs are retained in the mother cell during

adaptation and that this mechanism may contribute to the mother-daughter senescence asymmetry (reviewed in [150]).

5. Perspectives and future directions

DNA damage-induced foci of repair proteins are frequently used as markers of DNA damage and ongoing DNA repair. However, at present we know very little about the molecular architecture, regulation and even significance of these foci. The evidence suggests that the nucleation of foci is guided by a few proteins binding directly to the DNA lesion or to chromatin in its vicinity e.g. MRX(N) binding to DNA ends, RPA to single-stranded DNA, and MDC1 to γ -H2AX. These DNA damage sensors initiate a recruitment cascade to attract additional factors via a network of protein-protein interactions which, in some cases, are regulated by post-translational modifications such as phosphorylation, ubiquitylation and sumoylation that act as molecular switches (Fig. 5).

A recent genome-wide screen in *S. cerevisiae* identified several novel factors that impinge on Rad52 focus formation [151], indicating that repair factories respond to a variety of genome stress conditions. Among the gene disruptions that cause an increase in the percentage of cells exhibiting spontaneous Rad52 foci were many involved in DNA metabolism and cell cycle regulation as well as 22 uncharacterized open reading frames, *IRC2-11*, *13-16*, *18-25* (Increased Recombination Centers). Many of these ORFs are conserved throughout evolution. Similar screens in other organisms are expected to reveal additional DNA repair factors.

Acknowledgments

We thank members of the Lisby and Rothstein laboratories for helpful discussions concerning this work. We especially acknowledge the insightful comments and suggestions by Rebecca Burgess, Peter Thorpe and Simon Bekker-Jensen. This work was supported by The Danish Agency for Science, Technology and Innovation (ML), the Villum Kann Rasmussen Foundation (ML), and the NIH grants GM50237 and GM67055 (RR).

Abbreviation list

DSB, double-strand break
 DSB, double-strand break repair
 HR, homologous recombination
 IR, ionizing radiation
 MMS, methyl methanesulfonate
 NHEJ, non-homologous end-joining
 RPA, replication protein A
 GFP, green fluorescent protein
 CFP, cyan fluorescent protein
 FRAP, fluorescence recovery after photobleaching
 FLIP, fluorescence loss in photobleaching
 FA, Fanconi anemia
 NBS, Nijmegen breakage syndrome
 WS, Werner syndrome
 NPC, nuclear pore complex

References

- [1]. Shaner NC, Steinbach PA, Tsien RY. A guide to choosing fluorescent proteins. *Nat. Methods* 2005;2:905–909. [PubMed: 16299475]

- [2]. Bekker-Jensen S, Lukas C, Kitagawa R, Melander F, Kastan MB, Bartek J, Lukas J. Spatial organization of the mammalian genome surveillance machinery in response to DNA strand breaks. *J. Cell Biol* 2006;173:195–206. [PubMed: 16618811]
- [3]. Wyman C, Kanaar R. DNA double-strand break repair: all's well that ends well. *Annu. Rev. Genet* 2006;40:363–383. [PubMed: 16895466]
- [4]. Dudasova Z, Dudas A, Chovanec M. Non-homologous end-joining factors of *Saccharomyces cerevisiae*. *FEMS Microbiol. Rev* 2004;28:581–601. [PubMed: 15539075]
- [5]. Takata M, Sasaki MS, Sonoda E, Morrison C, Hashimoto M, Utsumi H, Yamaguchi-Iwai Y, Shinohara A, Takeda S. Homologous recombination and non-homologous end-joining pathways of DNA double-strand break repair have overlapping roles in the maintenance of chromosomal integrity in vertebrate cells. *EMBO J* 1998;17:5497–5508. [PubMed: 9736627]
- [6]. Moore JK, Haber JE. Cell cycle and genetic requirements of two pathways of nonhomologous end-joining repair of double-strand breaks in *Saccharomyces cerevisiae*. *Mol. Cell. Biol* 1996;16:2164–2173. [PubMed: 8628283]
- [7]. Mao Z, Bozzella M, Seluanov A, Gorbunova V. Comparison of nonhomologous end joining and homologous recombination in human cells. *DNA Repair (Amst)* 2008;7:1765–1771. [PubMed: 18675941]
- [8]. Lee SE, Paques F, Sylvan J, Haber JE. Role of yeast *SIR* genes and mating type in directing DNA double-strand breaks to homologous and non-homologous repair paths. *Curr. Biol* 1999;9:767–770. [PubMed: 10421582]
- [9]. Hefferin ML, Tomkinson AE. Mechanism of DNA double-strand break repair by non-homologous end joining. *DNA Repair (Amst)* 2005;4:639–648. [PubMed: 15907771]
- [10]. Essers J, Houtsmuller AB, van Veelen L, Paulusma C, Nigg AL, Pastink A, Vermeulen W, Hoeijmakers JH, Kanaar R. Nuclear dynamics of *RAD52* group homologous recombination proteins in response to DNA damage. *EMBO J* 2002;21:2030–2037. [PubMed: 11953322]
- [11]. Wu D, Topper LM, Wilson TE. Recruitment and dissociation of nonhomologous end joining proteins at a DNA double-strand break in *Saccharomyces cerevisiae*. *Genetics* 2008;178:1237–1249. [PubMed: 18245831]
- [12]. Martin SG, Laroche T, Suka N, Grunstein M, Gasser SM. Relocalization of telomeric Ku and SIR proteins in response to DNA strand breaks in yeast. *Cell* 1999;97:621–633. [PubMed: 10367891]
- [13]. Lisby M, Barlow JH, Burgess RC, Rothstein R. Choreography of the DNA damage response; spatiotemporal relationships among checkpoint and repair proteins. *Cell* 2004;118:699–713. [PubMed: 15369670]
- [14]. Milne GT, Jin S, Shannon KB, Weaver DT. Mutations in two Ku homologs define a DNA end-joining repair pathway in *Saccharomyces cerevisiae*. *Mol. Cell. Biol* 1996;16:4189–4198. [PubMed: 8754818]
- [15]. Hopfner KP, Karcher A, Craig L, Woo TT, Carney JP, Tainer JA. Structural biochemistry and interaction architecture of the DNA double-strand break repair Mre11 nuclease and Rad50-ATPase. *Cell* 2001;105:473–485. [PubMed: 11371344]
- [16]. Chen L, Trujillo K, Ramos W, Sung P, Tomkinson AE. Promotion of Dnl4-catalyzed DNA end-joining by the Rad50/Mre11/Xrs2 and Hdf1/Hdf2 complexes. *Mol. Cell* 2001;8:1105–1115. [PubMed: 11741545]
- [17]. Lee SE, Moore JK, Holmes A, Umezu K, Kolodner RD, Haber JE. *Saccharomyces* Ku70, Mre11/Rad50 and RPA proteins regulate adaptation to G2/M arrest after DNA damage. *Cell* 1998;94:399–409. [PubMed: 9708741]
- [18]. Mimitou EP, Symington LS. Sae2, Exo1 and Sgs1 collaborate in DNA double-strand break processing. *Nature* 2008;455:770–774. [PubMed: 18806779]
- [19]. Zhu Z, Chung WH, Shim EY, Lee SE, Ira G. Sgs1 helicase and two nucleases Dna2 and Exo1 resect DNA double-strand break ends. *Cell* 2008;134:981–994. [PubMed: 18805091]
- [20]. Trujillo KM, Sung P. DNA structure-specific nuclease activities in the *Saccharomyces cerevisiae* Rad50*Mre11 complex. *J. Biol. Chem* 2001;276:35458–35464. [PubMed: 11454871]
- [21]. Keeney S, Kleckner N. Covalent protein-DNA complexes at the 5' strand termini of meiosis-specific double-strand breaks in yeast. *Proc. Natl. Acad. Sci. USA* 1995;92:11274–11278. [PubMed: 7479978]

- [22]. Moreau S, Morgan EA, Symington LS. Overlapping functions of the *Saccharomyces cerevisiae* Mre11, Exo1 and Rad27 nucleases in DNA metabolism. *Genetics* 2001;159:1423–1433. [PubMed: 11779786]
- [23]. Lobachev KS, Gordenin DA, Resnick MA. The Mre11 complex is required for repair of hairpin-capped double-strand breaks and prevention of chromosome rearrangements. *Cell* 2002;108:183–193. [PubMed: 11832209]
- [24]. Limbo O, Chahwan C, Yamada Y, de Bruin RA, Wittenberg C, Russell P. Ctp1 is a cell-cycle-regulated protein that functions with Mre11 complex to control double-strand break repair by homologous recombination. *Mol. Cell* 2007;28:134–146. [PubMed: 17936710]
- [25]. Clerici M, Mantiero D, Lucchini G, Longhese MP. The *Saccharomyces cerevisiae* Sae2 protein promotes resection and bridging of double strand break ends. *J. Biol. Chem* 2005;280:38631–38638. [PubMed: 16162495]
- [26]. Lobachev K, Vitriol E, Stemple J, Resnick MA, Bloom K. Chromosome fragmentation after induction of a double-strand break is an active process prevented by the RMX repair complex. *Curr. Biol* 2004;14:2107–2112. [PubMed: 15589152]
- [27]. Lisby M, de Mayolo A, Antúnez, Mortensen UH, Rothstein R. Cell cycle-regulated centers of DNA double-strand break repair. *Cell Cycle* 2003;2:479–483. [PubMed: 12963848]
- [28]. Nakada D, Matsumoto K, Sugimoto K. ATM-related Tel1 associates with double-strand breaks through an Xrs2-dependent mechanism. *Genes Dev* 2003;17:1957–1962. [PubMed: 12923051]
- [29]. Rogakou EP, Pilch DR, Orr AH, Ivanova VS, Bonner WM. DNA double-stranded breaks induce histone H2AX phosphorylation on serine 139. *J. Biol. Chem* 1998;273:5858–5868. [PubMed: 9488723]
- [30]. Redon C, Pilch DR, Rogakou EP, Orr AH, Lowndes NF, Bonner WM. Yeast histone 2A serine 129 is essential for the efficient repair of checkpoint-blind DNA damage. *EMBO Rep* 2003;4:678–684. [PubMed: 12792653]
- [31]. Rogakou EP, Boon C, Redon C, Bonner WM. Megabase chromatin domains involved in DNA double-strand breaks in vivo. *J. Cell Biol* 1999;146:905–916. [PubMed: 10477747]
- [32]. Toh GW, O'Shaughnessy AM, Jimeno S, Dobbie IM, Grenon M, Maffini S, O'Rourke A, Lowndes NF. Histone H2A phosphorylation and H3 methylation are required for a novel Rad9 DSB repair function following checkpoint activation. *DNA Repair (Amst)*. 2006
- [33]. Huertas P, Cortes-Ledesma F, Sartori AA, Aguilera A, Jackson SP. CDK targets Sae2 to control DNA-end resection and homologous recombination. *Nature* 2008;455:689–692. [PubMed: 18716619]
- [34]. Deng C, Brown JA, You D, Brown JM. Multiple endonucleases function to repair covalent topoisomerase I complexes in *Saccharomyces cerevisiae*. *Genetics* 2005;170:591–600. [PubMed: 15834151]
- [35]. McKee AH, Kleckner N. A general method for identifying recessive diploid-specific mutations in *Saccharomyces cerevisiae*, its application to the isolation of mutants blocked at intermediate stages of meiotic prophase and characterization of a new gene *SAE2*. *Genetics* 1997;146:797–816. [PubMed: 9215888]
- [36]. Prinz S, Amon A, Klein F. Isolation of *COM1*, a new gene required to complete meiotic double-strand break-induced recombination in *Saccharomyces cerevisiae*. *Genetics* 1997;146:781–795. [PubMed: 9215887]
- [37]. Barlow JH, Lisby M, Rothstein R. Differential regulation of the cellular response to DNA double-strand breaks in G1. *Mol. Cell* 2008;30:73–85. [PubMed: 18406328]
- [38]. Ira G, Pelliccioli A, Balijja A, Wang X, Fiorani S, Carotenuto W, Liberi G, Bressan D, Wan L, Hollingsworth NM, et al. DNA end resection, homologous recombination and DNA damage checkpoint activation require CDK1. *Nature* 2004;431:1011–1017. [PubMed: 15496928]
- [39]. Bonilla CY, Melo JA, Toczyski DP. Colocalization of sensors is sufficient to activate the DNA damage checkpoint in the absence of damage. *Mol. Cell* 2008;30:267–276. [PubMed: 18471973]
- [40]. Soutoglou E, Misteli T. Activation of the cellular DNA damage response in the absence of DNA lesions. *Science* 2008;320:1507–1510. [PubMed: 18483401]

- [41]. Hays SL, Firmenich AA, Berg P. Complex formation in yeast double-strand break repair: participation of Rad51, Rad52, Rad55, and Rad57 proteins. *Proc. Natl. Acad. Sci. USA* 1995;92:6925–6929. [PubMed: 7624345]
- [42]. Milne GT, Weaver DT. Dominant negative alleles of *RAD52* reveal a DNA repair/recombination complex including Rad51 and Rad52. *Genes Dev* 1993;7:1755–1765. [PubMed: 8370524]
- [43]. Davis AP, Symington LS. The Rad52-Rad59 complex interacts with Rad51 and replication protein A. *DNA Repair (Amst)* 2003;2:1127–1134. [PubMed: 13679150]
- [44]. Kwon Y, Seong C, Chi P, Greene EC, Klein H, Sung P. ATP-dependent chromatin remodeling by the *Saccharomyces cerevisiae* homologous recombination factor Rdh54. *J. Biol. Chem* 2008;283:10445–10452. [PubMed: 18292093]
- [45]. Shinohara M, Shita-Yamaguchi E, Buerstedde JM, Shinagawa H, Ogawa H, Shinohara A. Characterization of the roles of the *Saccharomyces cerevisiae* *RAD54* gene and a homologue of *RAD54*, *RDH54/TID1*, in mitosis and meiosis. *Genetics* 1997;147:1545–1556. [PubMed: 9409820]
- [46]. Sung P. Yeast Rad55 and Rad57 proteins form a heterodimer that functions with replication protein A to promote DNA strand exchange by Rad51 recombinase. *Genes Dev* 1997;11:1111–1121. [PubMed: 9159392]
- [47]. de Jager M, van Noort J, van Gent DC, Dekker C, Kanaar R, Wyman C. Human Rad50/Mre11 is a flexible complex that can tether DNA ends. *Mol. Cell* 2001;8:1129–1135. [PubMed: 11741547]
- [48]. de Jager M, Wyman C, van Gent DC, Kanaar R. DNA end-binding specificity of human Rad50/Mre11 is influenced by ATP. *Nucleic Acids Res* 2002;30:4425–4431. [PubMed: 12384589]
- [49]. Mimori T, Hardin JA. Mechanism of interaction between Ku protein and DNA. *J. Biol. Chem* 1986;261:10375–10379. [PubMed: 3015926]
- [50]. Kim JS, Krasieva TB, Kurumizaka H, Chen DJ, Taylor AM, Yokomori K. Independent and sequential recruitment of NHEJ and HR factors to DNA damage sites in mammalian cells. *J. Cell Biol* 2005;170:341–347. [PubMed: 16061690]
- [51]. Spagnolo L, Rivera-Calzada A, Pearl LH, Llorca O. Three-dimensional structure of the human DNA-PKcs/Ku70/Ku80 complex assembled on DNA and its implications for DNA DSB repair. *Mol. Cell* 2006;22:511–519. [PubMed: 16713581]
- [52]. DeFazio LG, Stansel RM, Griffith JD, Chu G. Synapsis of DNA ends by DNA-dependent protein kinase. *EMBO J* 2002;21:3192–3200. [PubMed: 12065431]
- [53]. Yang YG, Saidi A, Frappart PO, Min W, Barrucand C, Dumon-Jones V, Michelon J, Herczeg Z, Wang ZQ. Conditional deletion of Nbs1 in murine cells reveals its role in branching repair pathways of DNA double-strand breaks. *EMBO J* 2006;25:5527–5538. [PubMed: 17082765]
- [54]. Bressan DA, Baxter BK, Petrini JH. The Mre11-Rad50-Xrs2 protein complex facilitates homologous recombination-based double-strand break repair in *Saccharomyces cerevisiae*. *Mol. Cell. Biol* 1999;19:7681–7687. [PubMed: 10523656]
- [55]. Moreau S, Ferguson JR, Symington LS. The nuclease activity of Mre11 is required for meiosis but not for mating type switching, end joining, or telomere maintenance. *Mol. Cell. Biol* 1999;19:556–566. [PubMed: 9858579]
- [56]. Stiff T, O'Driscoll M, Rief N, Iwabuchi K, Lohrich M, Jeggo PA. ATM and DNA-PK function redundantly to phosphorylate H2AX after exposure to ionizing radiation. *Cancer Res* 2004;64:2390–2396. [PubMed: 15059890]
- [57]. Paull TT, Rogakou EP, Yamazaki V, Kirchgesner CU, Gellert M, Bonner WM. A critical role for histone H2AX in recruitment of repair factors to nuclear foci after DNA damage. *Curr. Biol* 2000;10:886–895. [PubMed: 10959836]
- [58]. Celeste A, Fernandez-Capetillo O, Kruhlak MJ, Pilch DR, Staudt DW, Lee A, Bonner RF, Bonner WM, Nussenzweig A. Histone H2AX phosphorylation is dispensable for the initial recognition of DNA breaks. *Nat. Cell Biol* 2003;5:675–679. [PubMed: 12792649]
- [59]. Celeste A, Petersen S, Romanienko PJ, Fernandez-Capetillo O, Chen HT, Sedelnikova OA, Reina-San-Martin B, Coppola V, Meffre E, Difilippantonio MJ, et al. Genomic instability in mice lacking histone H2AX. *Science* 2002;296:922–927. [PubMed: 11934988]
- [60]. Stucki M, Clapperton JA, Mohammad D, Yaffe MB, Smerdon SJ, Jackson SP. MDC1 directly binds phosphorylated histone H2AX to regulate cellular responses to DNA double-strand breaks. *Cell* 2005;123:1213–1226. [PubMed: 16377563]

- [61]. Lou Z, Minter-Dykhouse K, Franco S, Gostissa M, Rivera MA, Celeste A, Manis JP, van Deursen J, Nussenzweig A, Paull TT, et al. MDC1 maintains genomic stability by participating in the amplification of ATM-dependent DNA damage signals. *Mol. Cell* 2006;21:187–200. [PubMed: 16427009]
- [62]. Bekker-Jensen S, Lukas C, Melander F, Bartek J, Lukas J. Dynamic assembly and sustained retention of 53BP1 at the sites of DNA damage are controlled by Mdc1/NFBD1. *J. Cell Biol* 2005;170:201–211. [PubMed: 16009723]
- [63]. Lee MS, Edwards RA, Thede GL, Glover JN. Structure of the BRCT repeat domain of MDC1 and its specificity for the free COOH-terminal end of the gamma-H2AX histone tail. *J. Biol. Chem* 2005;280:32053–32056. [PubMed: 16049003]
- [64]. Chapman JR, Jackson SP. Phospho-dependent interactions between NBS1 and MDC1 mediate chromatin retention of the MRN complex at sites of DNA damage. *EMBO Rep* 2008;9:795–801. [PubMed: 18583988]
- [65]. Spycher C, Miller ES, Townsend K, Pavic L, Morrice NA, Janscak P, Stewart GS, Stucki M. Constitutive phosphorylation of MDC1 physically links the MRE11-RAD50-NBS1 complex to damaged chromatin. *J. Cell Biol* 2008;181:227–240. [PubMed: 18411308]
- [66]. Melander F, Bekker-Jensen S, Falck J, Bartek J, Mailand N, Lukas J. Phosphorylation of SDT repeats in the MDC1 N terminus triggers retention of NBS1 at the DNA damage-modified chromatin. *J. Cell Biol* 2008;181:213–226. [PubMed: 18411307]
- [67]. Goldberg M, Stucki M, Falck J, D'Amours D, Rahman D, Pappin D, Bartek J, Jackson SP. MDC1 is required for the intra-S-phase DNA damage checkpoint. *Nature* 2003;421:952–956. [PubMed: 12607003]
- [68]. Stewart GS, Wang B, Bignell CR, Taylor AM, Elledge SJ. MDC1 is a mediator of the mammalian DNA damage checkpoint. *Nature* 2003;421:961–966. [PubMed: 12607005]
- [69]. Mailand N, Bekker-Jensen S, Fastrup H, Melander F, Bartek J, Lukas C, Lukas J. RNF8 ubiquitylates histones at DNA double-strand breaks and promotes assembly of repair proteins. *Cell* 2007;131:887–900. [PubMed: 18001824]
- [70]. Kolas NK, Chapman JR, Nakada S, Ylanko J, Chahwan R, Sweeney FD, Panier S, Mendez M, Wildenhain J, Thomson TM, et al. Orchestration of the DNA-damage response by the RNF8 ubiquitin ligase. *Science* 2007;318:1637–1640. [PubMed: 18006705]
- [71]. Huen MS, Grant R, Manke I, Minn K, Yu X, Yaffe MB, Chen J. RNF8 transduces the DNA-damage signal via histone ubiquitylation and checkpoint protein assembly. *Cell* 2007;131:901–914. [PubMed: 18001825]
- [72]. Sobhian B, Shao G, Lilli DR, Culhane AC, Moreau LA, Xia B, Livingston DM, Greenberg RA. RAP80 targets BRCA1 to specific ubiquitin structures at DNA damage sites. *Science* 2007;316:1198–1202. [PubMed: 17525341]
- [73]. Wu J, Huen MS, Lu LY, Ye L, Dou Y, Ljungman M, Chen J, Yu X. Histone ubiquitination associates with BRCA1-Dependent DNA Damage Response. *Mol. Cell. Biol.* 2008
- [74]. Yu X, Wu LC, Bowcock AM, Aronheim A, Baer R. The C-terminal (BRCT) domains of BRCA1 interact *in vivo* with CtIP, a protein implicated in the CtBP pathway of transcriptional repression. *J. Biol. Chem* 1998;273:25388–25392. [PubMed: 9738006]
- [75]. Kim H, Chen J, Yu X. Ubiquitin-binding protein RAP80 mediates BRCA1-dependent DNA damage response. *Science* 2007;316:1202–1205. [PubMed: 17525342]
- [76]. Wang B, Matsuoka S, Ballif BA, Zhang D, Smogorzewska A, Gygi SP, Elledge SJ. Abraxas and RAP80 form a BRCA1 protein complex required for the DNA damage response. *Science* 2007;316:1194–1198. [PubMed: 17525340]
- [77]. Wang B, Elledge SJ. Ubc13/Rnf8 ubiquitin ligases control foci formation of the Rap80/Abraxas/Brcal/Brc36 complex in response to DNA damage. *Proc. Natl. Acad. Sci. USA* 2007;104:20759–20763. [PubMed: 18077395]
- [78]. Wu LC, Wang ZW, Tsan JT, Spillman MA, Phung A, Xu XL, Yang MC, Hwang LY, Bowcock AM, Baer R. Identification of a RING protein that can interact *in vivo* with the BRCA1 gene product. *Nat. Genet* 1996;14:430–440. [PubMed: 8944023]

- [79]. Hashizume R, Fukuda M, Maeda I, Nishikawa H, Oyake D, Yabuki Y, Ogata H, Ohta T. The RING heterodimer BRCA1-BARD1 is a ubiquitin ligase inactivated by a breast cancer-derived mutation. *J. Biol. Chem* 2001;276:14537–14540. [PubMed: 11278247]
- [80]. Cantor SB, Bell DW, Ganesan S, Kass EM, Drapkin R, Grossman S, Wahrer DC, Sgroi DC, Lane WS, Haber DA, et al. BACH1, a novel helicase-like protein, interacts directly with BRCA1 and contributes to its DNA repair function. *Cell* 2001;105:149–160. [PubMed: 11301010]
- [81]. Kim H, Huang J, Chen J. CCDC98 is a BRCA1-BRCT domain-binding protein involved in the DNA damage response. *Nat. Struct. Mol. Biol* 2007;14:710–715. [PubMed: 17643122]
- [82]. Greenberg RA, Sobhian B, Pathania S, Cantor SB, Nakatani Y, Livingston DM. Multifactorial contributions to an acute DNA damage response by BRCA1/BARD1-containing complexes. *Genes Dev* 2006;20:34–46. [PubMed: 16391231]
- [83]. Yu X, Fu S, Lai M, Baer R, Chen J. BRCA1 ubiquitinates its phosphorylation-dependent binding partner CtIP. *Genes Dev* 2006;20:1721–1726. [PubMed: 16818604]
- [84]. Kim H, Chen J. New players in the BRCA1-mediated DNA damage responsive pathway. *Mol. Cells* 2008;25:457–461. [PubMed: 18443409]
- [85]. Botuyan MV, Lee J, Ward IM, Kim JE, Thompson JR, Chen J, Mer G. Structural basis for the methylation state-specific recognition of histone H4-K20 by 53BP1 and Crb2 in DNA repair. *Cell* 2006;127:1361–1373. [PubMed: 17190600]
- [86]. Kim J, Daniel J, Espejo A, Lake A, Krishna M, Xia L, Zhang Y, Bedford MT. Tudor, MBT and chromo domains gauge the degree of lysine methylation. *EMBO Rep* 2006;7:397–403. [PubMed: 16415788]
- [87]. Huyen Y, Zgheib O, Ditullio RA Jr. Gorgoulis VG, Zacharatos P, Petty TJ, Sheston EA, Mellert HS, Stavridi ES, Halazonetis TD. Methylated lysine 79 of histone H3 targets 53BP1 to DNA double-strand breaks. *Nature* 2004;432:406–411. [PubMed: 15525939]
- [88]. Joo WS, Jeffrey PD, Cantor SB, Finnin MS, Livingston DM, Pavletich NP. Structure of the 53BP1 BRCT region bound to p53 and its comparison to the Brca1 BRCT structure. *Genes Dev* 2002;16:583–593. [PubMed: 11877378]
- [89]. Hirao A, Kong YY, Matsuoka S, Wakeham A, Ruland J, Yoshida H, Liu D, Elledge SJ, Mak TW. DNA damage-induced activation of p53 by the checkpoint kinase Chk2. *Science* 2000;287:1824–1827. [PubMed: 10710310]
- [90]. Khanna KK, Jackson SP. DNA double-strand breaks: signaling, repair and the cancer connection. *Nat. Genet* 2001;27:247–254. [PubMed: 11242102]
- [91]. Caspari T. How to activate p53. *Curr. Biol* 2000;10:R315–317. [PubMed: 10801407]
- [92]. Xie A, Hartlerode A, Stucki M, Odate S, Puget N, Kwok A, Nagaraju G, Yan C, Alt FW, Chen J, et al. Distinct roles of chromatin-associated proteins MDC1 and 53BP1 in mammalian double-strand break repair. *Mol. Cell* 2007;28:1045–1057. [PubMed: 18158901]
- [93]. Wang B, Matsuoka S, Carpenter PB, Elledge SJ. 53BP1, a mediator of the DNA damage checkpoint. *Science* 2002;298:1435–1438. [PubMed: 12364621]
- [94]. Paull TT, Gellert M. The 3' to 5' exonuclease activity of Mre 11 facilitates repair of DNA double-strand breaks. *Mol. Cell* 1998;1:969–979. [PubMed: 9651580]
- [95]. Nimmonkar AV, Ozsoy AZ, Genschel J, Modrich P, Kowalczykowski SC. Human exonuclease 1 and BLM helicase interact to resect DNA and initiate DNA repair. *Proc. Natl. Acad. Sci. USA* 2008;105:16906–16911. [PubMed: 18971343]
- [96]. Sartori AA, Lukas C, Coates J, Mistrik M, Fu S, Bartek J, Baer R, Lukas J, Jackson SP. Human CtIP promotes DNA end resection. *Nature* 2007;450:509–514. [PubMed: 17965729]
- [97]. Chen L, Nievera CJ, Lee AY, Wu X. Cell cycle-dependent complex formation of BRCA1.CtIP.MRN is important for DNA double-strand break repair. *J. Biol. Chem* 2008;283:7713–7720. [PubMed: 18171670]
- [98]. Kobayashi J, Tauchi H, Sakamoto S, Nakamura A, Morishima K, Matsuura S, Kobayashi T, Tamai K, Tanimoto K, Komatsu K. NBS1 localizes to gamma-H2AX foci through interaction with the FHA/BRCT domain. *Curr. Biol* 2002;12:1846–1851. [PubMed: 12419185]
- [99]. Raderschall E, Golub EI, Haaf T. Nuclear foci of mammalian recombination proteins are located at single-stranded DNA regions formed after DNA damage. *Proc. Natl. Acad. Sci. USA* 1999;96:1921–1926. [PubMed: 10051570]

- [100]. Costanzo V, Shechter D, Lupardus PJ, Cimprich KA, Gottesman M, Gautier J. An ATR- and Cdc7-dependent DNA damage checkpoint that inhibits initiation of DNA replication. *Mol. Cell* 2003;11:203–213. [PubMed: 12535533]
- [101]. Zou L, Elledge SJ. Sensing DNA damage through ATRIP recognition of RPA-ssDNA complexes. *Science* 2003;300:1542–1548. [PubMed: 12791985]
- [102]. Zou L, Cortez D, Elledge SJ. Regulation of ATR substrate selection by Rad17-dependent loading of Rad9 complexes onto chromatin. *Genes Dev* 2002;16:198–208. [PubMed: 11799063]
- [103]. Zou L, Liu D, Elledge SJ. Replication protein A-mediated recruitment and activation of Rad17 complexes. *Proc. Natl. Acad. Sci. USA* 2003;100:13827–13832. [PubMed: 14605214]
- [104]. Majka J, Binz SK, Wold MS, Burgers PM. Replication protein A directs loading of the DNA damage checkpoint clamp to 5'-DNA junctions. *J. Biol. Chem* 2006;281:27855–27861. [PubMed: 16864589]
- [105]. Bermudez VP, Lindsey-Boltz LA, Cesare AJ, Maniwa Y, Griffith JD, Hurwitz J, Sancar A. Loading of the human 9-1-1 checkpoint complex onto DNA by the checkpoint clamp loader hRad17-replication factor C complex *in vitro*. *Proc. Natl. Acad. Sci. USA* 2003;100:1633–1638. [PubMed: 12578958]
- [106]. Ward IM, Chen J. Histone H2AX is phosphorylated in an ATR-dependent manner in response to replicational stress. *J. Biol. Chem* 2001;276:47759–47762. [PubMed: 11673449]
- [107]. Lukas C, Falck J, Bartkova J, Bartek J, Lukas J. Distinct spatiotemporal dynamics of mammalian checkpoint regulators induced by DNA damage. *Nat. Cell Biol* 2003;5:255–260. [PubMed: 12598907]
- [108]. Gasior SL, Wong AK, Kora Y, Shinohara A, Bishop DK. Rad52 associates with RPA and functions with Rad55 and Rad57 to assemble meiotic recombination complexes. *Genes Dev* 1998;12:2208–2221. [PubMed: 9679065]
- [109]. Tarsounas M, Davies D, West SC. BRCA2-dependent and independent formation of RAD51 nuclear foci. *Oncogene* 2003;22:1115–1123. [PubMed: 12606939]
- [110]. Liu Y, Maizels N. Coordinated response of mammalian Rad51 and Rad52 to DNA damage. *EMBO Rep* 2000;1:85–90. [PubMed: 11256631]
- [111]. Jacquemont C, Taniguchi T. The Fanconi anemia pathway and ubiquitin. *BMC Biochem* 2007;8 (Suppl 1):S10. [PubMed: 18047734]
- [112]. Yang H, Jeffrey PD, Miller J, Kinnucan E, Sun Y, Thoma NH, Zheng N, Chen PL, Lee WH, Pavletich NP. BRCA2 function in DNA binding and recombination from a BRCA2-DSS1-ssDNA structure. *Science* 2002;297:1837–1848. [PubMed: 12228710]
- [113]. Wong JM, Ionescu D, Ingles CJ. Interaction between BRCA2 and replication protein A is compromised by a cancer-predisposing mutation in BRCA2. *Oncogene* 2003;22:28–33. [PubMed: 12527904]
- [114]. Wang X, Andreassen PR, D'Andrea AD. Functional interaction of monoubiquitinated FANCD2 and BRCA2/FANCD1 in chromatin. *Mol. Cell. Biol* 2004;24:5850–5862. [PubMed: 15199141]
- [115]. Garcia SN, Pillus L. Net results of nucleolar dynamics. *Cell* 1999;97:825–828. [PubMed: 10399910]
- [116]. Xia B, Sheng Q, Nakanishi K, Ohashi A, Wu J, Christ N, Liu X, Jasin M, Couch FJ, Livingston DM. Control of BRCA2 cellular and clinical functions by a nuclear partner, PALB2. *Mol. Cell* 2006;22:719–729. [PubMed: 16793542]
- [117]. Vandenberg CJ, Gergely F, Ong CY, Pace P, Mallery DL, Hiom K, Patel KJ. BRCA1-independent ubiquitination of FANCD2. *Mol. Cell* 2003;12:247–254. [PubMed: 12887909]
- [118]. Garcia-Higuera I, Taniguchi T, Ganesan S, Meyn MS, Timmers C, Hejna J, Grompe M, D'Andrea AD. Interaction of the Fanconi anemia proteins and BRCA1 in a common pathway. *Mol. Cell* 2001;7:249–262. [PubMed: 11239454]
- [119]. Hussain S, Wilson JB, Medhurst AL, Hejna J, Witt E, Ananth S, Davies A, Masson JY, Moses R, West SC, et al. Direct interaction of FANCD2 with BRCA2 in DNA damage response pathways. *Hum. Mol. Genet* 2004;13:1241–1248. [PubMed: 15115758]
- [120]. O'Regan P, Wilson C, Townsend S, Thacker J. XRCC2 is a nuclear RAD51-like protein required for damage-dependent RAD51 focus formation without the need for ATP binding. *J. Biol. Chem* 2001;276:22148–22153. [PubMed: 11301337]

- [121]. Huber LJ, Yang TW, Sarkisian CJ, Master SR, Deng CX, Chodosh LA. Impaired DNA damage response in cells expressing an exon 11-deleted murine Brca1 variant that localizes to nuclear foci. *Mol. Cell. Biol* 2001;21:4005–4015. [PubMed: 11359908]
- [122]. Bhattacharyya A, Ear US, Koller BH, Weichselbaum RR, Bishop DK. The breast cancer susceptibility gene BRCA1 is required for subnuclear assembly of Rad51 and survival following treatment with the DNA cross-linking agent cisplatin. *J. Biol. Chem* 2000;275:23899–23903. [PubMed: 10843985]
- [123]. Tan TL, Essers J, Citterio E, Swagemakers SM, de Wit J, Benson FE, Hoeijmakers JH, Kanaar R. Mouse Rad54 affects DNA conformation and DNA-damage-induced Rad51 foci formation. *Curr. Biol* 1999;9:325–328. [PubMed: 10209103]
- [124]. Bishop DK, Ear U, Bhattacharyya A, Calderone C, Beckett M, Weichselbaum RR, Shinohara A. Xrcc3 is required for assembly of Rad51 complexes *in vivo*. *J. Biol. Chem* 1998;273:21482–21488. [PubMed: 9705276]
- [125]. Tarsounas M, Davies AA, West SC. RAD51 localization and activation following DNA damage. *Philos. Trans. R. Soc. Lond. B. Biol. Sci* 2004;359:87–93. [PubMed: 15065660]
- [126]. van Veelen LR, Essers J, van de Rakt MW, Odijk H, Pastink A, Zdzienicka MZ, Paulusma CC, Kanaar R. Ionizing radiation-induced foci formation of mammalian Rad51 and Rad54 depends on the Rad51 paralogs, but not on Rad52. *Mutat. Res* 2005;574:34–49. [PubMed: 15914205]
- [127]. Masson JY, Tarsounas MC, Stasiak AZ, Stasiak A, Shah R, McIlwraith MJ, Benson FE, West SC. Identification and purification of two distinct complexes containing the five RAD51 paralogs. *Genes Dev* 2001;15:3296–3307. [PubMed: 11751635]
- [128]. Schild D, Lio YC, Collins DW, Tsomondo T, Chen DJ. Evidence for simultaneous protein interactions between human Rad51 paralogs. *J. Biol. Chem* 2000;275:16443–16449. [PubMed: 10749867]
- [129]. Liu N, Lamerdin JE, Tebbs RS, Schild D, Tucker JD, Shen MR, Brookman KW, Siciliano MJ, Walter CA, Fan W, et al. XRCC2 and XRCC3, new human Rad51-family members, promote chromosome stability and protect against DNA cross-links and other damages. *Mol. Cell* 1998;1:783–793. [PubMed: 9660962]
- [130]. Forget AL, Bennett BT, Knight KL. Xrcc3 is recruited to DNA double strand breaks early and independent of Rad51. *J. Cell. Biochem* 2004;93:429–436. [PubMed: 15372620]
- [131]. Golub EI, Gupta RC, Haaf T, Wold MS, Radding CM. Interaction of human Rad51 recombination protein with single-stranded DNA binding protein, RPA. *Nucleic Acids Res* 1998;26:5388–5393. [PubMed: 9826763]
- [132]. Sleeth KM, Sorensen CS, Issaeva N, Dziegielewska J, Bartek J, Helleday T. RPA mediates recombination repair during replication stress and is displaced from DNA by checkpoint signalling in human cells. *J. Mol. Biol* 2007;373:38–47. [PubMed: 17765923]
- [133]. Tanaka K, Hiramoto T, Fukuda T, Miyagawa K. A novel human Rad54 homologue, Rad54B, associates with Rad51. *J. Biol. Chem* 2000;275:26316–26321. [PubMed: 10851248]
- [134]. Otterlei M, Bruheim P, Ahn B, Bussen W, Karmakar P, Baynton K, Bohr VA. Werner syndrome protein participates in a complex with RAD51, RAD54, RAD54B and ATR in response to ICL-induced replication arrest. *J. Cell Sci* 2006;119:5137–5146. [PubMed: 17118963]
- [135]. Cheng WH, Sakamoto S, Fox JT, Komatsu K, Carney J, Bohr VA. Werner syndrome protein associates with gamma H2AX in a manner that depends upon Nbs1. *FEBS Lett* 2005;579:1350–1356. [PubMed: 15733840]
- [136]. Baynton K, Otterlei M, Bjoras M, von Kobbe C, Bohr VA, Seeberg E. WRN interacts physically and functionally with the recombination mediator protein RAD52. *J. Biol. Chem* 2003;278:36476–36486. [PubMed: 12750383]
- [137]. Fujimori A, Tachiiri S, Sonoda E, Thompson LH, Dhar PK, Hiraoka M, Takeda S, Zhang Y, Reth M, Takata M. Rad52 partially substitutes for the Rad51 paralog XRCC3 in maintaining chromosomal integrity in vertebrate cells. *EMBO J* 2001;20:5513–5520. [PubMed: 11574483]
- [138]. Park MS, Ludwig DL, Stigger E, Lee SH. Physical interaction between human RAD52 and RPA is required for homologous recombination in mammalian cells. *J. Biol. Chem* 1996;271:18996–19000. [PubMed: 8702565]

- [139]. Lee K, Zhang Y, Lee SE. *Saccharomyces cerevisiae* ATM orthologue suppresses break-induced chromosome translocations. *Nature* 2008;454:543–546. [PubMed: 18650924]
- [140]. Costanzo V, Paull T, Gottesman M, Gautier J. Mre11 assembles linear DNA fragments into DNA damage signaling complexes. *PLoS Biol* 2004;2:600–609.
- [141]. Lisby M, Mortensen UH, Rothstein R. Colocalization of multiple DNA double-strand breaks at a single Rad52 repair centre. *Nat. Cell Biol* 2003;5:572–577. [PubMed: 12766777]
- [142]. Kruhlak MJ, Celeste A, Deltaille G, Fernandez-Capetillo O, Muller WG, McNally JG, Bazett-Jones DP, Nussenzweig A. Changes in chromatin structure and mobility in living cells at sites of DNA double-strand breaks. *J. Cell Biol* 2006;172:823–834. [PubMed: 16520385]
- [143]. Soutoglou E, Dorn JF, Sengupta K, Jasin M, Nussenzweig A, Ried T, Danuser G, Misteli T. Positional stability of single double-strand breaks in mammalian cells. *Nat. Cell Biol* 2007;9:675–682. [PubMed: 17486118]
- [144]. Aten JA, Stap J, Krawczyk PM, van Oven CH, Hoebe RA, Essers J, Kanaar R. Dynamics of DNA double-strand breaks revealed by clustering of damaged chromosome domains. *Science* 2004;303:92–95. [PubMed: 14704429]
- [145]. Dimitrova N, Chen YC, Spector DL, de Lange T. 53BP1 promotes non-homologous end joining of telomeres by increasing chromatin mobility. *Nature* 2008;524–528. [PubMed: 18931659]
- [146]. Weinstock DM, Brunet E, Jasin M. Formation of NHEJ-derived reciprocal chromosomal translocations does not require Ku70. *Nat. Cell Biol* 2007;9:978–981. [PubMed: 17643113]
- [147]. Torres-Rosell J, Sunjevaric I, De Piccoli G, Sacher M, Eckert-Boulet N, Reid R, Jentsch S, Rothstein R, Aragon L, Lisby M. The Smc5-Smc6 complex and SUMO modification of Rad52 regulates recombinational repair at the ribosomal gene locus. *Nat. Cell Biol* 2007;9:923–931. [PubMed: 17643116]
- [148]. Nagai S, Dubrana K, Tsai-Pflugfelder M, Davidson MB, Roberts TM, Brown GW, Varela E, Hediger F, Gasser SM, Krogan NJ. Functional targeting of DNA damage to a nuclear pore-associated SUMO-dependent ubiquitin ligase. *Science* 2008;322:597–602. [PubMed: 18948542]
- [149]. Shcheprova Z, Baldi S, Frei SB, Gonnet G, Barral Y. A mechanism for asymmetric segregation of age during yeast budding. *Nature* 2008;454:728–734. [PubMed: 18660802]
- [150]. Henderson KA, Gottschling DE. A mother's sacrifice: what is she keeping for herself? *Curr. Opin. Cell Biol* 2008;723–728. [PubMed: 18848886]
- [151]. Alvaro D, Lisby M, Rothstein R. Genome-wide analysis of Rad52 foci reveals diverse mechanisms impacting recombination. *PLoS Genet* 2007;3

Figure 1.

DNA damage-induced RPA foci. (A) Rfa1 foci in *S. cerevisiae*. A yeast strain expressing Rfa1-CFP was treated with 200 $\mu\text{g/ml}$ zeocin for 1 hour to induced DSBs. Six yeast nuclei are shown. (B) RPA foci in human cells. U2OS cells were sensitized with BrdU and laser microirradiated at 337 nm to generate a track of DSBs. Immunostaining with target specific antibodies against RPA. One nucleus is shown. Reproduced from [2] with permission from Journal of Cell Biology. Scale bars, 10 μm .

Figure 2.

DSB repair pathways in eukaryotes. Left, non-homologous end-joining pathway (NHEJ). See [3] and [4] for review of NHEJ in man and yeast, respectively. Right, homologous recombination pathway (HR). Depending on whether the NHEJ or HR pathway is used, DNA double-strand break repair proceeds through a number of distinct steps. NHEJ: (a) damage detection, (b) nucleolytic end-processing, (c) end-ligation, which can result in a deletion (shown as red Δ). HR: (a) damage detection, (d) extensive nucleolytic 5'-resection, (e) strand invasion (two-end invasion shown), (f) Holliday-junction formation, and (g) Holliday-junction resolution. For HR, damaged DNA in black and intact homologous sequences as well as newly synthesized DNA in red.

Functional class	<i>Saccharomyces cerevisiae</i>	Mammalian
DNA damage sensors	Mre11-Rad50-Xrs2 Rfa1-Rfa2-Rfa3	MRE11-RAD50-NBS1 RPA1-RPA2-RPA3
Checkpoint proteins	Rad24-RFC Ddc1-Mec3-Rad17 Mec1-Ddc2 Tel1 Dpb11	RAD17-RFC RAD9-HUS1-RAD1 ATR-ATRIP ATM TopBP1
Adaptors/transducers	Mrc1 Rad9 - - -	Claspin MDC1, 53BP1 BRCA1-BARD1 RNF8 RAP80-Abraxas-BRCC36
Effector kinases	Rad53 Chk1	CHK2 CHK1
Regulatory proteins	Sae2 - - -	CtIP FancACEFGL FancD2 c-Abl
Homologous recombination proteins	- Sem1 Rad52 Rad51, Rad55-Rad57 Rad54 Rad59 Rdh54 Sgs1	BRCA2-PALB2 DSS1 RAD52 RAD51, RAD51B, RAD51C RAD51D, XRCC2, XRCC3 RAD54 RAD52B RAD54B BLM, RTS, WRN
End-joining proteins	Yku70-Yku80 Dnl4-Lif1-Nej1 - Pso2	KU70-KU80 LIG4-XRCC4 DNA-PK _{CS} Artemis
Structural components	Smc5-Smc6 Smc1-Smc3 H2A	SMC5-SMC5 SMC1-SMC3 H2AX

Figure 3.

Evolutionary conservation of DSB response proteins. Homologues in *S. cerevisiae* and mammalian cells are shown. The assignments are based on functional and/or sequence similarities.

Figure 4. Order of recruitment of DSB repair proteins. Recruitments proceed from the top downwards. Solid black lines represent absolute requirements. Gray lines indicate putative requirements base on physical and/or genetic evidence (see text). Red dotted lines represent interactions with specific DNA substrates. Arrows indicate modifying functions and/or feedback loops. (A) Proteins in *S. cerevisiae*. (B) Proteins in mammalian cells.

Figure 5.

Model for the sequential assembly of several components of repair foci dictated by post-translational modifications. In this example, the DSB end is recognized by the MRN complex and DNA damage signaling is initiated by the ATM kinase, which phosphorylates chromatin in the vicinity of the DNA end. The phospho-epitope on the histone octamer is subsequently bound by MDC1, which itself is phosphorylated by ATM, generating a binding site for RNF8. The RNF8 ubiquitin E3 ligase ubiquitylates histones H2A and H2AX, which facilitates the recruitment of RAP80 and downstream DNA repair proteins.