available at www.sciencedirect.com

ScienceDirect

journal homepage: www.elsevier.com/locate/dnarepair

# DNA double-strand break repair: From mechanistic understanding to cancer treatment

*Thomas Helleday*[a,b], *Justin Lo*[c], *Dik C. van Gent*[d,*], *Bevin P. Engelward*[c]

[a] *Radiation Oncology & Biology, University of Oxford, Oxford OX3 7LJ, UK*
[b] *Department of Genetics, Microbiology and Toxicology, Stockholm University, S-106 91 Stockholm, Sweden*
[c] *Biological Engineering Division, Massachusetts Institute of Technology, Cambridge, MA 02139, USA*
[d] *Department of Cell Biology and Genetics, Erasmus Medical Center, P.O. Box 2040, 3000 CA Rotterdam, The Netherlands*

ARTICLE INFO

*Article history:*
Published on line 23 March 2007

*Keywords:*
Homologous recombination
DNA double-strand break
Non-homologous end joining
DNA damage response
Replication
DNA repair

ABSTRACT

Accurate repair of DNA double-strand breaks is essential to life. Indeed, defective DNA double-strand break repair can lead to toxicity and large scale sequence rearrangements that cause cancer and promote premature aging. Here, we highlight the two major repair systems for handling DNA double-strand breaks: homologous recombination and non-homologous end joining. To clarify recombination mechanisms, we present animations that illustrate DNA strand movements. In addition to describing how these pathways operate, we also describe why appropriate pathway choice is critical to genomic stability, and we summarize key pathway control features related to cell cycle checkpoint and apoptosis signaling. Importantly, recent progress in delineating the effects of specific defects in repair and checkpoint control has helped to explain several disease phenotypes, including cancer and premature aging. Improved understanding of these pathways has also sparked development of novel chemotherapeutic strategies that kill tumors with increased specificity and efficacy. This review aims to provide a foundational understanding of how the homologous recombination and non-homologous end joining pathways operate, and to demonstrate how a better understanding of these processes has advanced both our understanding of the underlying causes of cancer and our ability to innovate novel cancer treatment strategies.



## 1. DNA double-strand breaks and the replication connection

Faithful propagation of genetic material and transmission into daughter cells is critical to life, yet our genomes are incessantly exposed to environmental and endogenous agents that create thousands of DNA lesions per cell each day [1]. While some DNA lesions are considered to be relatively benign, other lesions can be quite toxic. The DNA double-strand break (DSB) is one of the most toxic and mutagenic DNA lesions experienced in human cells: a single DSB can potentially lead to loss of more than 100 million base pairs of genetic information (e.g., loss of an entire chromosome arm). Interestingly, despite the potential danger of DSBs, mammals have evolved clever ways of exploiting the intentional generation of DSBs to control biological processes. For example, programmed DSBs occur to initiate rearrangements during maturation of immunoglobulin genes [2], and DSBs are critical for genetic recombination between homologous chromosomes during meiosis [3]. Furthermore, DSBs also occur as transient intermediates when the topoisomerase II–DNA complex decatenates two DNA strands [4]. To combat the risk of

* *Corresponding author*. Tel.: +31 104087932; fax: +31 104089468.
E-mail address: d.vangent@erasmusmc.nl (D.C. van Gent).
1568-7864/$ – see front matter © 2007 Elsevier B.V. All rights reserved.
doi:10.1016/j.dnarep.2007.02.006

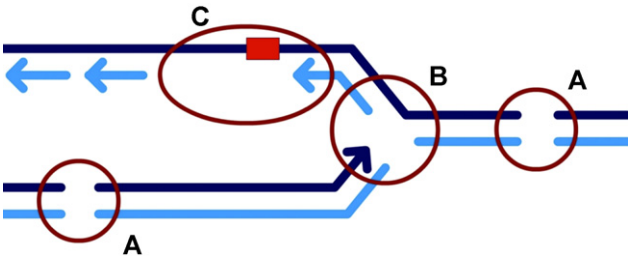


**Fig. 1 – Three major classes of recombinogenic structures. (A) Two-ended DNA double-strand break, created by direct fracture of a DNA duplex. (B) One-ended DNA double-strand break, created when a replication fork encounters a DNA single-strand break. (C) Daughter strand gap, created when lagging or leading strand progression is inhibited by a DNA lesion.**

large scale sequence rearrangements that could potentially result from both intentional and unintentional DSBs, mammals have evolved intricate DNA damage response and repair mechanisms. Here, we describe the major DSB sensor and repair processes, with a particular focus on the importance of accurate coordination among repair pathways in combating cancer and disease.

Historically, attention to DSBs has been focused primarily on two-ended DSBs that can be formed when a duplex molecule is fractured into two parts (Fig. 1A). Such two-ended breaks can be formed at any time during the cell cycle, and they can be accurately repaired by the non-homologous end joining in a process that rejoins the broken ends. While such two-ended DSBs are important DNA lesions, it is becoming increasingly clear that a significant portion of DSBs do not arise from direct fracture of a DNA duplex, but rather as a consequence of DNA replication. For example, one-ended DSBs can arise when the replication fork collides into an unrepaired DNA single-strand break (SSB) (Fig. 1B) [5]. Replication forks may also stall or breakdown when they run into certain base lesions. Homology directed repair provides a mechanism for accurate repair of such a broken replication fork [6,7] (Fig. 2, for a detailed description of this repair process, see Section 2.2.4). Importantly, non-homologous end joining of a one-ended DSB could be disadvantageous, since joining ends from independent loci will inevitably result in large scale sequence rearrangements (Fig. 3). Thus, DNA replication is associated with the risk of converting base damage and SSBs into highly toxic DSBs, and these one-ended breaks require complex signaling and processing in order to be accurately repaired.

In addition to the complexities of repair pathway choice, the cell also has to cope with a variety of DNA end structures. DNA ligase can readily rejoin juxtaposed broken ends with ligatable 3′ hydroxyl groups and 5′ phosphates. However, many conditions simultaneously introduce both strand breaks and covalent modifications to nearby nucleotides. For example, DSBs caused by ionizing radiation result in a large fraction of DNA ends that contain additional DNA lesions at or close to the end, so-called 'difficult' DSBs [8]. Here, we describe the non-homologous end joining and homology directed repair pathways required to repair different types of DSBs. In order

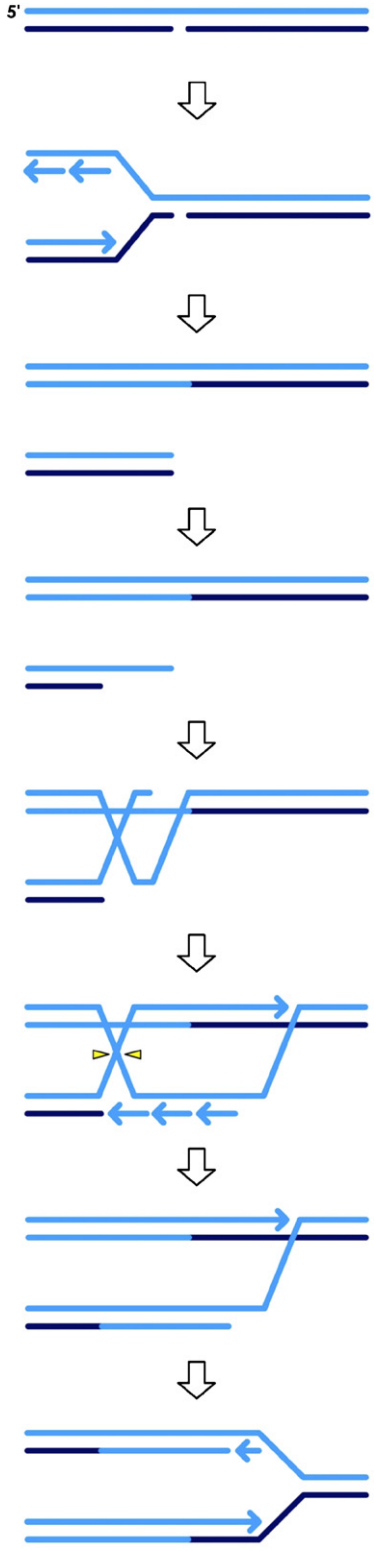


**Fig. 2 – Replication fork breakdown and repair. Upon encounter with a DNA single-strand break, the replication fork can breakdown to create a one-ended DNA double-strand break. Invasion of the 3′ overhang into what had been the sister chromatid creates a D-loop. Cleavage of the resulting Holliday Junction restores the replication fork. See animation at http://web.mit.edu/engelward-lab/animations/forkHR.html**

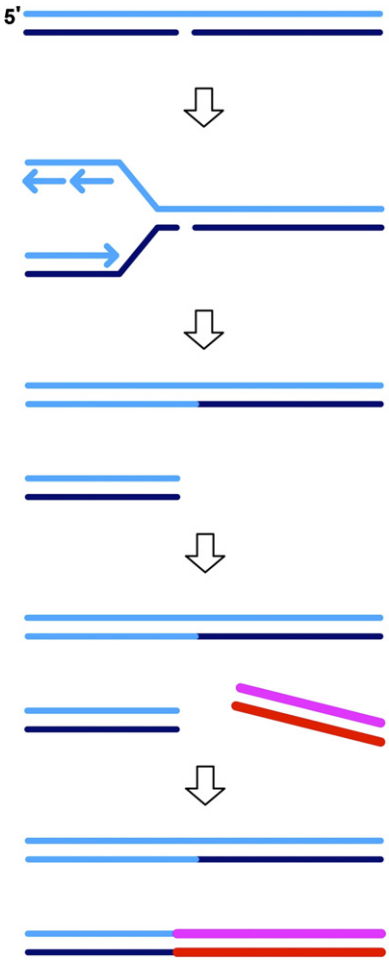


**Fig. 3 – Mis-joining resulting from non-homologous end joining of a one-ended DNA double-strand break. Replication fork encounter with a DNA single-strand break can lead to replication fork breakdown. The resulting one-ended DNA double-strand break is normally repaired by homologous recombination (see Fig. 2). Non-homologous end joining of this one-ended break to another double-strand end located elsewhere in the genome can result in a misjoining event. See animation at http://web.mit.edu/engelward-lab/animations/forkNHEJ.html**

to present strand exchange processes in their simplest form, we have created animations that demonstrate some of the key strand rearrangement processes associated with homologous recombination.

## 2. Non-homologous end joining and homologous recombination—partners in repair

### 2.1. *Non-homologous end joining*

#### 2.1.1. *The core non-homologous end joining machinery*

The simplest repair mechanism for a DSB is non-homologous end joining. In essence, this DSB repair pathway directly rejoins the two severed DNA ends in a sequence independent fashion [9]. This DSB repair pathway is mostly precise for simple breaks, such as blunt ends [10], but can lead to sequence alterations at the breakpoint when the ends are not compatible. Although the term "non-homologous" is used to describe this repair pathway, a tiny 1–6 bp region of sequence homology (microhomology) near the DNA end often facilitates rejoining. In contrast to non-homologous end joining, homology directed repair is guided by much longer stretches of homology, generally encompassing 100 bp or more. Thus, a major difference between non-homologous end joining and homology directed repair is the span of homologous sequences associated with repair processing.

Many proteins are required to efficiently perform non-homologous end joining. The core machinery consists of DNA-dependent protein kinase (DNA-PK) and the ligase IV/XRCC4/XLF complex (Fig. 4). The Ku70/80 heterodimer is the DNA binding component of DNA-PK, which forms a ring that can specifically bind to DNA ends [11]. This DNA–Ku complex

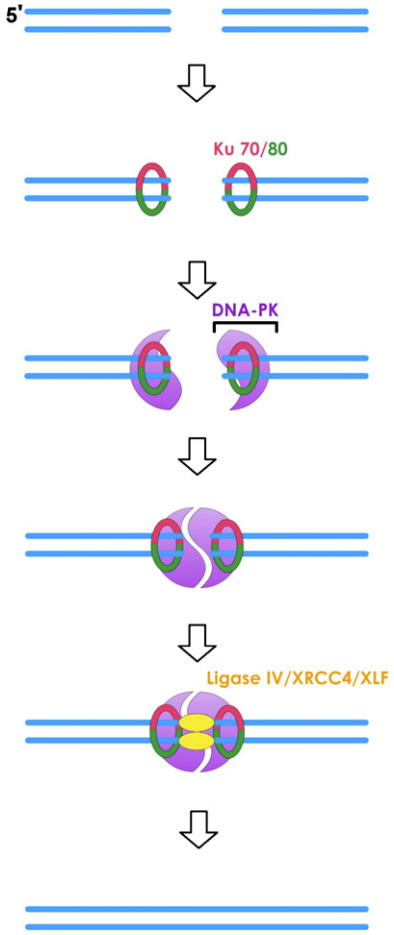


**Fig. 4 – Model of the key steps required for non-homologous end joining of two ends. DNA ends are first bound by the Ku70/80 heterodimer, which then attracts DNA-PKcs to form the DNA-PK complex. DNA-PK then attracts the ligase IV complex (comprised of ligase IV, XRCC4 and XLF), which together seal the DNA ends. Note that in some cases the DNA ends require covalent modification prior to ligation, which is not shown in this model (see text for details). See animation at http://web.mit.edu/engelward-lab/animations/NHEJ.html**

then attracts and activates the catalytic subunit (DNA-$PK_{CS}$), a serine/threonine protein kinase. After juxtaposition of the two DNA ends, DNA-$PK_{CS}$ is autophosphorylated [12–14] and the ends become available for ligation by the ligase IV complex, which also contains the XRCC4 and XLF cofactors that are probably required for proper targeting of the ligase to DNA ends [15–18]. The Mre11, Rad50 and Nbs1 protein complex may facilitate tethering of the two DNA ends, and may be less critical under conditions where ends can be directly ligated than under conditions when ends require processing [19–21].

#### 2.1.2. *Non-homologous end joining of 'difficult' DSBs*

As a result of associated lesions, not all DNA ends are readily ligatable. DNA ends can contain aberrant 3′ phosphate groups, 5′ hydroxyl groups, damaged backbone sugar residues and damaged DNA bases. Such DNA ends require processing before proper joining can proceed. DNA ends carrying 3′ phosphates or 5′ hydroxyl groups can be polished by polynucleotide kinase, which interacts with XRCC4 [22,23]. Another subclass of incompatible DNA end structures can be polished by the structure-specific Artemis nuclease, which can cleave both DNA hairpins (which are intermediates in V(D)J recombination) and 3′ overhanging single-stranded regions [24,25]. Furthermore, the WRN protein, which is mutated in Werner syndrome patients, may polish another subset of DNA ends with its exonuclease activity [26]. Finally, several DNA polymerases, including polymerases μ and λ, can fill in 5′ single-stranded extensions [27]. It is to be expected that additional processing factors will surface in the future.

Indeed, in addition to these relatively well-defined activities, several other genes have been found to be required for efficient repair of a subset of ill-defined 'difficult' breaks (also referred to as 'dirty' or 'complex' breaks). Although the mechanistic details of such non-homologous end joining subpathways are not yet fully elucidated, they probably involve the ATM, 53BP1 and Mre11/Rad50/Nbs1 proteins and require phosphorylation of histone H2AX [28].

### 2.2. *Homology directed repair*

While non-homologous end joining operates in a template-independent fashion by rejoining two broken ends (and this process is often error-prone), homology directed repair has the capacity to accurately resynthesize damaged or missing sequence information at the break site by using a template located elsewhere in the genome. This error-free process can be accomplished by finding homologous sequences, preferably in the sister chromatid, and inserting a 3′ end so that repair synthesis occurs across the breakpoint. All homology directed repair pathways are initiated by 5′–3′ resection at the DSB end, which is facilitated by the Mre11/Rad50/Nbs1 complex [29]. From here on, several possible homology directed repair subpathways have been identified. Here, we discuss the synthesis-dependent strand annealing pathway, the classical double-Holliday Junction model for DSB repair [30], and single-strand annealing, all of which contribute to the repair of two-ended DSBs. In addition, we will also describe how homology directed repair can mend a one-ended DSB in replication fork repair. Finally, in addition to these traditional models of homology directed repair subpathways, we also briefly discuss template switching events, which are potentially initiated by single-stranded gaps.

#### 2.2.1. *Synthesis-dependent strand annealing at two-ended DSBs*

Synthesis-dependent strand annealing is thought to be the predominant mechanism by which homology directed repair handles two-ended DSBs. To demonstrate the inherent simplicity of synthesis-dependent strand annealing, an animation accompanies the text below. In addition, we have separated the text description of this process into two parts: the first section focuses on the movement of DNA strands, whereas the latter describes some key features of the proteins that catalyze each of the major steps in this process.

In common with all homology directed repair subpathways, the synthesis-dependent strand annealing pathway is initiated by resection of a broken end to create single-stranded DNA (Fig. 5). After resection and protein binding, the resulting nucleoprotein filament invades the sister chromatid, forming heteroduplex DNA wherever it base pairs. This process of strand invasion and formation of heteroduplex DNA displaces a DNA strand, forming a so-called D-loop. Strand invasion is then followed by DNA synthesis beyond the original break site to restore the missing sequence information at the break point. The sister chromatid provides an ideal template for such error-free repair synthesis, and indeed it is the preferred template for homology directed repair [31]. On the other side of the D-loop, an "X" shaped structure called a Holliday Junction is formed at the border between hetero- and homoduplex [32]. Several proteins can bind Holliday Junctions and modulate the ability of these junctions to slide in either direction (this process is often called 'branch migration'). If the Holliday Junction is transported in the same direction as replication, it will release the newly synthesized strand. It is noteworthy that only one DNA end needs to invade the template DNA, as long as replication extends beyond the gap and into the region that is homologous to the opposite DNA end. Thus, by sliding the Holliday Junction, the invading strand can be released, and the newly synthesized 3′ single-stranded end can then anneal to the other side of the DSB. Final processing to remove flaps, fill in gaps, and ligate remaining nicks then completes this pathway. It is important to point out that repair synthesis requires that sequence information be copied into the breakpoint in the synthesis-dependent strand annealing model for two-ended homology directed repair. This associated transfer of sequence information, termed gene conversion, has indeed been demonstrated experimentally in mammalian cells [33–37].

The central player in almost all homology directed repair events is Rad51. With the help of a series of associated proteins (i.e., BRCA2, RAD52, RAD54, RAD54B, and likely also the RAD51 paralogues RAD51B, RAD51C, RAD51D, XRCC2 and XRCC3), Rad51 forms the nucleoprotein filament that facilitates homology searching and strand invasion [38–44]. Interestingly, vertebrate cells rapidly accumulate chromosome aberrations and cease to divide when Rad51 expression is suppressed [45] and mice lacking Rad51 are inviable [46,47]. These studies clearly demonstrate that Rad51 is an essential protein and call attention to the critical role that homology directed repair plays in maintaining genomic integrity.

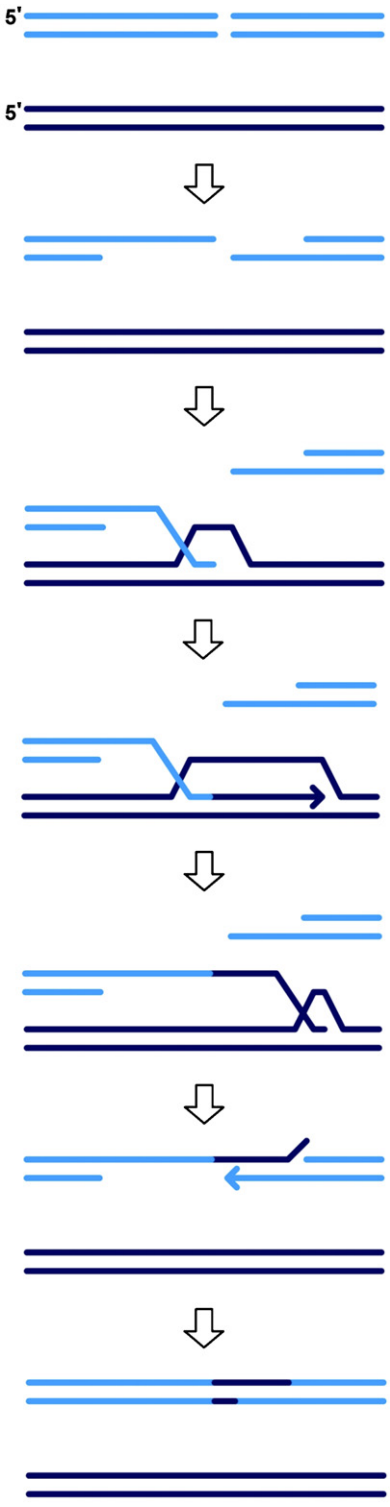


**Fig. 5 – Synthesis dependent strand annealing. This pathway is initiated by a two-ended DNA double-strand break. After resection to create single-stranded 3′ overhangs, strand invasion allows for 3′ extension. Branch migration of the resulting Holliday Junction allows for release of the invading strand, which subsequently anneals to the opposite side of the original break. See animation at http://web.mit.edu/engelward-lab/animations/SDSA.html**

After D-loop formation, the annealed 3′ end is then extended by repair synthesis. Recent studies show that DNA polymerase η (eta) can perform 3′ end extension at a D-loop [48], which is consistent with the observation that cells lacking polymerase η showed a defect in homologous recombination [49]. Although Pol η, clearly affects homology directed repair, it remains likely that other DNA polymerases can compensate in absence of Pol η, since humans with mutant Pol η, are alive and mostly healthy, which likely would not be the case if they were completely defective in homology directed repair.

Once repair synthesis is complete, the next step in this pathway is to release the newly synthesized end, which can be accomplished simply by sliding the Holliday Junction toward the 3′ end. Many proteins have been shown to bind and/or modulate Holliday Junctions in vitro (e.g., WRN, BLM, p53, RAD54, BLAP75 and hMSH2-hMSH6) [50–54], but exactly how these proteins are coordinated during synthesis-dependent strand annealing is not yet fully elucidated. For example, while it is clear that RAD54, WRN and BLM facilitate Holliday Junction migration [53,55,56], it is not clear how the direction of migration is controlled, nor is it clear whether these proteins are involved in all homologous recombination events or only in certain subpathways. Following branch migration, the freed 3′ end likely becomes rapidly bound by RPA. If the opposite end of the DSB was similarly recessed, then simple annealing is all that is required to reconnect the two broken ends, and this annealing step can be facilitated by Rad52 or possibly p53 [57–59]. Depending on the degree to which the 3′ end was extended during repair synthesis, there may or may not be a flap following the annealing step. If such a flap is formed, it can potentially be removed by structure specific endonucleases, such as XPF/ERCC1 [60,61]. Finally, remaining gaps are filled and ligase seals the nicks. It is to be expected that the normal cadre of proteins involved in repair synthesis would be recruited for these final steps (e.g., polymerase δ/ε, PCNA, and DNA ligase I) [62].

#### 2.2.2. *Double-Holliday Junction model for DSB repair*

The double-Holliday Junction model for DSB repair was initially designed to explain gene conversion and crossover events occurring simultaneously following a DSB during meiosis [30]. In this model, both DNA ends invade the homologous DNA template and form a double Holliday Junction that may be resolved to create a crossover or a non-crossover product (Fig. 6). The model is very elegant and gained popularity to also explain repair of DSBs during mitosis. However, there are several complications that arise when applying this model to explain how mammalian mitotic DSB repair occurs. Importantly, when resolution products are analyzed following introduction of a site-specific double-strand break, crossover products are rarely observed [33]. Furthermore, there is little direct evidence to support the possibility that this model accurately reflects strand processing in vivo. Nevertheless, it is important to point out that the human BLM and topoisomerase III proteins may resolve double Holliday Junctions to avoid cross-over products [54,63], which is consistent with the possibility that such double crossover intermediates indeed can form in mitotic cells. Furthermore, extensive studies of polymorphism patterns associated with sites of loss of heterozygosity in human and rodent cells have shown that

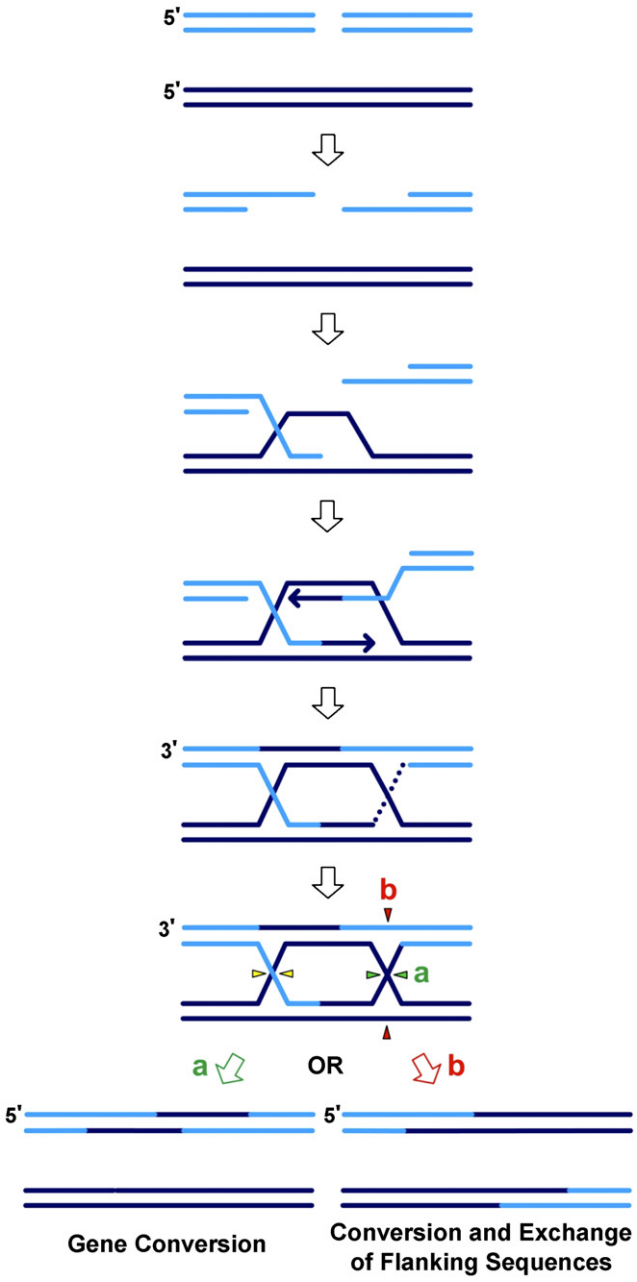


**Fig. 6 – Classical double Holliday Junction model of two-ended DNA double-strand break repair. Ends of the two-ended break are recessed and one DNA end invades a homologous duplex to create a D-loop. The opposite DNA end of the break then anneals to the D-loop to create a double Holliday Junction. Depending on the orientation of cleavage, resolution of the Holliday Junctions either preserves flanking sequence continuity or results in a crossover event. Note that regardless of the resolution pattern, repair synthesis results in gene conversion at the location of the break point. See animation at http://web.mit.edu/engelward-lab/animations/DSBR.html**

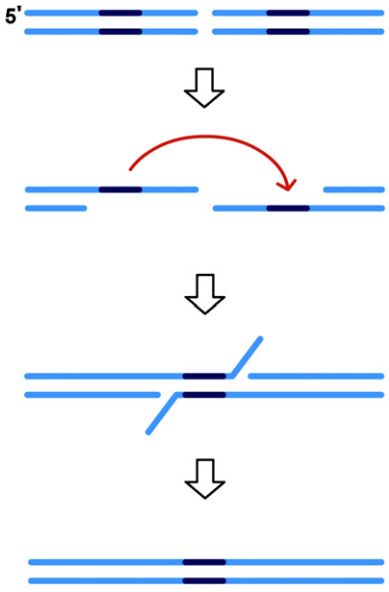


**Fig. 7 – Single-strand annealing model for repair of a two-ended DNA double-strand break. Darkened regions indicate stretches of homologous sequence. See animation at http://web.mit.edu/engelward-lab/animations/SSA.html**

large stretches of chromosomes can be exchanged in mitotic cells [64–67]. Although the underlying mechanism of such large scale exchanges is not yet known, it remains possible that cleavage of double Holliday Junctions could drive these events.

### 2.2.3. *Single-strand annealing in repair of two-ended DSBs*

If two adjacent repeat sequences are present, single-strand annealing may be utilised to repair a two-ended DSB. In this case the two 3′ overhangs are simply aligned and annealed (Fig. 7). This process is facilitated by RPA and RAD52 in a RAD51-independent manner [57,68]. It is noteworthy that single-strand annealing is associated with inevitable loss of the sequences between the repeats, as well as one of the repeats. As such, single-strand annealing is always error-associated, since it leads to permanent large deletions. Haber has made a strong case that single-strand annealing is actually a 'spandrel' [69] (a term applied by S.J. Gould to describe an unintentional consequence [70]) in this case referring to the possibility that single-strand annealing is an unintentional consequence of the need to create single-stranded DNA in order to initiate synthesis-dependent strand annealing. Despite the inevitable loss of sequence information, single-strand annealing may play a role in DSB repair. The human genome is repleat with repetitive elements, e.g., there are $>10^6$ Alu repeats in the human genome [71], and more than 10% of the human genome is comprised of repeat sequences [72]. However, it is noteworthy that these repeats exhibit high sequence diversity [73], and mismatches between the repeat elements can dramatically suppress single-strand annealing in mammalian cells [74]. Thus, although single-strand annealing between Alu elements can occur at repeat sequences in the human genome, this pathway is likely to play a

fairly limited role in the repair of DSBs in human mitotic cells.

### 2.2.4. *Replication fork repair of one-ended DSBs*

One-ended DSBs arise when replication forks break down, for example upon encounter with a SSB [5]. Resection at this DNA end provides a 3′ overhang which is a substrate for RAD51-mediated strand invasion (Fig. 2). Strand invasion is then followed by Holliday Junction cleavage, which allows resumption of DNA replication. This recombination pathway has been called break-induced replication, as suggested by Haber [75,76], or replication fork repair. If the replication fork breaks down when the leading strand encounters a single-strand break (as shown in Fig. 2), then the leading strand template can potentially become covalently joined to the newly forming lagging strand (this depends on the direction by which the single Holliday Junction behind the replication fork is resolved) [31,77]. In this fashion, cleavage of the Holliday Junction can result in a sister chromatid exchange (SCE) that can be detected by BrdU labeling. Indeed, consistent with this model, mammalian cells with a defect in SSB repair show increased susceptibility to SCEs. For example, high levels of SCEs have been observed in cells harboring mutations in XRCC1 or Poly(ADP-ribose) polymerase 1 (PARP-1), presumably as a result of deficiencies in key proteins necessary to assemble repair factors at SSBs or to prevent replication fork encounter with SSBs [78–81]. Furthermore, one-ended DSBs at replication forks trigger a RAD51-dependent homologous recombination event that can result in a SCE [6,34]. It is interesting to consider the possibility that the need to repair broken replication forks may be the driving force behind the evolution of homology directed repair, which is supported by the observation that a complete lack of homology directed repair is lethal at the single cell level [45].

### 2.2.5. *Template switching*

Emerging evidence suggests that homologous recombination is also part of a bypass mechanism for handling replication-blocking lesions. If replication on the lagging strand is obstructed by lesions, persistent gaps between Okazaki fragments (shown in Fig. 1C) could potentially stimulate template switching (Fig. 8). Indeed, the RAD51 paralog proteins have been shown to assist formation of a RAD51 filament on gapped DNA [42], suggesting that homologous recombination can be initiated in the absence of a DNA end. Lesions obstructing leading strand synthesis may similarly induce template switching in the absence of a DSB. It is important to note that template-switching is transient, and it only facilitates bypass of DNA lesions; other repair pathways are needed to actually remove the offending lesion.

### 2.2.6. *A case for one-ended DSBs as the critical endogenous substrate for homologous recombination*

While it is clear that two-ended DSBs are preferentially repaired by non-homologous end joining [82], it remains unclear to what extent two-ended breaks drive spontaneous recombination, which is a question that is important to address if we are to understand the role of homologous recombination in disease. There is now substantial data supporting the notion that homology directed repair is critical for repair at the replication fork [83]. We have discussed several potentially recombinogenic lesions that can be formed during replication (e.g., one- or two-ended DSBs, as well as single-stranded gaps) [84]. Here, we argue that one-ended DSBs are likely to be the underlying cause of a significant portion of spontaneous homology directed repair events. First, it has been shown that inability to efficiently repair SSBs increases the frequency of one-ended DSBs [34,85] and also increases the spontaneous levels of both SCEs and RAD51 foci in mammalian cells, in some cases by more than an order of magnitude [34,79,86–88]. Second, analysis of recombination products shows that approximately a third of spontaneous recombination events in mouse embryonic stem cells are consistent with repair of broken replications forks [35]. Finally, most spontaneous recombination events in human cells showed similar resolution products as those created when cells were subjected to increased levels of single-strand breaks [34]. Thus, it is clear that single-strand breaks induce replication fork breakdown, and it has been shown that a significant portion of recombination products show resolution patterns that are consistent with the repair of broken replication forks.

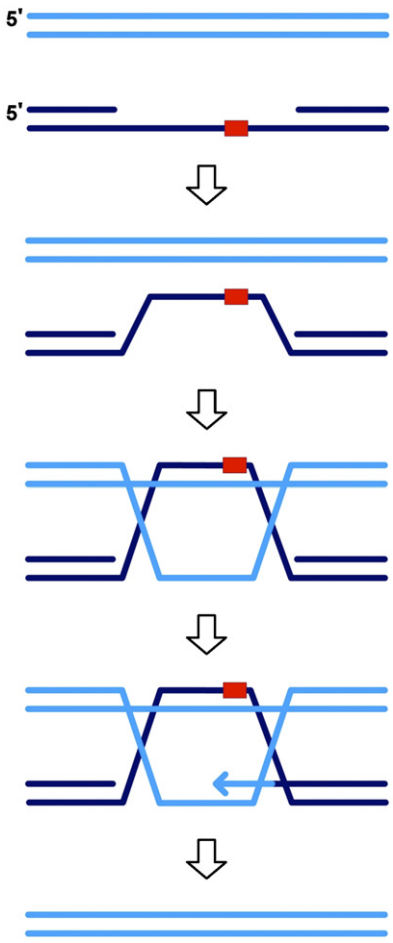


**Fig. 8 – Template-switching model to bypass lesions with homologous recombination. Homologous recombination is initiated by a DNA single-strand gap that allow replication using opposite template to bypass replication lesion.**

## 3. How does the cell choose between homology directed repair and non-homologous end joining?

Choosing between homology directed repair and non-homologous end joining depends on several factors. One quite obvious factor is the cell cycle stage at which the DSB is generated. Most homologous recombination events occur between sister chromatids and should therefore be largely confined to the S and G2 phases of the cell cycle [89,90]. A question thus arises: how does the cell know whether a certain part of the genome has already been replicated? Although the complete answer to this question is not yet known, several findings suggest that the initiation of homologous recombination is tightly cell-cycle regulated. One way to restrict homologous recombination activity to the S/G2 phases is to link initiation of this pathway to cyclin-dependent kinases that are specifically active during these stages of the cell cycle. Indeed, it has been demonstrated that generation of the 3′ ssDNA overhang is regulated by CDK activity, which prevents resection of DNA ends outside the S and G2 phases of the cell cycle in eukaryotic cells [91,92]. By preventing homologous recombination outside of S and G2, exchanges between homologous chromosomes can be reduced, thus suppressing loss of heterozygosity events that potentially could result from such exchanges [89].

In addition to confining homology directed repair to S/G2, the cell needs to ensure that non-homologous end joining does not act on one-ended DSBs, since this activity could promote misjoining between different loci (see Section 1, Fig. 3). Interestingly, the Ku70/80 heterodimer appears at DNA ends much more quickly than homologous recombination factors [18,93]. This observation is consistent with the possibility that non-homologous end joining is the preferred DSB repair pathway for two-ended DSBs, even in S phase, and also implies that mechanisms must exist to prevent non-homologous end joining from acting at broken replication forks. Possibilities for ensuring that one-ended breaks are acted upon by homologous recombination include the fact that a one-ended break in the lagging strand could have a relatively long 3′ single-stranded extension that could prevent Ku70/80 binding [94]. In addition, the leading strand may exploit a hand-off mechanism from the replication machinery to the homologous recombination pathway.

The DSB repair pathway choice may also be influenced by the structure of the DNA end. A simple, directly ligatable DSB makes a good substrate for non-homologous end joining, whereas more difficult breaks may be more prone to attract the homologous recombination machinery. A more detailed investigation of the influence of DNA end structure on DSB repair has been hampered by the absence of agents that can specifically produce one type of DNA end. However, it is clear that difficult DSBs require more time for repair [28], which is consistent with the possibility that they are somehow shunted toward homologous recombination.

An additional layer of control over DSB repair pathway choice is provided by DNA damage signaling pathways. It has been clear for many years that ionizing radiation directly impairs replication origin firing through ATM signaling [95,96], in a process that is facilitated by the MRN complex [97]. This intra-S phase checkpoint suppresses creation of new replication forks, and thus diminishes the odds that replication forks will run into lesions that might otherwise stimulate fork breakdowns. Furthermore, an intra-S checkpoint may also help to provide the time required to perform homologous recombination.

Nevertheless, even under optimal conditions for DNA replication, replication forks are likely to encounter DNA lesions, and these encounters require signaling and appropriate pathway choice to prevent catastrophe in S phase. Mammalian cells sustain more than 10,000 abasic sites per day [1] and it is estimated that hundreds of thousands of damaged bases are formed each day [98]. Therefore, one would expect the replication fork to run into DNA lesions multiple times per round of replication, which is consistent with the observations that normal human cells undergo about 10 sister chromatid exchanges per round of replication [99] (see Fig. 2), and that recombination events accumulate throughout the lifespan of mammals [100].

Conceptually, one could envision two ways to counteract the problem of encounters with DNA lesions and single-strand breaks during replication: repairing the broken replication fork and preventing the replication fork from advancing through DNA lesions. Among researchers interested in homologous recombination, most research has focussed on the first scenario, repairing one-ended DSBs to restore replication fork integrity. Clearly, this process requires homology directed repair, as elaborated above. Equally important is the question of how cells prevent replication forks from encountering DNA lesions in the first place. Many signaling proteins, such as ATR and Chk1 [101–103] are required for maintenance of replication fork integrity under conditions that cause replication stress (e.g., hydroxyurea treatment, which depletes the nucleotide pool). Furthermore, depletion of the MRN complex or ATM and ATR from Xenopus egg extracts causes accumulation of DSBs during normal replication [104,105], suggesting that replication is not properly regulated under these conditions. Interestingly, exposure to an alkylating agent has been shown to inhibit origin firing and also to slow down replication fork progression [106], which may result from direct inhibition of replication fork progression by 3-methyladenine [107,108]. It is tempting to speculate that DNA damage ahead of the replication fork might lead to signals not only to suppress origin firing (as described above), but also possibly to slow down or halt replication fork progression in order to repair the damage before a DSB might be formed. It will be interesting to learn the extent to which DNA lesions that slow replication fork progression do so via active signaling versus passive inhibition of DNA polymerases.

## 4. Defective DSB repair in association with cancer and aging

### 4.1. *Non-homologous end joining defects are associated with cancer*

Chromosomal instability is a hallmark of many tumors. Interestingly, careful analysis of translocation breakpoints in lymphoid malignancies has revealed that most junctions have

characteristics of normal non-homologous end joining [109]. Therefore, one might expect that non-homologous end joining deficient mice would have a reduced incidence of transformed cells containing chromosomal translocations. However, the opposite effect has been observed in several non-homologous end joining mouse models [110,111]. Deficiencies in non-homologous end joining most often lead to an increased risk of cancer, with enhanced chromosomal instability, including translocations. This phenomenon was particularly obvious in combination with a mutation in the tumor suppressor gene p53 [111], probably because of a decreased apoptosis rate. Strikingly, a relatively subtle decrease in non-homologous end joining capacity (caused by heterozygosity at the ligase IV locus) resulted in a cancer-prone phenotype in an Ink4a deficient background, suggesting that subtle variations in non-homologous end joining capacity in the population might contribute to carcinogenesis [112]. Indeed, patients with hypomorphic mutations in the Artemis gene have been found to develop thymic lymphomas, showing that a decrease in non-homologous end joining capacity can increase the risk of cancer in humans as well as mice [113].

### 4.2. *Deregulated homologous recombination leads to cancer and aging*

Given the high stakes involved in assuring accurate rejoining of two-ended breaks, and accurate reinsertion of one-ended breaks during replication fork repair, it is not surprising that defects in key proteins involved in homologous recombination are also associated with an increased risk of cancer [114]. While many proteins that modulate homologous recombination are known to be cancer genes (e.g., BRCA1 and FANC genes, MMR genes, p53, and ATM [115–118]), these genes have pleiotropic effects, so it is difficult to dissect out the importance of homologous recombination in these cases. On the other hand, there are also cancer genes where a direct link to homologous recombination is more apparent. For example, BRCA2 plays a central role in displacing RPA and facilitating loading of Rad51 onto single-stranded DNA [119]. The resulting homology directed repair deficiency in BRCA2 null cells results in the accumulation of chromosome aberrations [120], which is quite similar to what has been observed in vertebrate cells depleted of Rad51 [45]. Thus, a defect in the ability to initiate homologous recombination is associated with an increased cancer risk. Another interesting example is the BLM helicase. Unlike BRCA2, BLM mutant cells are proficient in initiating homologous recombination, but the outcome of these repair events is apparently shifted toward exchange-associated events [63,121]. The resulting increase in exchanges between homologous chromosomes leads to increased rates of loss of heterozygosity, which has been proposed to be the driving force behind the increased risk of cancer in Bloom's syndrome patients [122]. Finally, as another example, defects in the WRN helicase are associated with accelerated aging and increased cancer risk. In this case, the rate of initiation of homologous recombination appears to be increased, but there are problems in resolution of homologous recombination events, which apparently increases the risk of cell death [123,124]. These three examples demonstrate that deficiencies in both homologous recombination initiation and resolution lead to disease and call attention to the fundamental role of homologous recombination in genome maintenance. Undoubtedly research in the next decade will reveal additional connections between homologous recombination, genomic stability, and disease.

It is important to emphasize that loss of homologous recombination in a normal cell is generally more toxic than it is mutagenic. For example, conditional knock out of Rad51 in vertebrate cells leads to increased levels of chromosome aberrations, but within a short time, no cells survive [45]. In other words, severe deficiencies in homologous recombination are as toxic as they are genome destabilizing. One might then ask how loss of function of BRCA2 promotes cancer. At least two possible explanations exist. First, cells might tolerate a partial deficiency in homologous recombination more readily than complete loss of function. Indeed, cells lacking BRCA2 retain some homologous recombination capacity [125,126]. Another possibility is that suppression of homologous recombination capacity is a late event in tumorigenesis (as has been observed in the case of pancreatic cancer [127]), preceded by mutations that confer resistance to apoptosis, thus allowing highly unstable cells to survive.

## 5. Exploiting DSB repair defects in cancer therapy

Most cancer cells have acquired several mutations in key regulatory genes, such as those that control growth factors, growth factor receptors or apoptosis. In order to accelerate the rate of mutation accumulation, a large fraction of cancers display genomic instability, a condition that is exacerbated by defects in the DNA damage response. In principle, one could exploit these inherent weaknesses to attack the cancer cell, potentially without causing excessive damage to the surrounding healthy cells that are proficient in DNA damage responses. Recently, this idea has been put into practice for cells that are mutated in the breast cancer susceptibility genes BRCA1 or BRCA2. Heterozygous carriers of a mutation in one of these genes have a dramatically increased risk of breast or ovarian cancers that arise from cells that have lost the wild type copy. After loss of heterozygosity, their capacity to carry out homology directed repair is impaired, rendering cells highly prone to spontaneous chromosomal aberrations [120,128]. In addition to being prone to genomic instability, BRCA2 deficient cells are also highly vulnerable to treatments that increase the level of SSBs [129,130], which is entirely consistent with a model wherein BRCA2 cells are deficient in the ability to repair the one-ended DSBs that arise when replication forks encounter SSBs. Therefore, inhibition of appropriate processing of SSBs by interfering with Poly(ADP-ribose) polymerase (PARP) activity should result in an increased dependence on homology directed repair. Two recent studies have indeed found that BRCA1 or BRCA2 deficiency leads to a dramatic hypersensitivity to PARP inhibitors, raising hopes for developing a powerful, targeted therapy for these tumors [85,131].

Importantly, we do not yet know how common it is for tumor cells to be deficient in homologous recombination. Thus, it is to be expected that a subset of other tumors will also show PARP inhibitor sensitivity because of mutations in

other genes that are required for efficient repair of one-ended DSBs. A major challenge is therefore to identify tumors that are sensitized to PARP inhibitors. Developments in expression profiling of tumors using microarray technology will undoubtedly contribute to identification of expression patterns that render a 'BRCA-like' phenotype, which may help identify other PARP inhibitor sensitive tumors.

Although most tumors are genetically unstable, they do not all show the same type of genome maintenance defect. The challenge for the next decade will be to pinpoint the molecular defects in DNA damage response mechanisms and to use this knowledge to specifically attack the cancer cell's Achilles' heel. This will require a profound knowledge of all aspects of the DNA damage response, as well as development of specific inhibitors of key regulatory enzymes of the various DNA repair and cell cycle checkpoint pathways. Identification of the Achilles' heels of various tumors will be aided tremendously by the recent development of RNA interference screens, which are able to identify genes and pathways that are indispensable for tumor cell survival, but not for normal cells [132]. In combination with the increasing basic knowledge of the intricate interplay between repair, replication and cell cycle machinery, these developments may revolutionize cancer treatment in the 21st century.

## REFERENCES


[1] T. Lindahl, Instability and decay of the primary structure of DNA, Nature 362 (1993) 709–715.
[2] M. Gellert, J.E. Hesse, K. Hiom, M. Melek, M. Modesti, T.T. Paull, D.A. Ramsden, D.C. van Gent, V(D)J recombination: links to transposition and double-strand break repair, Cold Spring Harb. Symp. Quant. Biol. 64 (1999) 161–167.
[3] M.J. Neale, S. Keeney, Clarifying the mechanics of DNA strand exchange in meiotic recombination, Nature 442 (2006) 153–158.
[4] A.J. Schoeffler, J.M. Berger, Recent advances in understanding structure-function relationships in the type II topoisomerase mechanism, Biochem. Soc. Trans. 33 (2005) 1465–1470.
[5] D. Strumberg, A.A. Pilon, M. Smith, R. Hickey, L. Malkas, Y. Pommier, Conversion of topoisomerase I cleavage complexes on the leading strand of ribosomal DNA into 5′-phosphorylated DNA double-strand breaks by replication runoff, Mol. Cell. Biol. 20 (2000) 3977–3987.
[6] C. Arnaudeau, C. Lundin, T. Helleday, DNA double-strand breaks associated with replication forks are predominantly repaired by homologous recombination involving an exchange mechanism in mammalian cells, J. Mol. Biol. 307 (2001) 1235–1245.
[7] B. Michel, M.J. Flores, E. Viguera, G. Grompone, M. Seigneur, V. Bidnenko, Rescue of arrested replication forks by homologous recombination, Proc. Natl. Acad. Sci. U.S.A. 98 (2001) 8181–8188.
[8] M. O'Driscoll, P.A. Jeggo, The role of double-strand break repair—insights from human genetics, Nat. Rev. Genet. 7 (2006) 45–54.
[9] E. Weterings, D.C. van Gent, The mechanism of non-homologous end joining: a synopsis of synapsis, DNA Repair (Amst.) 3 (2004) 1425–1435.
[10] D. van Heemst, L. Brugmans, N.S. Verkaik, D.C. van Gent, End joining of blunt DNA double-strand breaks in mammalian fibroblasts is precise and requires DNA-PK and XRCC4, DNA Repair (Amst.) 3 (2004) 43–50.
[11] J.R. Walker, R.A. Corpina, J. Goldberg, Structure of the Ku heterodimer bound to DNA and its implications for double-strand break repair, Nature 412 (2001) 607–614.
[12] Q. Ding, Y.V. Reddy, W. Wang, T. Woods, P. Douglas, D.A. Ramsden, S.P. Lees-Miller, K. Meek, Autophosphorylation of the catalytic subunit of the DNA-dependent protein kinase is required for efficient end processing during DNA double-strand break repair, Mol. Cell. Biol. 23 (2003) 5836–5848.
[13] Y.V. Reddy, Q. Ding, S.P. Lees-Miller, K. Meek, D.A. Ramsden, Non-homologous end joining requires that the DNA-PK complex undergo an autophosphorylation-dependent rearrangement at DNA ends, J. Biol. Chem. 279 (2004) 39408–39413.
[14] E. Weterings, N.S. Verkaik, H.T. Bruggenwirth, J.H. Hoeijmakers, D.C. van Gent, The role of DNA dependent protein kinase in synapsis of DNA ends, Nucleic Acids Res. 31 (2003) 7238–7246.
[15] S.A. Nick McElhinny, C.M. Snowden, J. McCarville, D.A. Ramsden, Ku recruits the XRCC4-ligase IV complex to DNA ends, Mol. Cell. Biol. 20 (2000) 2996–3003.
[16] P. Ahnesorg, P. Smith, S.P. Jackson, XLF interacts with the XRCC4-DNA ligase IV complex to promote DNA non-homologous end joining, Cell 124 (2006) 301–313.
[17] D. Buck, L. Malivert, R. de Chasseval, A. Barraud, M.C. Fondaneche, O. Sanal, A. Plebani, J.L. Stephan, M. Hufnagel, F. le Deist, A. Fischer, A. Durandy, J.P. de Villartay, P. Revy, Cernunnos, a novel non-homologous end joining factor, is mutated in human immunodeficiency with microcephaly, Cell 124 (2006) 287–299.
[18] P.O. Mari, B.I. Florea, S.P. Persengiev, N.S. Verkaik, H.T. Brüggenwirth, M. Modesti, G. Giglia-Mari, K. Bezstarosti, J.A.A. Demmers, T.M. Luider, A.B. Houtsmuller, D.C. van Gent, Dynamic assembly of end joining complexes requires interaction between Ku70/80 and XRCC4, Proc. Natl. Acad. Sci. U.S.A. 103 (2006) 18597–18602.
[19] F. Moreno-Herrero, M. de Jager, N.H. Dekker, R. Kanaar, C. Wyman, C. Dekker, Mesoscale conformational changes in the DNA-repair complex Rad50/Mre11/Nbs1 upon binding DNA, Nature 437 (2005) 440–443.
[20] K.P. Hopfner, L. Craig, G. Moncalian, R.A. Zinkel, T. Usui, B.A. Owen, A. Karcher, B. Henderson, J.L. Bodmer, C.T. McMurray, J.P. Carney, J.H. Petrini, J.A. Tainer, The Rad50 zinc-hook is a structure joining Mre11 complexes in DNA recombination and repair, Nature 418 (2002) 562–566.
[21] M. de Jager, J. van Noort, D.C. van Gent, C. Dekker, R. Kanaar, C. Wyman, Human Rad50/Mre11 is a flexible complex that can tether DNA ends, Mol. Cell 8 (2001) 1129–1135.
[22] C. Chappell, L.A. Hanakahi, F. Karimi-Busheri, M. Weinfeld, S.C. West, Involvement of human polynucleotide kinase in double-strand break repair by non-homologous end joining, EMBO J. 21 (2002) 2827–2832.
[23] C.A. Koch, R. Agyei, S. Galicia, P. Metalnikov, P. O'Donnell, A. Starostine, M. Weinfeld, D. Durocher, Xrcc4 physically links DNA end processing by polynucleotide kinase to DNA ligation by DNA ligase IV, EMBO J. 23 (2004) 3874–3885.
[24] Y. Ma, U. Pannicke, K. Schwarz, M.R. Lieber, Hairpin opening and overhang processing by an Artemis/DNA-dependent protein kinase complex in non-homologous end joining and V(D)J recombination, Cell 108 (2002) 781–794.
[25] D. Moshous, I. Callebaut, R. de Chasseval, B. Corneo, M. Cavazzana-Calvo, F. Le Deist, I. Tezcan, O. Sanal, Y. Bertrand, N. Philippe, A. Fischer, J.P. de, Villartay, Artemis, a novel DNA double-strand break repair/V(D)J recombination protein, is mutated in human severe combined immune deficiency, Cell 105 (2001) 177–186.
[26] J.J. Perry, S.M. Yannone, L.G. Holden, C. Hitomi, A. Asaithamby, S. Han, P.K. Cooper, D.J. Chen, J.A. Tainer, WRN exonuclease structure and molecular mechanism imply an

editing role in DNA end processing, Nat. Struct. Mol. Biol. 13 (2006) 414–422.
[27] S.A. Nick McElhinny, J.M. Havener, M. Garcia-Diaz, R. Juarez, K. Bebenek, B.L. Kee, L. Blanco, T.A. Kunkel, D.A. Ramsden, A gradient of template dependence defines distinct biological roles for family × polymerases in non-homologous end joining, Mol. Cell 19 (2005) 357–366.
[28] E. Riballo, M. Kuhne, N. Rief, A. Doherty, G.C. Smith, M.J. Recio, C. Reis, K. Dahm, A. Fricke, A. Krempler, A.R. Parker, S.P. Jackson, A. Gennery, P.A. Jeggo, M. Lobrich, A pathway of double-strand break rejoining dependent upon ATM, Artemis, and proteins locating to gamma-H2AX foci, Mol. Cell 16 (2004) 715–724.
[29] T.T. Paull, M. Gellert, The 3′ to 5′ exonuclease activity of Mre 11 facilitates repair of DNA double-strand breaks, Mol. Cell 1 (1998) 969–979.
[30] J.W. Szostak, T.L. Orr-Weaver, R.J. Rothstein, F.W. Stahl, The double-strand-break repair model for recombination, Cell 33 (1983) 25–35.
[31] C. Richardson, M.E. Moynahan, M. Jasin, Double-strand break repair by interchromosomal recombination: suppression of chromosomal translocations, Genes Dev. 12 (1998) 3831–3842.
[32] R. Holliday, A mechanism for gene conversion in fungi, Genet. Res. 5 (1964) 282–304.
[33] R.D. Johnson, M. Jasin, Sister chromatid gene conversion is a prominent double-strand break repair pathway in mammalian cells, EMBO J. 19 (2000) 3398–3407.
[34] N. Saleh-Gohari, H.E. Bryant, N. Schultz, K.M. Parker, T.N. Cassel, T. Helleday, Spontaneous homologous recombination is induced by collapsed replication forks that are caused by endogenous DNA single-strand breaks, Mol. Cell. Biol. 25 (2005) 7158–7169.
[35] V.S. Jonnalagadda, T. Matsuguchi, B.P. Engelward, Interstrand crosslink-induced homologous recombination carries an increased risk of deletions and insertions, DNA Repair (Amst.) 4 (2005) 594–605.
[36] B. Elliott, C. Richardson, J. Winderbaum, J.A. Nickoloff, M. Jasin, Gene conversion tracts from double-strand break repair in mammalian cells, Mol. Cell. Biol. 18 (1998) 93–101.
[37] R.M. Liskay, J.L. Stachelek, Evidence for intrachromosomal gene conversion in cultured mouse cells, Cell 35 (1983) 157–165.
[38] Z. Shen, K.G. Cloud, D.J. Chen, M.S. Park, Specific interactions between the human RAD51 and RAD52 proteins, J. Biol. Chem. 271 (1996) 148–152.
[39] P. Baumann, F.E. Benson, S.C. West, Human Rad51 protein promotes ATP-dependent homologous pairing and strand transfer reactions in vitro, Cell 87 (1996) 757–766.
[40] G. Petukhova, S. Stratton, P. Sung, Catalysis of homologous DNA pairing by yeast Rad51 and Rad54 proteins, Nature 393 (1998) 91–94.
[41] S. Sigurdsson, S. Van Komen, W. Bussen, D. Schild, J.S. Albala, P. Sung, Mediator function of the human Rad51B-Rad51C complex in Rad51/RPA-catalyzed DNA strand exchange, Genes Dev. 15 (2001) 3308–3318.
[42] J.Y. Masson, M.C. Tarsounas, A.Z. Stasiak, A. Stasiak, R. Shah, M.J. McIlwraith, F.E. Benson, S.C. West, Identification and purification of two distinct complexes containing the five RAD51 paralogs, Genes Dev. 15 (2001) 3296–3307.
[43] T. Sugiyama, S.C. Kowalczykowski, Rad52 protein associates with replication protein A (RPA)-single-stranded DNA to accelerate Rad51-mediated displacement of RPA and presynaptic complex formation, J. Biol. Chem. 277 (2002) 31663–31672.
[44] J. Essers, R.W. Hendriks, J. Wesoly, C.E. Beerens, B. Smit, J.H. Hoeijmakers, C. Wyman, M.L. Dronkert, R. Kanaar, Analysis of mouse Rad54 expression and its implications for homologous recombination, DNA Repair (Amst.) 1 (2002) 779–793.
[45] E. Sonoda, M.S. Sasaki, J.M. Buerstedde, O. Bezzubova, A. Shinohara, H. Ogawa, M. Takata, Y. Yamaguchi-Iwai, S. Takeda, Rad51-deficient vertebrate cells accumulate chromosomal breaks prior to cell death, EMBO J. 17 (1998) 598–608.
[46] D.S. Lim, P. Hasty, A mutation in mouse rad51 results in an early embryonic lethal that is suppressed by a mutation in p53, Mol. Cell. Biol. 16 (1996) 7133–7143.
[47] T. Tsuzuki, Y. Fujii, K. Sakumi, Y. Tominaga, K. Nakao, M. Sekiguchi, A. Matsushiro, Y. Yoshimura, T. Morita, Targeted disruption of the Rad51 gene leads to lethality in embryonic mice, Proc. Natl. Acad. Sci. U.S.A. 93 (1996) 6236–6240.
[48] M.J. McIlwraith, A. Vaisman, Y. Liu, E. Fanning, R. Woodgate, S.C. West, Human DNA polymerase eta promotes DNA synthesis from strand invasion intermediates of homologous recombination, Mol. Cell 20 (2005) 783–792.
[49] T. Kawamoto, K. Araki, E. Sonoda, Y.M. Yamashita, K. Harada, K. Kikuchi, C. Masutani, F. Hanaoka, K. Nozaki, N. Hashimoto, S. Takeda, Dual roles for DNA polymerase eta in homologous DNA recombination and translesion DNA synthesis, Mol. Cell 20 (2005) 793–799.
[50] S. Lee, L. Cavallo, J. Griffith, Human p53 binds Holliday junctions strongly and facilitates their cleavage, J. Biol. Chem. 272 (1997) 7532–7539.
[51] P. Mohaghegh, J.K. Karow, R.M. Brosh Jr., V.A. Bohr, I.D. Hickson, The Bloom's and Werner's syndrome proteins are DNA structure-specific helicases, Nucleic Acids Res. 29 (2001) 2843–2849.
[52] D. Subramanian, J.D. Griffith, Interactions between p53, hMSH2-hMSH6 and HMG I(Y) on Holliday Junctions and bulged bases, Nucleic Acids Res. 30 (2002) 2427–2434.
[53] D.V. Bugreev, O.M. Mazina, A.V. Mazin, Rad54 protein promotes branch migration of Holliday Junctions, Nature 442 (2006) 590–593.
[54] S. Raynard, W. Bussen, P. Sung, A double Holliday Junction dissolvasome comprising BLM, topoisomerase IIIalpha, and BLAP75, J. Biol. Chem. 281 (2006) 13861–13864.
[55] A. Constantinou, M. Tarsounas, J.K. Karow, R.M. Brosh, V.A. Bohr, I.D. Hickson, S.C. West, Werner's syndrome protein (WRN) migrates Holliday Junctions and co-localizes with RPA upon replication arrest, EMBO Rep. 1 (2000) 80–84.
[56] J.K. Karow, A. Constantinou, J.L. Li, S.C. West, I.D. Hickson, The Bloom's syndrome gene product promotes branch migration of Holliday Junctions, Proc. Natl. Acad. Sci. U.S.A. 97 (2000) 6504–6508.
[57] E. Van Dyck, A.Z. Stasiak, A. Stasiak, S.C. West, Visualization of recombination intermediates produced by RAD52-mediated single-strand annealing, EMBO Rep. 2 (2001) 905–909.
[58] J.H. New, T. Sugiyama, E. Zaitseva, S.C. Kowalczykowski, Rad52 protein stimulates DNA strand exchange by Rad51 and replication protein A, Nature 391 (1998) 407–410.
[59] P. Oberosler, P. Hloch, U. Ramsperger, H. Stahl, p53-catalyzed annealing of complementary single-stranded nucleic acids, EMBO J. 12 (1993) 2389–2396.
[60] G.M. Adair, R.L. Rolig, D. Moore-Faver, M. Zabelshansky, J.H. Wilson, R.S. Nairn, Role of ERCC1 in removal of long non-homologous tails during targeted homologous recombination, EMBO J. 19 (2000) 5552–5561.
[61] L.J. Niedernhofer, H. Odijk, M. Budzowska, E. van Drunen, A. Maas, A.F. Theil, J. de Wit, N.G. Jaspers, H.B. Beverloo, J.H. Hoeijmakers, R. Kanaar, The structure-specific endonuclease Ercc1-Xpf is required to resolve DNA interstrand cross-link-induced double-strand breaks, Mol. Cell. Biol. 24 (2004) 5776–5787.
[62] D.P. Batty, R.D. Wood, Damage recognition in nucleotide excision repair of DNA, Gene 241 (2000) 193–204.

[63] L. Wu, I.D. Hickson, The Bloom's syndrome helicase suppresses crossing over during homologous recombination, Nature 426 (2003) 870–874.
[64] R.G. Shao, C.X. Cao, H. Zhang, K.W. Kohn, M.S. Wold, Y. Pommier, Replication-mediated DNA damage by camptothecin induces phosphorylation of RPA by DNA-dependent protein kinase and dissociates RPA:DNA-PK complexes, EMBO J. 18 (1999) 1397–1406.
[65] A.A. Morley, S.A. Grist, D.R. Turner, A. Kutlaca, G. Bennett, Molecular nature of in vivo mutations in human cells at the autosomal HLA-A locus, Cancer Res. 50 (1990) 4584–4587.
[66] X. Zhu, J.M. Dunn, A.D. Goddard, J.A. Squire, A. Becker, R.A. Phillips, B.L. Gallie, Mechanisms of loss of heterozygosity in retinoblastoma, Cytogenet. Cell. Genet. 59 (1992) 248–252.
[67] P.K. Gupta, A. Sahota, S.A. Boyadjiev, S. Bye, C. Shao, J.P. O'Neill, T.C. Hunter, R.J. Albertini, P.J. Stambrook, J.A. Tischfield, High frequency in vivo loss of heterozygosity is primarily a consequence of mitotic recombination, Cancer Res. 57 (1997) 1188–1193.
[68] F. Prado, A. Aguilera, Role of reciprocal exchange, one-ended invasion crossover and single-strand annealing on inverted and direct repeat recombination in yeast: different requirements for the RAD1, RAD10, and RAD52 genes, Genetics 139 (1995) 109–123.
[69] J.E. Haber, Transpositions and translocations induced by site-specific double-strand breaks in budding yeast, DNA Repair (Amst.) 5 (2006) 998–1009.
[70] S.J. Gould, The exaptive excellence of spandrels as a term and prototype, Proc. Natl. Acad. Sci. U.S.A. 94 (1997) 10750–10755.
[71] M.A. Batzer, P.L. Deininger, Alu repeats and human genomic diversity, Nat. Rev. Genet. 3 (2002) 370–379.
[72] C.W. Schmid, Alu: structure, origin, evolution, significance and function of one-tenth of human DNA, Prog. Nucleic Acid Res. Mol. Biol. 53 (1996) 283–319.
[73] A.F. Smit, The origin of interspersed repeats in the human genome, Curr. Opin. Genet. Dev. 6 (1996) 743–748.
[74] B. Elliott, C. Richardson, M. Jasin, Chromosomal translocation mechanisms at intronic alu elements in mammalian cells, Mol. Cell 17 (2005) 885–894.
[75] J.E. Haber, Lucky breaks: analysis of recombination in *Saccharomyces*, Mutat. Res. 451 (2000) 53–69.
[76] E. Kraus, W.Y. Leung, J.E. Haber, Break-induced replication: a review and an example in budding yeast, Proc. Natl. Acad. Sci. U.S.A. 98 (2001) 8255–8262.
[77] C. Richardson, M. Jasin, Frequent chromosomal translocations induced by DNA double-strand breaks, Nature 405 (2000) 697–700.
[78] K.W. Caldecott, J.D. Tucker, L.H. Thompson, Construction of human XRCC1 minigenes that fully correct the CHO DNA repair mutant EM9, Nucleic Acids Res. 20 (1992) 4575–4579.
[79] Z.Q. Wang, L. Stingl, C. Morrison, M. Jantsch, M. Los, K. Schulze-Osthoff, E.F. Wagner, PARP is important for genomic stability but dispensable in apoptosis, Genes Dev. 11 (1997) 2347–2358.
[80] C.M. Simbulan-Rosenthal, B.R. Haddad, D.S. Rosenthal, Z. Weaver, A. Coleman, R. Luo, H.M. Young, Z.Q. Wang, T. Ried, M.E. Smulson, Chromosomal aberrations in PARP(−/−) mice: genome stabilization in immortalized cells by reintroduction of poly(ADP-ribose) polymerase cDNA, Proc. Natl. Acad. Sci. U.S.A. 96 (1999) 13191–13196.
[81] J.M. de Murcia, C. Niedergang, C. Trucco, M. Ricoul, B. Dutrillaux, M. Mark, F.J. Oliver, M. Masson, A. Dierich, M. LeMeur, C. Walztinger, P. Chambon, G. de Murcia, Requirement of poly(ADP-ribose) polymerase in recovery from DNA damage in mice and in cells, Proc. Natl. Acad. Sci. U.S.A. 94 (1997) 7303–7307.
[82] R.G. Sargent, M.A. Brenneman, J.H. Wilson, Repair of site-specific double-strand breaks in a mammalian chromosome by homologous and illegitimate recombination, Mol. Cell. Biol. 17 (1997) 267–277.
[83] M.M. Cox, M.F. Goodman, K.N. Kreuzer, D.J. Sherratt, S.J. Sandler, K.J. Marians, The importance of repairing stalled replication forks, Nature 404 (2000) 37–41.
[84] C. Lundin, N. Schultz, C. Arnaudeau, A. Mohindra, L.T. Hansen, T. Helleday, RAD51 is involved in repair of damage associated with DNA replication in mammalian cells, J. Mol. Biol. 328 (2003) 521–535.
[85] H.E. Bryant, N. Schultz, H.D. Thomas, K.M. Parker, D. Flower, E. Lopez, S. Kyle, M. Meuth, N.J. Curtin, T. Helleday, Specific killing of BRCA2-deficient tumours with inhibitors of poly(ADP-ribose)polymerase, Nature 434 (2005) 913–917.
[86] L.H. Thompson, K.W. Brookman, L.E. Dillehay, A.V. Carrano, J.A. Mazrimas, C.L. Mooney, J.L. Minkler, A CHO-cell strain having hypersensitivity to mutagens, a defect in DNA strand-break repair, and an extraordinary baseline frequency of sister-chromatid exchange, Mutat. Res. 95 (1982) 427–440.
[87] V. Schreiber, D. Hunting, C. Trucco, B. Gowans, D. Grunwald, G. De Murcia, J.M. De Murcia, A dominant-negative mutant of human poly(ADP-ribose) polymerase affects cell recovery, apoptosis, and sister chromatid exchange following DNA damage, Proc. Natl. Acad. Sci. U.S.A. 92 (1995) 4753–4757.
[88] N. Schultz, E. Lopez, N. Saleh-Gohari, T. Helleday, Poly(ADP-ribose) polymerase (PARP-1) has a controlling role in homologous recombination, Nucleic Acids Res. 31 (2003) 4959–4964.
[89] J.M. Stark, M. Jasin, Extensive loss of heterozygosity is suppressed during homologous repair of chromosomal breaks, Mol. Cell. Biol. 23 (2003) 733–743.
[90] K. Rothkamm, I. Kruger, L.H. Thompson, M. Lobrich, Pathways of DNA double-strand break repair during the mammalian cell cycle, Mol. Cell. Biol. 23 (2003) 5706–5715.
[91] G. Ira, A. Pellicioli, A. Balijja, X. Wang, S. Fiorani, W. Carotenuto, G. Liberi, D. Bressan, L. Wan, N.M. Hollingsworth, J.E. Haber, M. Foiani, DNA end resection, homologous recombination and DNA damage checkpoint activation require CDK1, Nature 431 (2004) 1011–1017.
[92] Y. Aylon, B. Liefshitz, M. Kupiec, The CDK regulates repair of double-strand breaks by homologous recombination during the cell cycle, EMBO J. 23 (2004) 4868–4875.
[93] J.S. Kim, T.B. Krasieva, H. Kurumizaka, D.J. Chen, A.M. Taylor, K. Yokomori, Independent and sequential recruitment of NHEJ and HR factors to DNA damage sites in mammalian cells, J. Cell. Biol. 170 (2005) 341–347.
[94] D. Ristic, M. Modesti, R. Kanaar, C. Wyman, Rad52 and Ku bind to different DNA structures produced early in double-strand break repair, Nucleic Acids Res. 31 (2003) 5229–5237.
[95] J.M. Larner, H. Lee, J.L. Hamlin, Radiation effects on DNA synthesis in a defined chromosomal replicon, Mol. Cell. Biol. 14 (1994) 1901–1908.
[96] J. Falck, N. Mailand, R.G. Syljuasen, J. Bartek, J. Lukas, The ATM-Chk2-Cdc25A checkpoint pathway guards against radioresistant DNA synthesis, Nature 410 (2001) 842–847.
[97] T. Uziel, Y. Lerenthal, L. Moyal, Y. Andegeko, L. Mittelman, Y. Shiloh, Requirement of the MRN complex for ATM activation by DNA damage, EMBO J. 22 (2003) 5612–5621.
[98] G.P. Holmquist, Endogenous lesions, S-phase-independent spontaneous mutations, and evolutionary strategies for base excision repair, Mutat. Res. 400 (1998) 59–68.
[99] W.F. Morgan, P.E. Crossen, The incidence of sister chromatid exchanges in cultured human lymphocytes, Mutat. Res. 42 (1977) 305–311.
[100] D.M. Wiktor-Brown, C.A. Hendricks, W. Olipitz, B.P. Engelward, Age-dependent accumulation of recombinant

cells in the mouse pancreas revealed by in situ fluorescence imaging, Proc. Natl. Acad. Sci. U.S.A. 103 (2006) 11862–11867.
[101] E. Petermann, K.W. Caldecott, Evidence that the ATR/Chk1 pathway maintains normal replication fork progression during unperturbed S phase, Cell Cycle 5 (2006) 2203–2209.
[102] E. Petermann, A. Maya-Mendoza, G. Zachos, D.A. Gillespie, D.A. Jackson, K.W. Caldecott, Chk1 requirement for high global rates of replication fork progression during normal vertebrate S phase, Mol. Cell. Biol. 26 (2006) 3319–3326.
[103] R.G. Syljuåsen, C.S. Sørensen, L.T. Hansen, K. Fugger, C. Lundin, F. Johansson, T. Helleday, M. Sehested, J. Lukas, J. Bartek, Inhibition of human Chk1 causes increased initiation of DNA replication, phosphorylation of ATR targets, and DNA breakage, Mol. Cell. Biol. 25 (2005) 3553–3562.
[104] V. Costanzo, K. Robertson, M. Bibikova, E. Kim, D. Grieco, M. Gottesman, D. Carroll, J. Gautier, Mre11 protein complex prevents double-strand break accumulation during chromosomal DNA replication, Mol. Cell 8 (2001) 137–147.
[105] D. Shechter, V. Costanzo, J. Gautier, ATR and ATM regulate the timing of DNA replication origin firing, Nat. Cell. Biol. 6 (2004) 648–655.
[106] C.J. Merrick, D. Jackson, J.F. Diffley, Visualization of altered replication dynamics after DNA damage in human cells, J. Biol. Chem. 279 (2004) 20067–20075.
[107] B.P. Engelward, J.M. Allan, A.J. Dreslin, J.D. Kelly, M.M. Wu, B. Gold, L.D. Samson, A chemical and genetic approach together define the biological consequences of 3-methyladenine lesions in the mammalian genome, J. Biol. Chem. 273 (1998) 5412–5418.
[108] K. Larson, J. Sahm, R. Shenkar, B. Strauss, Methylation-induced blocks to in vitro DNA replication, Mutat. Res. 150 (1985) 77–84.
[109] Y. Zhang, J.D. Rowley, Chromatin structural elements and chromosomal translocations in leukemia, DNA Repair (Amst.) 5 (2006) 1282–1297.
[110] G.C. Li, H. Ouyang, X. Li, H. Nagasawa, J.B. Little, D.J. Chen, C.C. Ling, Z. Fuks, C. Cordon-Cardo, Ku70: a candidate tumor suppressor gene for murine T cell lymphoma, Mol. Cell 2 (1998) 1–8.
[111] D.C. van Gent, J.H. Hoeijmakers, R. Kanaar, Chromosomal stability and the DNA double-stranded break connection, Nat. Rev. Genet. 2 (2001) 196–206.
[112] N.E. Sharpless, D.O. Ferguson, R.C. O'Hagan, D.H. Castrillon, C. Lee, P.A. Farazi, S. Alson, J. Fleming, C.C. Morton, K. Frank, L. Chin, F.W. Alt, R.A. DePinho, Impaired non-homologous end joining provokes soft tissue sarcomas harboring chromosomal translocations, amplifications, and deletions, Mol. Cell 8 (2001) 1187–1196.
[113] D. Moshous, C. Pannetier, R. Chasseval Rd, F. Deist Fl, M. Cavazzana-Calvo, S. Romana, E. Macintyre, D. Canioni, N. Brousse, A. Fischer, J.L. Casanova, J.P. Villartay, Partial T and B lymphocyte immunodeficiency and predisposition to lymphoma in patients with hypomorphic mutations in Artemis, J. Clin. Invest. 111 (2003) 381–387.
[114] L.H. Thompson, D. Schild, Recombinational DNA repair and human disease, Mutat. Res. 509 (2002) 49–78.
[115] D. Gebow, N. Miselis, H.L. Liber, Homologous and non-homologous recombination resulting in deletion: effects of p53 status, microhomology, and repetitive DNA length and orientation, Mol. Cell. Biol. 20 (2000) 4028–4035.
[116] M.E. Moynahan, J.W. Chiu, B.H. Koller, M. Jasin, Brca1 controls homology-directed DNA repair, Mol. Cell 4 (1999) 511–518.
[117] E. Bolderson, J. Scorah, T. Helleday, C. Smythe, M. Meuth, ATM is required for the cellular response to thymidine induced replication fork stress, Hum. Mol. Genet. 31 (2004) 2937–2945.
[118] A.D. D'Andrea, M. Grompe, The Fanconi anaemia/BRCA pathway, Nat. Rev. Cancer 3 (2003) 23–34.
[119] A.A. Davies, J.Y. Masson, M.J. Mcllwraith, A.Z. Stasiak, A. Stasiak, A.R. Venkitaraman, S.C. West, Role of BRCA2 in control of the RAD51 recombination and DNA repair protein, Mol. Cell 7 (2001) 273–282.
[120] K.J. Patel, V.P. Yu, H. Lee, A. Corcoran, F.C. Thistlethwaite, M.J. Evans, W.H. Colledge, L.S. Friedman, B.A. Ponder, A.R. Venkitaraman, Involvement of Brca2 in DNA repair, Mol. Cell 1 (1998) 347–357.
[121] R.S. Chaganti, S. Schonberg, J. German, A manyfold increase in sister chromatid exchanges in Bloom's syndrome lymphocytes, Proc. Natl. Acad. Sci. U.S.A. 71 (1974) 4508–4512.
[122] G. Luo, I.M. Santoro, L.D. McDaniel, I. Nishijima, M. Mills, H. Youssoufian, H. Vogel, R.A. Schultz, A. Bradley, Cancer predisposition caused by elevated mitotic recombination in Bloom mice, Nat. Genet. 26 (2000) 424–429.
[123] Y. Saintigny, K. Makienko, C. Swanson, M.J. Emond, R.J. Monnat Jr., Homologous recombination resolution defect in werner syndrome, Mol. Cell. Biol. 22 (2002) 6971–6978.
[124] M. Lebel, Increased frequency of DNA deletions in pink-eyed unstable mice carrying a mutation in the Werner syndrome gene homologue, Carcinogenesis 23 (2002) 213–216.
[125] N. Saleh-Gohari, T. Helleday, Strand invasion involving short tract gene conversion is specifically suppressed in BRCA2-deficient hamster cells, Oncogene 23 (2004) 9136–9141.
[126] M. Tarsounas, D. Davies, S.C. West, BRCA2-dependent and independent formation of RAD51 nuclear foci, Oncogene 22 (2003) 1115–1123.
[127] N. Bardeesy, N.E. Sharpless, R.A. DePinho, G. Merlino, The genetics of pancreatic adenocarcinoma: a roadmap for a mouse model, Semin. Cancer Biol. 11 (2001) 201–218.
[128] M.E. Moynahan, A.J. Pierce, M. Jasin, BRCA2 is required for homology-directed repair of chromosomal breaks, Mol. Cell 7 (2001) 263–272.
[129] W.J. Overkamp, M.A. Rooimans, I. Neuteboom, P. Telleman, F. Arwert, M.Z. Zdzienicka, Genetic diversity of mitomycin C-hypersensitive Chinese hamster cell mutants: a new complementation group with chromosomal instability, Somat. Cell. Mol. Genet. 19 (1993) 431–437.
[130] M. Kraakman-van der Zwet, W.J. Overkamp, R.E. van Lange, J. Essers, A. van Duijn-Goedhart, I. Wiggers, S. Swaminathan, P.P. van Buul, A. Errami, R.T. Tan, N.G. Jaspers, S.K. Sharan, R. Kanaar, M.Z. Zdzienicka, Brca2 (XRCC11) deficiency results in radioresistant DNA synthesis and a higher frequency of spontaneous deletions, Mol. Cell. Biol. 22 (2002) 669–679.
[131] H. Farmer, N. McCabe, C.J. Lord, A.N. Tutt, D.A. Johnson, T.B. Richardson, M. Santarosa, K.J. Dillon, I. Hickson, C. Knights, N.M. Martin, S.P. Jackson, G.C. Smith, A. Ashworth, Targeting the DNA repair defect in BRCA mutant cells as a therapeutic strategy, Nature 434 (2005) 917–921.
[132] V.N. Ngo, R.E. Davis, L. Lamy, X. Yu, H. Zhao, G. Lenz, L.T. Lam, S. Dave, L. Yang, J. Powell, L.M. Staudt, A loss-of-function RNA interference screen for molecular targets in cancer, Nature 441 (2006) 106–110.