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Mini-review

Coping with DNA double strand breaks

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ABSTRACT

The repair of DNA double strand breaks (dsb) is important for maintaining the physical and genetic integrity of the genome. Moreover, in humans it is associated with the prevention of diseases such as immune deficiencies and cancer. This review briefly explores the fundamental strategies for repairing dsb, examines how cells maximize the fidelity of dsb repair in the cell cycle and discusses the requirements for dsb repair in the context of chromatin.

Contents

1. Introduction

Cells are subjected to a wide range of DNA damage that pose a constant threat to the structural and genetic integrity of the genome. A single base change or physical discontinuity in an important gene may have ongoing and catastrophic effects on the health and viability of an organism. To minimize this threat cells have evolved a comprehensive toolbox for tolerating, reversing and repairing genomic insults (reviewed in [1]).

One of the most toxic lesions in DNA is the double-stranded break (dsb) where the phosphate backbones of the two complementary DNA strands are broken simultaneously. Not only does this cause a discontinuity in the genetic code but broken DNA ends are also vulnerable to further physical and chemical assault resulting in lost or damaged bases or the formation of abnormal DNA structures, all of which can result in loss of genetic information.

The failure to repair dsb can have dire genetic consequences. The physical discontinuity of dsb present a serious challenge for cell division as the equal partitioning of replicated genomes into daughter cells during mitosis relies on the segregation of intact chromosomes. The inaccurate repair of dsb, although sometimes unavoidable, may also result in the loss of genetic information and mutation. Alternatively, inappropriate repair of broken DNA ends may result in the generation of harmful genomic rearrangements such as the DNA translocations common in many cancers. These are particularly well characterized for breaks associated with the generation of antibody diversity in the immune system (see later), for example in Burkitts Lymphoma where the strong immunoglobulin heavy chain promoter becomes juxtaposed to the cellular oncogene c-myc [1]. Finally, since DNA breaks are a potent signal for the initiation of cell-cycle checkpoints, failure to repair a single dsb prior to cell division may lead to prolonged cell cycle arrest, failure to undergo cell division and ultimately cell death. Cells that escape arrest and continue to divide with unrepaired breaks may eventually succumb to mitotic catastrophe.

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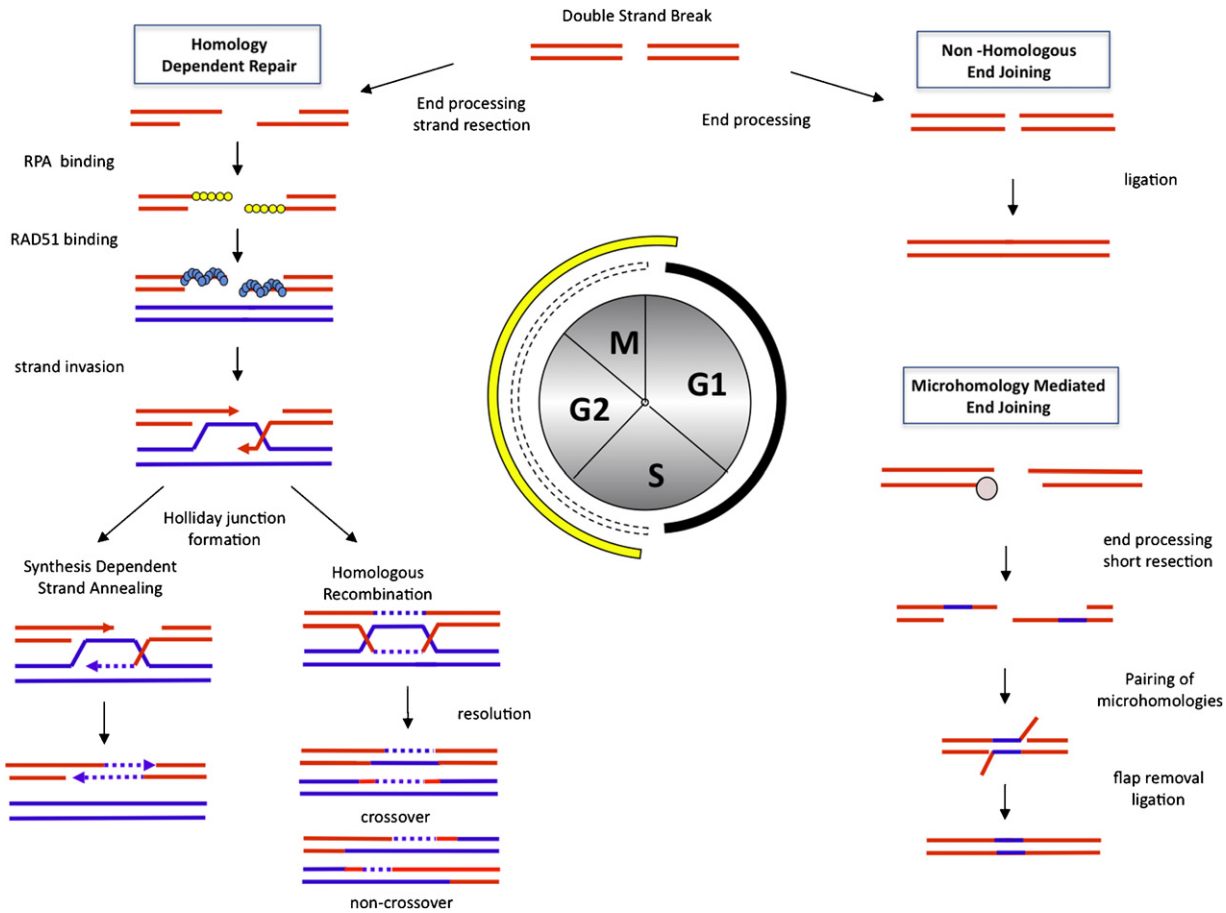

Fig. 1. Pathways for repairing DNA double strand breaks. Dsb can be repaired throughout the cell cycle by non-homologous end joining (NHEJ) or microhomology mediated end joining (MMEJ), which repair breaks regardless of DNA sequence and therefore are error prone. In NHEJ DNA ends are ligated directly, sometimes after trimming by nucleases or filling in by polymerases. NHEJ is initiated by the binding of Ku70/80 which recruits downstream factors including DNAPKcs and Artemis. Ligation of ends is carried out by the XRCC4–LigaseIV complex. In some circumstances one or more of the broken ends is refractory to Ku mediated NHEJ. In this case repair can proceed by nucleolytic processing and resection of the 3′-end until a short region of complimentary bases is revealed (in blue). Pairing of this microhomology stabilizes the broken ends, displaced flaps are removed (by Fen1 or Rad1/Rad10) and ligation can occur. During S and G2 phases dsb can also be repaired by homology directed repair. This requires extensive 5′ to 3′ resection of DNA to generate a 3′ single stranded tail, which is stabilized by the single stranded binding protein RPA (yellow circles). This is then displaced by the RAD51 recombinase (light blue circles), which forms a nucleoprotein filament which invades a homologous DNA duplex. This process named strand exchange forms a DNA crossover or Holliday junction which provides a primer to initiate new DNA synthesis. At this point there can be several outcomes. In synthesis dependent strand annealing the newly synthesized DNA reverts back to its original partner where it can be used as a template to complete repair. Alternatively for homologous recombination the Holliday junction migrates away from the initial point of exchange (branch migration) until the junction is resolved by nucleolytic cleavage of either the crossed strands or non-crossed strands of the junction. Resolution of the two Holliday junctions in different orientations results in the exchange of flanking markers (crossover), whereas resolution in the same orientation does not result in exchange of flanking markers (non-crossover). The availability of end joining (black) and homology dependent repair (yellow) for repair of dsb is shown.

Dsb can be generated in cells in a variety of ways. They may occur as a consequence of direct exposure to harmful exogenous agents, such as ionizing radiation, or indirectly by drugs like camptothecin, which inhibits the DNA breakage and rejoining cycle of topoisomerases required to release DNA torsion [2]. Dsb may also arise as by products of normal aerobic metabolism, which generates large numbers of potentially harmful oxygen free-radicals that can damage DNA [3]. DNA replication is another common source of dsb, either through errors in this process or through the replication of single stranded DNA breaks. Finally, there are a number of specialized cellular processes that generate and repair dsb as part of normal programmed genomic rearrangements. Two classic examples of this are V(D)J recombination and class-switch recombination, both associated with the generation of a diverse antibody repertoire in the immune system [reviewed in [4]]. In both these processes the generation and repair of dsb is 'managed' to avoid the potentially toxic effects of the lesion. These specialized chromosome breakage functions will be discussed specifically later on.

To counter the threat of dsb, organisms from unicellular bacteria to complex eukaryotes, have developed essentially two mechanisms for their repair, DNA end-joining and homologous recombination. While these mechanisms have evolved to account for differences in genomic complexity and multicellularity, the fundamental principles for repair of dsb are similar throughout evolution [5,6]. Accordingly, a huge contribution to our understanding of dsb repair has been made through studies on bacteria and fungi. However, the discussion here will largely be confined to the repair of DNA breaks in complex organisms.

2. DNA end-joining

2.1. Non homologous end-joining (NHEJ)

The most straightforward way to repair a dsb is simply to rejoin the broken ends regardless of the genetic sequence at the break. This process is called non-homologous end joining (NHEJ). Dsb with flushed 5′-phosphorylated ends or complementary overhangs, can be re-ligated efficiently with relatively high fidelity by NHEJ [7]. However, if, as is often the case, nucleotides have been lost from the break site or DNA ends have become altered and are in need of pro-

cessing before ligation can take place, then NHEJ is often mutagenic and is therefore considered to be error-prone. Consequently, while NHEJ plays a critical role in maintaining the structural integrity of DNA, it does not guarantee the preservation of genetic integrity.

The basic pathway for NHEJ is outlined in Fig. 1. DNA breaks are recognized and targeted for NHEJ through the rapid high affinity binding of the heterodimeric Ku70/80 complex to each of the broken ends [8,9]. The association of Ku70/80 with DNA end binding has no discernable sequence specificity, but rather DNA is threaded through a central fissure in the Ku70/80 complex, which enables it to diffuse away from the broken terminus leaving it accessible for other factors [10]. Ku70/80 binding serves two purposes. Firstly, Ku heterodimers at different DNA ends can interact and contribute to the tethering of broken DNA ends. This is particularly important for pairs of broken ends that are less able to associate, for example blunt ends, where there is no complimentary base pairing to facilitate the interaction of broken DNA ends [11]. Secondly, Ku70/80 serves as a platform for the recruitment of additional NHEJ factors (reviewed in [12]).

Once bound to DNA, Ku is thought to undergo a conformational change, which promotes the recruitment of the large DNA-dependent protein kinase catalytic subunit (DNAPKcs), which binds both to DNA and to Ku70/80 [13]. It is the association of DNAPKcs with DNA bound-Ku that activates its serine/threonine kinase activity. This is a rapid event as DNAPKcs kinase activity contributes to the phosphorylation of the histone variant H2AX at DNA breaks, a very early event in the repair of dsb [14]. Beyond this, the contribution of DNAPKcs-dependent phosphorylation in dsb repair is unclear although it is thought that autophosphorylation of DNAPKcs might also be important for NHEJ.

Another complex recruited to DNA ends is Mre11-Rad50-Nbs1 (MRN). Our understanding of the role of MRN in DNA end-joining is complicated by the fact that it is also required for dsb repair by homologous recombination. Although MRN has endo- and exonucleolytic activities these do not appear to be important for NHEJ in complex organisms [15]. Rather MRN is thought to assist Ku70/80 and DNAPK in tethering broken ends together by virtue of the zinc hook domains of RAD50 [16].

As mentioned above, not all broken DNA ends are immediately compatible for ligation. For example, loss of nucleotides at the break may lead to ends with incompatible termini (for example one end with a 3′ overhang and one with a 5′ overhang). Alternatively, one or more of the ends may have an abnormal structure such as a hairpin (this is particularly important in V(D)J recombination as we will see later). In these cases the DNA ends must first be processed to facilitate ligation. In yeast, but not higher eukaryotes, the endo- and exonuclease functions of MRN are thought to be important for end processing [15]. However, in mammals another nuclease called Artemis is recruited to DNA breaks where it is phosphorylated and activated by DNAPKcs [17]. Biochemical studies have shown that Artemis itself has endonuclease activity, which can trim DNA overhangs and cleave hairpins at the transition between double and single stranded DNA in preparation for end-ligation. It may also associate with exonucleases, which contribute to this processing function [18,19]. Alternatively, DNA ends with incompatible overhangs might also be 'filled in' by one of several polymerases including polμ and polλ, which are recruited for NHEJ through the interaction of their N-terminal BRCT domains with Ku70/80 [19].

Once processed, the trimmed ends can be ligated through the activity of the DNA ligase IV–XRCC4–XLF complex [20,21]. Although XRCC4 has no discernable biochemical activity it is required for the stable interaction of LigaseIV with DNA ends. Moreover XRCC4–LigIV, in keeping with the wide variety of different DNA end structures, is very versatile, able to ligate a wide variety of DNA ends, including single stranded DNA and ends associated with gaps.

2.2. Alternative end joining/microhomology mediated end joining

On occasion, polypeptides may be covalently attached to a broken DNA end, for example after treatment of cells with the topoisomerase inhibiting drug camptothecin. Consequently, these DNA ends cannot be bound by Ku70/80 and be repaired by normal NHEJ but require a Ku-independent repair pathway. This pathway is called alternative end-joining (altNHEJ) or sometimes microhomology mediated end-joining (MMEJ) (reviewed in [22]). In this case the blocked DNA end is removed by nucleolytic processing and one strand of the break is resected until a small region of complimentary base pairs (5–20 nt) is revealed. The broken ends are then stabilized through base-pairing, the displaced DNA flap is removed and ligation occurs. Like NHEJ, MMEJ does not take account of lost genetic information and therefore is also an error-prone pathway. The exact mechanism through which processing and resection of these ends is performed is not completely understood. However, both the MRN complex and another factor called CtIP, which was also reported to have endonuclease activity, play an important role [23]. Moreover some clues to this process may be gleaned from the function of the HerA and NurA proteins in the thermophilic archae *Pyrococcus furiosus*, which appear to perform an analogous function [24]. CtIP shares sequence similarities and considerable functional similarities with Sae2 protein in *S. cerevisae* [25,26] and Ctp1 in *S. pombe* [27], which also contribute to repair by MMEJ [28]. Biochemical studies on these proteins are likely to provide insight into the molecular function of their mammalian orthologues.

Both MRN and CtIP also play an important role in the resection of DNA ends during the early stages of homologous recombination, which as we shall see later makes an important contribution to the cell cycle regulation and pathway choice in dsb repair.

3. Specialized break repair functions for NHEJ

As mentioned earlier, dsb occur as normal intermediates in the pathways for generating antibody repertoire in the immune system. V(D)J recombination is the process whereby the mature gene coding for the antibody variable region is assembled from multiple arrays of V (variable), D (diversity) and J (joining) gene coding segments. Once assembled the coding DNA for the variable region of the immunoglobulin heavy chain is combined with DNA encoding one of several constant domains, which determine the isotype of the antibody (IgM, IgD, IgG, IgE or IgA). This process is called class switch recombination (CSR). Both these rearrangements involve the generation of broken DNA intermediates and their subsequent rejoining without eliciting the toxic effects associated with a sporadic dsb.

3.1. V(D)J recombination

The ordered assembly of the coding sequences for the variable domain of immunoglobulin and T-cell receptors is achieved through a cut and paste mechanism, in which dsb are introduced at a specific pair of gene segments separated by up to a megabase and the subsequent joining of the coding sequences. The incredible choreography of this process owes much to the function of the RAG1/RAG2 protein complex, which bind to specific gene segments, makes the dsb and holds the broken ends together for joining. However, we will limit ourselves to the repair of the dsb, the early steps of V(D)J recombination will not be discussed here. For a more in depth treatment see [29].

What is particularly interesting in the context of dsb repair is the nature of the broken DNA ends generated by RAG1/RAG2. These breaks are made at the boundary between the gene coding sequences and a specific recombination signal sequence (RSS) to

which RAG1 and RAG2 bind. However, while the broken signal end is flush and 5′-ends phosphorylated, the broken coding end is closed in a hairpin structure. This owes much to the fact that the RAG proteins are related to transposons and generate the dsb through the transesterification chemistry that is common to transposases and retroviral integrases [30].

Ligation of the two signal ends by NHEJ is straightforward, often resulting in the generation of a covalently closed circular molecule which excises the intervening DNA sequence at the immunoglobulin or T cell receptor loci and is lost from the genome. However, productive assembly of the coding sequence for the antibody variable domain requires the joining of the two hairpin coding ends and therefore first requires processing [29]. As discussed above the two NHEJ factors with the capacity to deal with hairpins are DNAPKcs and Artemis, both of which have an important role in V(D) J recombination. This is supported in genetic studies which show that while mice lacking Ku exhibit defects in formation of signal joints, they can form coding joints albeit dependent on microhomologies [31]. Mice defective in either DNAPKcs or Artemis are defective in the formation coding joints and exhibit severe immune deficiencies.

It is important to note that NHEJ not only makes a quantitative contribution to the production of functional antibody genes, but also a qualitative contribution to the coding sequence. Firstly, the asymmetric opening of DNA hairpins by DNAPKcs/Artemis generates small nucleotide insertions, which may change both the quality and the reading frame of the coding DNA. Secondly, in cells expressing terminal deoxynucleotide transferase additional nucleotides may be added to the 3′ end of the DNA prior to ligation adding further sequence diversity to the variable domain gene (reviewed in [32]). Clearly, in B and T cells the capacity of NHEJ to mediate error-prone rejoining is of great utility.

3.2. Class switch recombination

Class switch recombination (CSR) is less well understood at a mechanistic level. Like V(D)J recombination CSR occurs through a cut and paste mechanism where dsb are introduced at distantly positioned sequences in the genome, this time adjacent to the DNA coding for the different heavy chain constant regions. In contrast with V(D)J recombination the exact position of the dsb is less well defined occurring within regions which may be several hundred base pairs long comprising multiple repeats of conserved G/C rich sequences.

In CSR, breaks are introduced by the action of a series of proteins more commonly associated with base excision repair pathways [12,33]. This pathway is initiated by the specific deamination of cytosine bases to uracil within the switch region by AID, a member of the Apobec group of proteins involved in functions as diverse as RNA editing and viral restriction. The altered base is subjected to excision by subsequent action of the UNG DNA glycosylase and AP endonuclease resulting in the generation of single-stranded breaks in the DNA. Presumably the formation of a dsb arises through the very close proximity of AID initiated single strand breaks on complimentary DNA strands generated [34]. Since the exact nature of the switch break is difficult to determine, the mechanism through which broken DNA ends are co-located and ligated is also unclear. However, genetic evidence showing that mice defective in either Ku or DNA-PK are impaired, but not completely defective for CSR supports the involvement of the NHEJ pathway in this process [35–38].

For CSR the error-prone nature of NHEJ is not important as the fusion of the newly juxtaposed constant region to the variable domain coding sequence occurs by normal splicing of the mRNA message. Nevertheless for both V(D)J recombination and CSR the joining of appropriate ends appears to be tightly regulated since loss of this regulation and inappropriate joining of DNA ends to dsb elsewhere in the genome can result in the generation of harmful translocations associated with progression to cancer.

4. Homology directed DSB repair (HR)

NHEJ and MMEJ are functional throughout G1, S and G2 phase of the cell cycle. As cells progress through S-phase and into G2 replication produces a second copy of the genome in the form of a sister chromatid, providing a template to accurately repair the genetic information missing in the broken copy through homologous recombination/homology directed repair (HR) (Fig. 1). HR can occur in a variety of different forms but most share fundamental steps that are essential for accurate dsb repair. As with NHEJ and MMEJ, the first step in HR is the identification of the broken DNA ends. In human cells the order of early events is not completely clear but the recruitment of the MRN complex [16,39] and the phosphorylation of histone H2AX [40] are two early events. But, as we have seen earlier, these are not specific to HR since they occur also with end-joining. As with NHEJ, MRN may play a role in tethering the broken DNA ends for repair although in HR there is some evidence that the cohesin complex SMC5/6 may be important for maintaining the proximity of the homologous sister chromatids. MRN is also important in HR for the recruitment and activation of the DNA damage response-signaling kinase ATM [41], one function of which is to phosphorylate H2AX (this modified form referred to as γH2AX). The importance of this modification for dsb repair is not clear, because although γH2AX is an early marker for the generation of dsb in cells, mice lacking functional H2AX are only mildly defective in dsb repair.

What is clear is that the processing of broken DNA ends plays a key role in channeling DNA breaks into repair by HR. Whereas NHEJ and MMEJ require relatively local processing of DNA ends for ligation, a prerequisite for HR is that broken ends are extensively resected to a generate 3′ single-stranded DNA tail onto which the central recombinase protein RAD51 can load. Although the exact mechanics of resection are unclear key players in this process include the helicase/nuclease functions of MRN and the BRCA1-interacting protein CtIP [26], (also reported to posses nuclease functions in vitro), both of which also function in MMEJ. It is likely that MRN and CtIP collaborate in the initial processing of ends in preparation for more extensive resection by the processive single-strand exonuclease Exo1 [42,43]. As with NHEJ genetic information at a broken DNA end may be lost before or during DNA end processing, but with HR this is retrieved in subsequent steps.

Initially, the 3′-ssDNA tail is bound and stabilized by binding of the single strand binding protein, RPA. However, RPA is soon displaced by RAD51 with the help of RAD52 and BRCA2 to form a nucleoprotein filament along the ssDNA tail [43,44]. It is assembly of this RAD51 nucleoprotein filament which initiates the search for homology in the sister chromatid and promotes the exchange of homologous DNA strands to form heteroduplex DNA (DNA duplex formed between complementary strands of DNA from different molecules).

At this point the HR pathway can diverge towards a number of different outcomes (for a fuller treatment of the mechanics of HR see [45]). One possibility is that the invading strand is replicated using the genetic information on its intact sister chromatid, to include the DNA sequence missing on the broken chromatid. Afterwards the replicated strand reverts to its original position having replaced the missing information and can be used to complete the repair synthesis on the broken strand. This pathway is called synthesis dependent strand annealing (SDSA).

Alternatively the two sisters can exchange strands to form a Holliday junction, which can then branch migrate to extend the regions of heteroduplex a process which may involve the RAD51

paralogues RAD51B, RAD51C and RAD51D. In this pathway nucleolytic resolution of the Holliday junction is required to separate the recombined sister chromatids. In mammalian cells resolution of the Holliday junction is most likely achieved through the action of the Slx1–Slx4 nuclease complex [46–48]. Mammalian cells also have another Holliday junction resolving protein called Gen1 (the orthologue of Yen1 in yeast) but its role is less clear [49]. The symmetry of Holliday junctions enabled them to be resolved in either of two orientations, which determines whether the DNA sequences flanking the Holliday junction are exchanged or not (see Fig. 1). Nevertheless in each case the dsb and the genetic information lost at a break in one chromatid is faithfully restored using its sister chromatid.

5. End joining or HR: the battle for dsb

Although NHEJ and MMEJ repair pathways carry the risk of mutation the requirement for an intact genome is critically important for the two major cell cycle decisions, whether to replicate the genome and whether to undergo cell division? Consequently the NHEJ and MMEJ pathways are functional throughout G1, S and G2 phases of the cell cycle. Nevertheless in S and G2 phase when HR is also available it is of significant benefit to repair dsb, where possible, by accurate HR. How then might this be achieved? Recent evidence has highlighted a critical role for CtIP and DNA end-resection in the regulation of dsb repair through the cell cycle.

As described earlier, CtIP plays an important role in the resection of DNA ends for both MMEJ in G1 and HR during S-phase [23,25,26]. Interestingly, CtIP protein is present at relatively low levels in G1, presumably reflecting the limited need for DNA resection during end joining. However, in S-phase, when there is an increased requirement for ssDNA to initiate HR, CtIP protein not only becomes more abundant but also becomes phosphorylated at several sites, including two consensus sequences for cyclin dependent kinase (serine 327 and threonine 847) an important cell-cycle regulator [23,25]. Genetic studies revealed that phosphorylation at both putative CDK sites upregulates DNA end-resection with a concomitant increase in the repair of dsb by HR.

Phosphorylation of S327 is also required for the interaction of CtIP with the BRCT domains of BRCA1. Studies in DT40 confirmed that resection is not upregulated in BRCA1 defective cells even when CtIP is phosphorylated, establishing a mechanistic link between BRCA1 and DNA end-resection via CtIP [23]. It is of note that in yeast, which does not have a BRCA1 orthologue, only the CDK corresponding to T847 in CtIP is conserved [26]. Why vertebrates have an extra level of regulation is a question of considerable interest. Nevertheless, it seems likely that in simple and complex eukaryotes the regulation of DNA end resection is an important mechanism for shifting the balance in dsb repair from error-prone end-joining to accurate HR as cells progress through S and G2 phases.

A second important role for DNA end resection is the generation of RPA coated ssDNA, which acts as a signal for the G2/M cell cycle checkpoint [50]. It appears that by coupling dsb repair with cell cycle arrest through DNA resection, cells ensure that repair is completed before cell division occurs. It is perhaps not surprising therefore that defects in factors involved in HR such as BRCA1 and CtIP also compromise the G2/M checkpoint and may also be associated with cancer predisposition.

6. NHEJ and HR: good intention, bad outcome

The balance between repair of dsb by end-joining and HR can be absolutely critical for the maintenance of genome integrity and cell viability. It is increasingly apparent that inappropriate intervention of NHEJ can have a considerable negative impact on genomic fidelity. Several examples of this have been published recently [51,52]. One relates to the repair of DNA crosslinks by the Fanconi anaemia (FA) pathway. Two groups showed independently that in cells defective in the FA pathway, both the loss of viability and the increased chromosomal abnormalities associated with exposure to DNA damage is alleviated by the additional impairment of NHEJ. Or to put it another way, the NHEJ pathway is toxic to cells lacking a functional FA pathway. The implication is that lesions, which cannot be fully repaired by the FA pathway result in dsb that are released and acted upon by NHEJ in a manner that is deleterious to the cell, perhaps through incomplete or inappropriate repair resulting in chromosome abnormalities and reduced viability.

Another example of the battle for DNA ends has been provided by two further studies, which reported that disruption of 53BP1 reverses the effects of BRCA1 deficiency in mice [53,54]. Previous studies had shown that impairment of 53BP1 reduced tumour formation in BRCA1 deficient mice. The new studies provide a mechanistic explanation for this observation. First, these groups showed that the sensitivity of BRCA1 defective cells to DNA damage and to PARP inhibitors, as well as the accumulation of chromosome aberrations in these cells are also reversed by disrupting 53BP1. This, they go on to show, is due to partial restoration of HR in these cells. As described above, the HR defect in BRCA1 cells arises through a defect in the processing and resection of dsb. Accordingly Bunting et al. [53] demonstrate the restoration of DNA end resection in BRCA1$^{\Delta 11/\Delta 11}$ 53BP1$^{-/-}$ cells.

53BP1 is a mediator of the DNA damage response, which has been implicated in NHEJ, including that associated with CSR. Bunting and colleagues proposed a model where 53BP1 inhibits resection of DNA ends, which can be overcome by BRCA1. In the absence of BRCA1 one-ended dsb are channeled into NHEJ with the outcome that DNA ends are incorrectly or inappropriately repaired leading to chromosome aberrations and potentially to tumour formation (Fig. 2) [55,56]. However, in the absence of both factors, the requirement for BRCA1 is circumvented, resection can occur and accurate repair, using a sister chromatid as a template for HR, is no longer blocked. Again it appears that HR and NHEJ exist in a delicate equilibrium which when unbalanced may have deleterious consequences for genome stability and viability.

It is pertinent to mention that this model suggests that inhibition of 53BP1 might have therapeutic possibilities by reversing the HR defect in BRCA1-defective tumour cells. However, Bouwman et al. [54,57] have shown that many of the most aggressive triple negative breast cancer tumours also have mutations in 53BP1 as do many tumours lacking BRCA1. This suggests that there are other factors are at work and that in the context of a tumour cell the simple restoration of HR may not be of therapeutic benefit.

Genetic interactions between dsb repair and other DNA repair pathways may be exploited in therapy. A fine example of this is the use of drugs that inhibit the function of poly-ADP ribose polymerase (PARP), a component of the pathway for the repair of DNA base damage. Inhibition of PARP results in the accumulation of single strand breaks at sites of base damage, which after replication are converted to dsb in S phase. Whereas in normal cells, these dsb will be dealt with by HR, in cancer cells that are defective in HR by virtue of mutations in BRCA1 or BRCA2 they are less well repaired and their toxic effects are more likely to kill the tumour cell [57,58]. This clever exploitation of dsb repair pathways has opened up a new paradigm for therapy by exploiting this synthetic lethality (where individual genetic defects are viable but in combination are lethal in a given environment) for disease treatment.

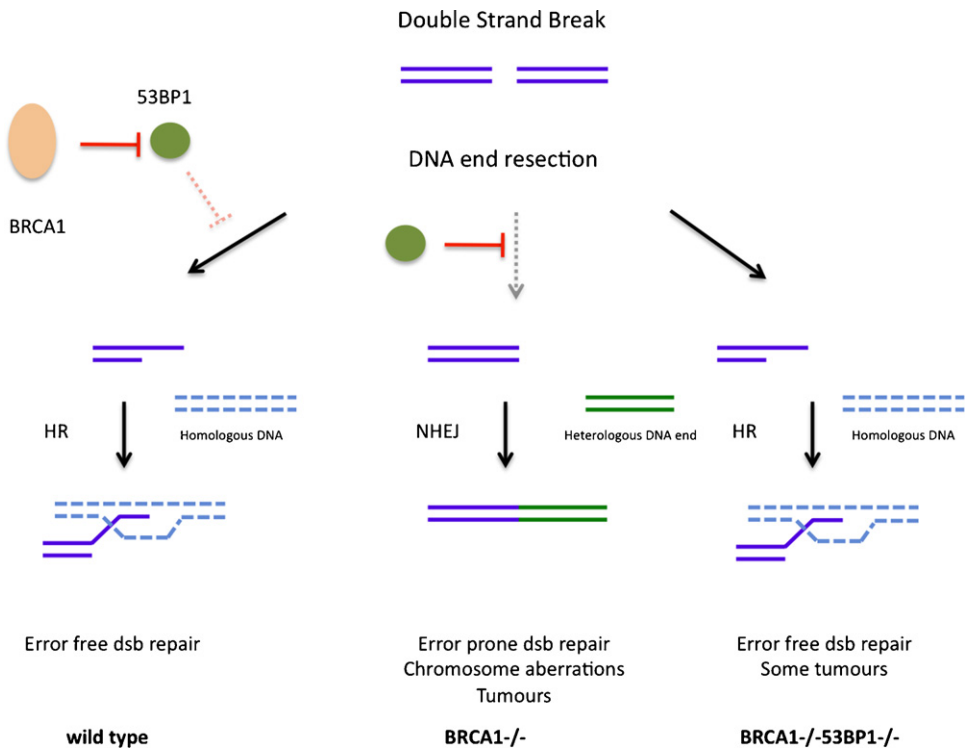

Fig. 2. Competition for dsb by BRCA1 and 53BP1. In wild type cells (left) the inhibitory effect of 53BP1 on DNA end resection is suppressed by the function of BRCA1 (presumably through its association with CtIP). In wild type cells dsb are repaired accurately by homology dependent repair, using homologous DNA (usually a sister chromatid) as a template. In BRCA1 defective cells 53bp1 inhibits DNA end resection and repair of an individual (one-ended) dsb proceeds only by error-prone NHEJ to another broken end, regardless of its origin. This leads to misrepair, the generation of chromosome abnormalities and an increase in tumour formation. In cells lacking both BRCA1 and 53BP1 (right) resection and therefore HR, is partially restored because the inhibitory effect of 53BP1 on these processes is missing. Dsb are repaired more accurately resulting in reduced tumour susceptibility.

7. Dsb repair in the context of chromatin

So far the discussion of dsb repair has carefully ignored the fact that DNA is not naked but is wrapped up in the nucleoprotein structure that is chromatin. Consequently, the detection, processing and mechanics of dsb repair require the manipulation of DNA within a chromatin context. The details of how this occurs are not well understood but it is thought to involve chromatin remodeling factors such as INO80, a yeast protein also conserved in mammals, which has been specifically linked with dsb repair [59].

In recent years attention has also turned towards the role of histone modifications in the signaling and repair of DNA damage. These modifications come in a wide variety, including mono-, di- and tri-methylation, acetylation, phosphorylation and ubiquitylation. It is thought that some provide a chemical mark for the recruitment of other factors, perhaps including chromatin-remodeling proteins, while other modifications might directly affect the local folding of chromatin. The most common histone modification associated with dsb is phosphorylation of H2AX, which is required for the recruitment of another repair factor, MDC1, to dsb. In addition the tri-methylation of histone H3 on lysine 4 and monomethylation of histone H4 lysine 20 have been shown to contribute to the regulation of V(D)J recombination and CSR respectively [60,61].

It now appears that histone ubiquitylation also plays an important role in the repair of dsb. Several studies have reported the involvement of an ubiquitin signaling cascade for the recruitment of factors involved in HR and in NHEJ. Key players in this pathway are the ubiquitin ligases RNF8, RNF168 and BRCA1, the ubiquitin conjugating factor UbcH13 and the deubiquitylating protein BRCC36. Each of these E3 proteins has been shown to be required for the accumulation of ubiquitin at sites of DNA damage. Moreover each has been shown to ubiquitylate histone H2A in vitro. The involvement of UbcH13 implies a likely role for polyubiquitin chains, joined through lysine 63. These are thought to be required for the recruitment of downstream proteins including, RAP80, Abraxas, BRCA1 and 53BP1. An in depth discussion of this signaling pathway can be found in [62].

The detailed mechanism of ubiquitin-mediated signaling is unclear and whether any of these modifications have a direct effect on chromatin structure is still to be determined. However, it is intriguing that mice lacking the RNF8 component of this pathway are defective in the removal of nucleosomes during spermatogenesis. Nevertheless, the potential importance of the ubiquitin signaling pathway derives from the fact that it is required for the recruitment of BRCA1 and 53BP1 providing a mechanistic link between NHEJ and HR. It too may play an important role in determining pathway choice in dsb repair.

Although our understanding of histone modification and modifying enzymes has advanced greatly, our knowledge about the contribution of chromatin architecture to dsb is lacking. Indeed very little is known about the specific factors required for the repair of dsb in chromatin. However, Goodarzi et al. recently addressed the issue of how the repair of dsb in heterochromatic regions of the genome might differ from that in euchromatic regions. They demonstrated that dsb formed in densely packed heterochromatin after treatment with ionizing radiation are repaired more slowly than those in euchromatin. Moreover, they identified an important role for the signaling kinase ATM specifically in the repair of heterochromatic dsb but not for euchromatic breaks. They went on to show that the requirement for ATM is for the phosphorylation of the heterochromatin associated factor KAP-1, which alters its affinity diminishing its association with heterochromatin [63].

In a subsequent study the repair of dsb in heterochromatin was been shown to require additional factors implicated in the ubiquitin mediated signaling cascade described above, including MDC1, RNF8 and RNF168 [64]. As we have seen before this pathway involves several histone-specific ubiquitin ligases and is also required for recruitment of BRCA1 and 53BP1. Again this places the ubiquitylation of histone H2A as a potentially key event in chromatin dynamics associated with dsb repair. Furthermore this provides further evidence that this interesting pathway, which was previously thought to be involved in the recruitment of factors involved in dsb repair by HR during s-phase, also appears to be important for the repair of heterochromatin associated dsb in G0/G1. Clearly the contribution of chromatin structure in both the generation and repair of dsb is a very complex and an interesting area for future study.

It seems that despite the considerable research effort studying the repair of dsb across the evolutionary landscape, new important and interesting avenues for investigation arise. Perhaps, after many years, the most exciting of these relate specifically to dsb repair in complex organisms, in particular those that directly relate to human disease and may impact on disease therapy. That said, our humble unicellular relatives often have a way of making us eat our words.

Conflict of interest

The author declares that there is no conflict of interest.

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