Chapter 20

Double-Strand Break Repair: Homologous Recombination in Mammalian Cells

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Chapter Outline

1. INTRODUCTION

DNA double–strand breaks (DSBs) are one of the most injurious lesions that can generate genomic rearrangements and challenge cell fate. DSBs are produced through exposure to exogenous treatments (such as ionizing radiation), the byproducts of endogenous cellular metabolism and arrested replication forks. DSB repair is essential for the maintenance of DNA integrity, but can also trigger profound genomic rearrangements. Conversely, DSBs can also generate genetic diversity in essential biological processes, such as meiosis and the establishment of the immune repertoire (discussed in Refs. [1,2]). Therefore, DSB repair must be tightly controlled.

Two major strategies are used to repair DSBs: homologous recombination (HR), which requires an intact homologous sequence and nonhomologous end joining (NHEJ), which joins the DNA double–stranded ends (DSEs) without requiring any extended homologous sequence [3], and is a prominent process in mammalian cells. The canonical NHEJ (C-NHEJ) pathway is Ku70/Ku80- and XRCC4-DNA ligase 4-dependent. During early 2000s, an additional highly mutagenic alternative end-joining pathway(s) (A-EJ) that is Ku70/Ku80-and XRCC4-DNA ligase 4-independent was described (for review, see Refs. [1,4]).

Here, we focus on HR in mammalian cells. HR is evolutionarily conserved in all organisms. The main roles of HR are the protection and reactivation of replication forks that have been blocked (reviewed in Refs. [2,5]), the gap filling of single-stranded DNA (ssDNA) and the repair of DSBs [3]. Therefore, HR also plays essential and pivotal roles in genome stability, diversity and plasticity.

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2. THE ROLE OF HR IN THE EQUILIBRIUM OF GENETIC STABILITY VERSUS DIVERSITY

The products of HR are gene conversions (GC, nonreciprocal exchange of genetic material) associated with or without crossing over (CO, reciprocal exchange of the adjacent sequences), which allows HR to generate new combinations of genetic material or eliminate mutations (Fig. 20.1A). This function combined with the ability to repair DNA breakage places HR at the heart of the equilibrium controlling the balance between genetic stability and variability. Therefore, HR is implicated in many fundamental biological processes (see Fig. 20.1B). Indeed, due to its versatility, HR is involved in essential biological processes ranging from molecular evolution to DNA repair and meiotic differentiation and is also relevant to the application of targeted gene replacement (Fig. 20.1B).

Other examples of the diverse roles of HR in genome plasticity are as follows (Fig. 20.1B):

- HR is a driving force for the evolution of multigene families. In some families of repeated genes, the duplicated genes co-evolved via a phenomenon called concerted evolution [6,7].
- During meiosis, HR favors allelic recombination between two homologous chromosomes. Because the two homologous chromosomes are not fully identical, this process ensures allele mixing and creates genetic diversity (for review, see Ref. [8]).
- HR participates in neurogenesis during embryonic and postnatal neural development [9]. Heterozygous mutations in *RAD51*, which is the central HR component, have been found in individuals with congenital mirror movements (CMM) [10].

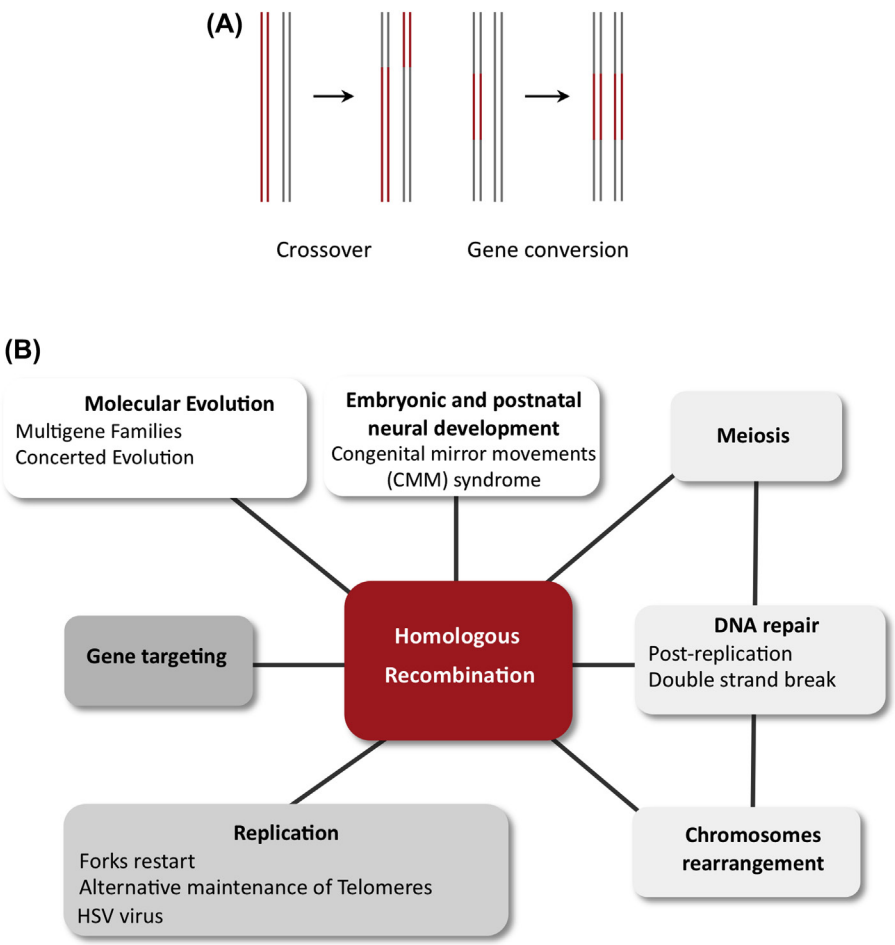

FIGURE 20.1 The outcomes and roles of HR in mammalian cells. (A) The products of HR. Crossover (left): Reciprocal exchange of the adjacent sequences. Gene conversion (right): Nonreciprocal transfer of genetic material.(B) The involvement of HR in many biological processes. HR participates in numerous fundamental processes controlled by the equilibrium between the stability and instability/diversity of the genome.

3. MOLECULAR MECHANISMS AND REGULATION OF HR

HR refers to different molecular mechanisms (Fig. 20.2). DSB repair by HR acts through several successive steps that need to be precisely coordinated to secure genome integrity. All of the different HR processes are initiated by a 5′–3′-single-strand break.

3.1 DSB Sensing and Chromatin Remodeling

The initial sensing of HR is mediated by the MRN complex (MRE11/RAD50/NBS1) in cooperation with the ATM (ataxia telangiectasia–mutated) kinase, which transduces the DNA-damage response (DDR). Inaccessible areas of the chromatin cannot be supported by the DDR. Thus, immediate changes in the DNA structure that result from the detection of the affected region are needed [11,12]. Indeed, the state of the chromatin changes after the recognition of DSBs, particularly through the phosphorylation of histone variant H2A.X in the vicinity of the lesion [13]. This change facilitates the accumulation of repair proteins at the damaged areas. The MDC1 mediator is recruited to the damage after its phosphorylation by ATM. MDC1 stabilizes the MRN complex, and its accumulation leads to remodeling of the chromatin by the ubiquitin ligases RNF8 and RNF168 (for review, see Ref. [2]).

3.2 Initiation of DNA Resection

After chromatin decondensation, the 53BP1 and Rap80–BRCA1 complex is recruited to the DSB. BRCA1 ("breast cancer type 1 susceptibility protein") is an HR mediator, and multiple roles of BRCA1 have been described in HR and DDR [14,15]. HR is initiated by the resection of the 5′-end toward the 3′-end to obtain ssDNA with a 3′-extension. This step is performed by nucleases and DNA helicases. One essential role of BRCA1 (in association with CtIP) during HR initiation is the removal of 53BP1 from the DNA ends, thereby making them accessible for resection initiation [16,17].

The resection occurs during two substeps and is modulated by the MNR complex. BRCA1 has a BRCT domain that enables interactions with phosphorylated proteins. BRCA1 forms a heterodimer with its BARD1 cofactor ("BRCA1-associated ring domain 1"), which possesses a RING domain [18] and gives the BRCA1/BARD1 complex E3 ubiquitin ligase activity. Thus, this complex allows the ubiquitination of the CtIP nuclease (exonuclease activity in the 3′- to 5′-direction) that cooperates with MRN to initiate the resection of the DSB [19–21]. Studies in 2015 showed that the MCM8–9 helicase complex was essential for DNA resection by the MRN complex at DSBs and was required for the proper localization of the MRN complex to the DSBs [22]. Then, the exonuclease 1 (Exo1) and/or the BLM/DNA2 complex ensures the elongation of the 3′-strand to generate a long 3′-overhang [23] (Fig. 20.2A); BLM is a member of the Rec Q helicase family and is mutated in Bloom syndrome [24]. Finally, the ssDNA is protected and stabilized by RPA.

3.3 Loading of RAD51 and Strand Exchange

BRCA2 ("breast cancer type 2 susceptibility protein") in association with Palb2 (which is also mutated in breast cancer familial cases) replaces RPA by RAD51, creating a presynaptic filament. RPA contributes to the polarity of the process. The RAD51/ssDNA filament promotes homologous pairing, through microhomologies scanning [24a] and strand invasion of a homologous duplex sequence, initiating then copy of the homologous matrix, and generating cruciform intermediates called Holliday junctions (HJs) (Fig. 20.2A). Rad54, which is a protein from the SWI2/SNF2 family, interacts with Rad51, thereby facilitating strand invasion [25]. Rad54 catalyzes the migration of the branches that takes place between the two strands.

The invading 3′-ssDNA allows the priming of DNA synthesis. This priming displaces the complementary strand creating a D-loop (displacing loop), which is then captured by the other broken DNA end. RAD54 also stabilizes this HR intermediate. This intermediate generates the cruciform HJs that are resolved by nucleases or dissolved, leading to the HR outcomes (Fig. 20.2A).

A family of six proteins (RAD51B, RAD51C, RAD51D, XRCC2, XRCC3, and RAD51AP1) known as the RAD51 paralogs (ie, proteins that share sequence homology with RAD51) has been identified. Genes encoding these paralogs may have derived from RAD51 gene duplication, and share at least 20% identity at the amino acid level with RAD51 and each other [26]. Two distinct complexes have been identified: RAD51B–RAD51C–RAD51D–XRCC2 (BCDX2) and RAD51C–XRCC3 (CX3) [28]. The early role of the RAD51 paralogs in HR is to promote the formation and stabilization of the RAD51 nucleoprotein filament, most likely by counteracting the disruption of the filament by the helicases. A 2004 work showed that the BCDX2 complex (but not the CX3 complex) was responsible

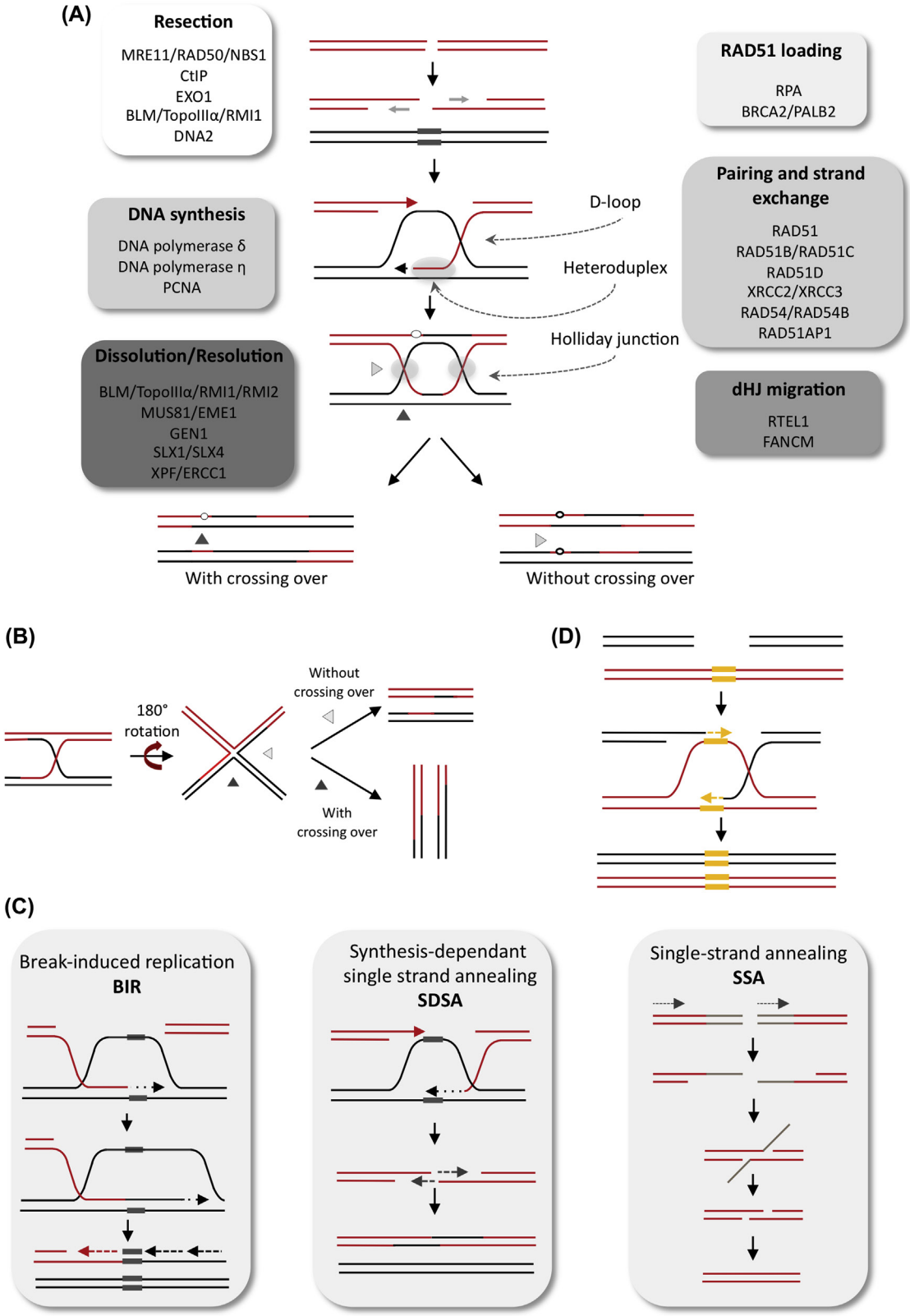

FIGURE 20.2 **The different HR models.** (A) The DSB repair model [100]. DSB resection (*gray arrows*) generates ssDNA tails that invade an intact homologous duplex DNA and initiate DNA synthesis (*black arrow*). This strand invasion displaces the complementary strand and creates a D-loop that anneals to the complementary strand of the recipient molecule. DNA synthesis fills in the gaps, and the processes results in two cruciform junctions (Holliday junctions) that can be resolved/dissolved or migrate. The final outcome is gene conversion associated with (*black arrow*) or without (*gray arrow*) crossing over. Mismatch forming heteroduplexe (*white circles*). The proteins involved in these steps are noted on both sides of the schematic. (B) Resolution of the Holliday junctions. The products of HR issue from a 180° rotation of the HJ followed by its resolution that leads to a crossing over (*black arrow*) or a non-crossing over (*white arrow*) event. (C) Other HR models. In the absence of resolution/dissolution of the dHJ, synthesis can be prolonged up to the end of the chromosome (left panel; BIR: break-induced replication). Alternatively, the invading strand can flip back to its parental molecule (middle panel; SDSA: synthesis-dependent single-strand annealing). Finally, another process results in the annealing of the complementary strand revealed by resection (right panel; SSA: single-strand annealing). SSA does not act through strand invasion of the duplex DNA, does not generate a dHJ, and is a nonconservative process because it leads to the deletion of the intervening sequence. (D) Gap filling. The broken molecule (black) invades an intact homologous molecule (red) containing a heterologous sequence (yellow). DNA synthesis initiated by HR copies the heterologous sequence (yellow) and transfers it to the acceptor molecule (black).

for RAD51 recruitment to DNA-damage sites in human cells. After RAD51-mediated strand invasion, the RAD51 paralogs influence GC tract length. Moreover, the RAD51 paralogs can bind to Y-shaped replication-like intermediates and synthetic HJ suggesting a role for the RAD51 paralogs in DNA repair during replication and the resolution of HR intermediary structures [29].

3.4 Resolution of the HJ and HR Outcomes

The nucleases GEN1 ("gen endonuclease homolog 1"), the heterodimer Mus81/Eme1 ("essential meiotic endonuclease 1"), and SLX1 and SLX4 [30–32] are among the factors that resolve HJ through cleavage. Topoisomerase III (TopoIII) and BLM resolve the double HJ (dHJ) substrate via the convergent migration of the two dHJs toward one another, leading to their collapse. TopoIII alpha recruits Rmi1 to catalyze dHJ dissolution. Finally, Rmi2 (an essential member of the "dissolvasome" complex) stimulates dHJ resolution [33] (Fig. 20.2A).

According to the orientation of the HJ resolution, the process may result in an exchange of adjacent sequences (Fig. 20.2B). Therefore, the products of HR are GCs with or without CO (depending on the resolution of the intermediate structure). However, the absence of HJ resolution will lead to break-induced replication (BIR) or synthesis-dependent strand annealing (SDSA) [3] (Fig. 20.2C).

Pairing and strand exchange of homologous DNA strands tolerate some differences between the molecules involved, thereby allowing the creation of a hybrid double-stranded DNA molecule called a heteroduplex that carries mismatches (Fig. 20.2A). Mismatch repair will result in the nonreciprocal transfer of genetic information from one DNA molecule to the other (ie, GC). In addition, the 3′-end of the invading strand allows DNA synthesis by copying the recipient molecule. In this process, a sequence absent from the invading strand can be copied, leading to the nonreciprocal transfer of genetic information from the invaded molecule to the invading molecule and therefore GC (Fig. 20.2D). Of note, all models are initiated by common steps beginning with the resection of the ssDNA, followed by the invasion and exchange of a homologous DNA strand that is a pivotal step in HR. These models are considered to be prominent for mitotic and meiotic recombination without crossover. Furthermore, BIR seems to be the mechanism underlying telomere maintenance by the ALT system in the absence of telomerase [34].

The last model (single-strand annealing or SSA) can occur between two sequences in tandem and is initialized by a single-strand resection. However, in contrast with the models described previously, this step is not followed by invasion of the DNA duplex and strand exchange. In fact, when the two sequences are in direct orientation, the ssDNA sequences revealed are complementary and can hybridize to form a branched structure. The following concerns should be noted in relation to the SSA model: it is a nonconservative process leading inevitably to a deletion of the intervening sequence; it cannot occur between inverted repeat sequences because the strands revealed by the resection are not complementary but identical; and finally, SSA can generate translocations if two breaks occur simultaneously in ectopic homologous sequences [35].

4. ROLES OF HR IN REPLICATION FORK REACTIVATION AND DSB REPAIR

4.1 Fork Stability/Restart by HR Upon Replication Stress

Replication fork progression is routinely challenged by diverse exogenous or endogenous stresses that ultimately lead to replication fork stalling, collapse, or breakage and trigger the DDR [5,36–39].

A crucial role for HR in genome stability maintenance is to escort replication fork progression. Indeed, HR is involved in the recovery of arrested replication forks (Fig. 20.3) [2,5,38,40].

Because the newly synthesized DNA strands produced by replication are complementary, reversion of the blocked fork can take place (Fig. 20.3) [41]. Notably, RAD51 participates in this replication fork reversion process [42].

The resumption of replication forks can be initiated by the loading of HR factors onto the single-strand DNA present at the stalled fork. Several different restart pathways have been proposed: (1) fork restart after repriming (ie, the loading of the replisome after a lesion) (Fig. 20.3A1); (2) restart after a fork reversion, when the newly synthesized DNA strand is homologous to the parental DNA downstream and creates a "chicken foot" structure (Fig. 20.3A2); and (3) restart using the ssDNA formed after fork regression (Fig. 20.3C) in a process analogous to BIR (Fig. 20.3C).

In some cases, single-ended DSBs are formed by either the passage of replication forks through a nick or an ssDNA gap (Fig. 20.3B) or the cleavage of the reversed forks by structure-specific endonucleases, such as MUS81 (Fig. 20.3A2). Then, HR can use the sister chromatid to prime DNA synthesis, thereby allowing the resumption of replication (Fig. 20.3).

In addition BRCA2 and RAD51 can protect the DNA ends of an arrested replication fork from resection by MRE, without leading to a recombination outcome [42a,42b,42c].

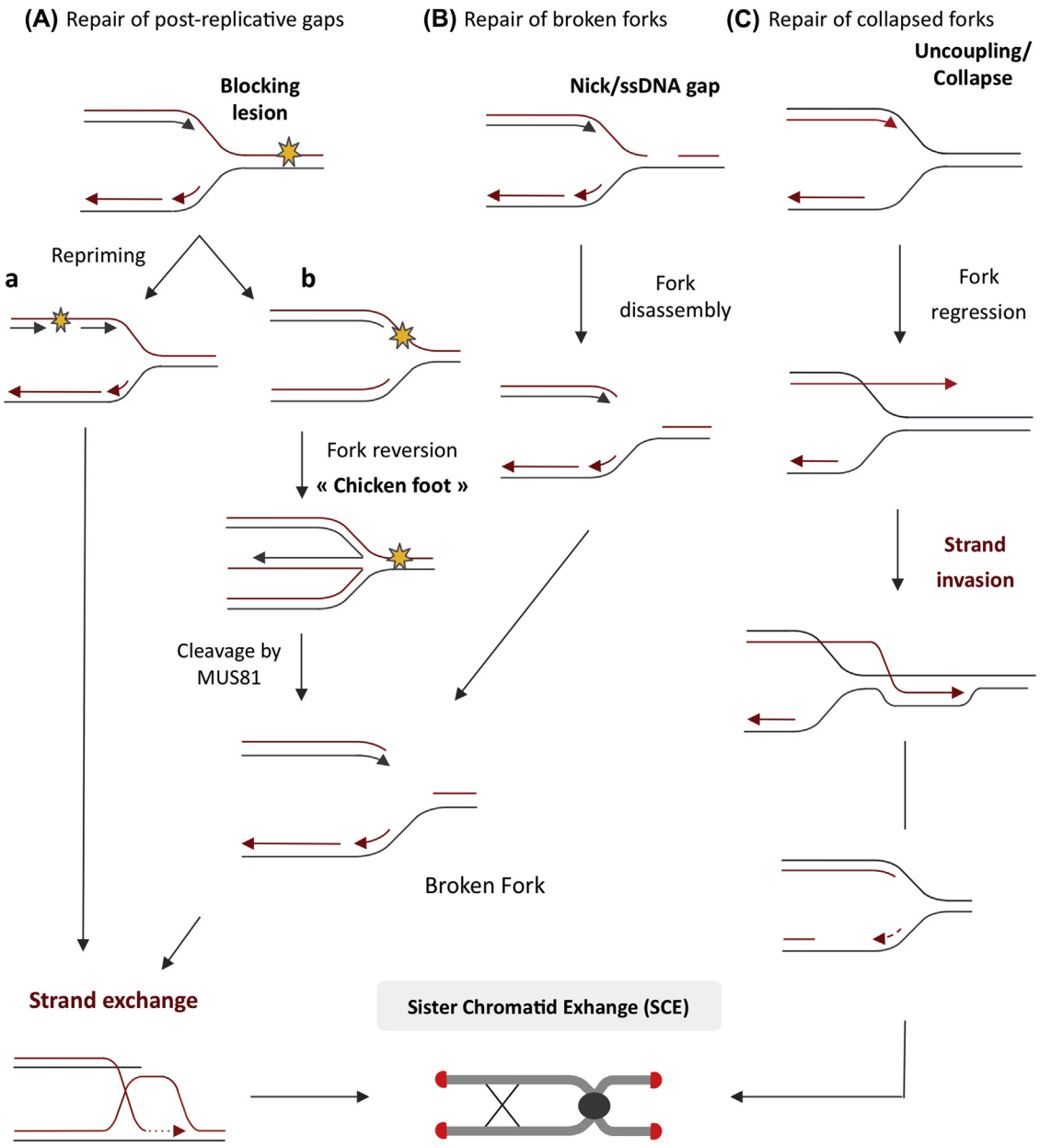

FIGURE 20.3 The role of HR in the reactivation of arrested replication forks. (A) Repair of post-replicative gaps. The replication fork (*arrows*) reaches a blocking DNA lesion (*yellow star*). DNA synthesis is primed downstream of the lesion to produce a single-strand gap bearing the lesion. This gap is filled in via sister chromatid exchange (SCE) by a copy of the intact sister chromatid. Reversion of the blocked fork leads to a "chicken foot" structure. Cleavage of this cruciform structure generates a DSB with only one end. SCE allows replication to resume. (B) Repair of broken forks. One replication fork reaching a ssDNA gap or nick is converted into a DSB. SCE can resume replication. (C) Repair of a collapsed fork. If a replication fork collapses, an uncoupling between the leading and lagging strand can occur generating a ssDNA strand that can invade the duplex matrix and restart DNA synthesis.

Because HR plays a pivotal role in the resumption of arrested replication forks, defects in HR lead to spontaneous slowed replication fork progression [43,44]. Replication defects in *HR*$^{-}$ cell lead to mitosis and chromosome defects, including anaphase bridges, common fragile sites, and supernumerary centrosomes, which result in multipolar mitosis and aneuploidy [44–50].

Thus, HR is an essential mechanism for the protection, recovery, and restart of replication forks. Consistent with the role in replication fork reactivation, HR-deficient cells are highly sensitive to agents that block the progression of replication forks, such as cisplatin or mitomycin C that generate interstrand cross-links in the DNA [51].

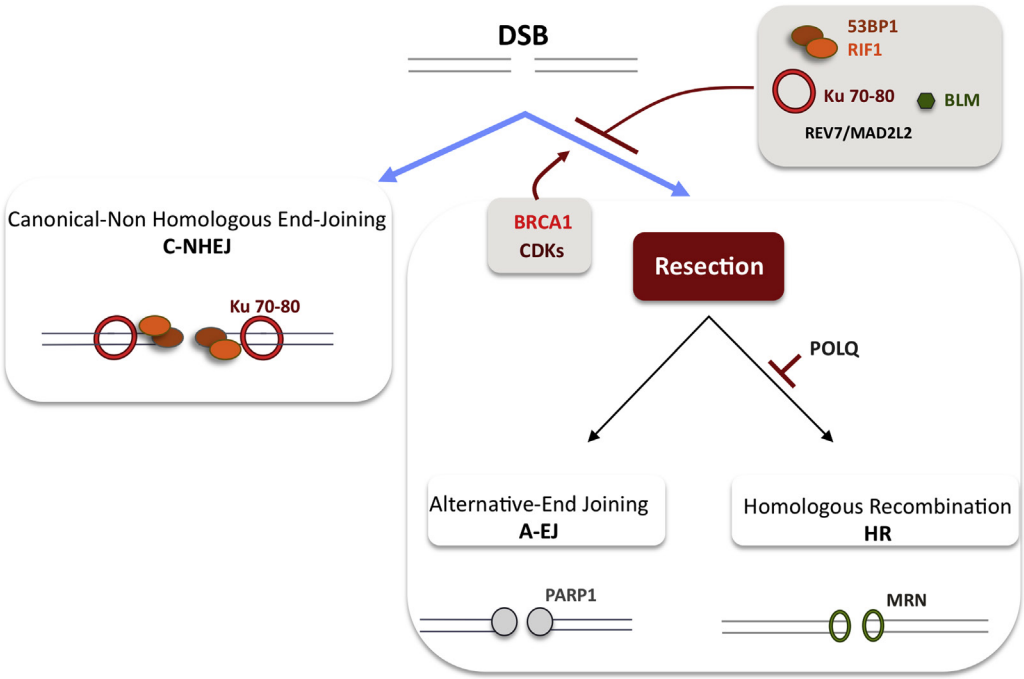

FIGURE 20.4 **The two-step model for the choice of the repair pathway.** The first alternative is the choice between C-NHEJ versus a resection producing ssDNA. The second alternative is competition between alternative end joining versus HR on the resected DNA. Some essential components of these processes are noted.

4.2 Competition for the DNA DSB–Repair Pathway Choice and Consequences for Meiosis and Genome Manipulation

4.2.1 Competition Between HR and End Joining for DSB Repair: A Two-Step Model

Several competing processes can repair DSBs: C-NHEJ, which is not initiated by DNA end resection, and HR and A-EJ, which are both initiated by resection involving common components, such as MRN and CtIP. Therefore, we propose a model in two steps [1,4,20] for the choice of the DSB-repair process (Fig. 20.4). First, competition occurs between C-NHEJ and resection. NHEJ is active throughout the cell cycle and resection is favored at S-phase entry, although A-EJ is also active throughout the cell cycle [52,53]. Second, when resection is initiated, competition between HR and A-EJ takes place. This competition can be modulated by the cell-cycle phase and the extent of the resection.

4.2.2 Meiosis

Defects in HR lead to sterility linked with meiotic division issues. The role of the meiotic program is to generate gametes with half of the chromosome content of the original progenitor cell. This task is accomplished by the occurrence of a single round of DNA replication, followed by two successive rounds of chromosome segregation. During meiosis, which aims to generate genetic diversity, sister-chromatid exchanges (SCEs) are repressed and HR between homologous chromosomes (which are not fully identical) is favored. HR plays a double role during meiosis division. The first is to assure the balance of the segregation of homologous chromosomes, and the second is to ensure the mixture of alleles to create genetic diversity. During reductional division, the cell must segregate the two homologous chromosomes into the two different daughter cells. However, these chromosomes do not have a physical link that would distinguish them (in mitosis, the chromosomes are linked by centromeres). This physical link is assured in meiosis by HR, which generates HJs; additionally, the generation of crossovers allows the rearrangement of alleles, thereby ensuring genetic diversity. Meiotic recombination is initiated by a DSB generated by the enzyme SPO11; the repair of this break essentially utilizes the same systems used for the repair of breaks induced by ionizing radiation (for review, see Refs. [8,54]).

4.2.3 Genome Manipulation

Due to its requirement for sequence homology, HR can be used for gene targeting (GT)—that is, the targeted modification (correction or insertional alteration) of a nuclear sequence by an exogenous sequence (Fig. 20.5). Drs. M. Cappecchi and

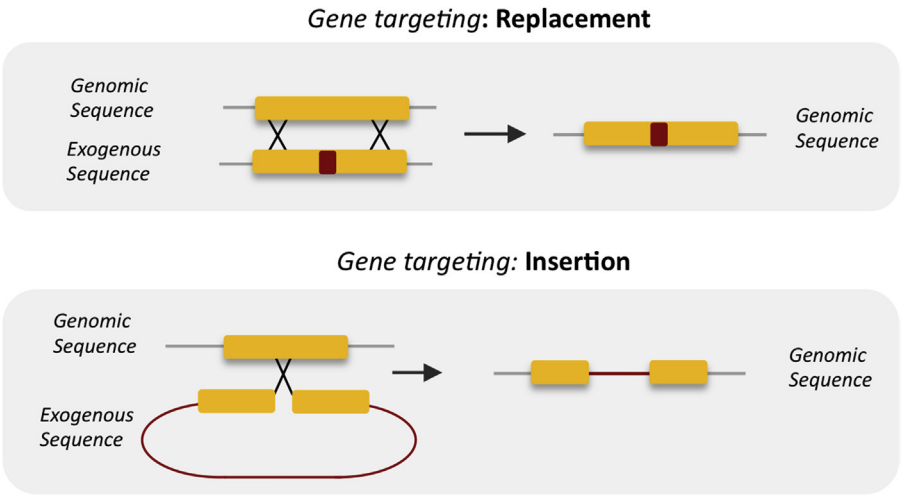

FIGURE 20.5 **Basic gene-targeting strategies via HR.** Upper panel: Replacement vector. Lower panel: Insertion vector. Yellow: Homologous sequences. Red: Modified sequence.

O. Smithies, who developed GT in mammalian cells, were awarded the Noble Prize in 2007. GT represents a promising strategy for gene therapy and the development of new biological models of interest for both academic and applied medical, biotechnological, and agronomic research. Importantly, GT allows the correction of a mutated nuclear gene, leading to the restoration of normal gene functions and thus targeted in situ gene therapy.

However, the efficiency of HR remains disappointing resulting in low efficiency of GT compared to the random integration of the correcting DNA. Because HR can repair DSBs, one promising strategy to increase the frequency of GT is to generate a DSB in the target sequence. The development of engineered sequence-specific nucleases (ie, a zinc finger, TALE, or sgRNA-Cas9 nuclease) allows the generation of the required DSBs in a given loci of the mammalian genome, thereby stimulating HR by several orders of magnitude [55]. Several studies have reported the feasibility of this ex vivo approach in human stem cells and primary cells [56] and in the liver of a hemophilia B mouse model [57], providing a proof of concept for the treatment of monogenic disease by genome editing with engineered nucleases.

5. THE DARK SIDE OF HR: PROMOTION OF GENOME INSTABILITY

HR contributes to the maintenance of genome stability/diversity through the combination of its different products and its ability to repair DNA. Because it copies an intact homologous DNA, HR is frequently classified as an error-free DNA-repair process. Indeed, HR-deficient cells exhibit increased genetic instability [58]. However, careful examination of the data can challenge this strict view (for review, see Ref. [2]):

1. CO between ectopic homologous sequences (nonallelic HR, NAHR) generates profound genome rearrangements leading to genetic instability (Fig. 20.6B). Moreover, BIR can also induce genome instability in mammalian cells. Indeed, it was reported in 2014 that replication stress induced by the overexpression of cyclin E in human cells leads to copy number alterations (CNAs). One-third of these genome alterations (duplications of less than 200 kb) have been attributed to BIR events. The authors propose that BIR repair of damaged replication forks may explain the presence of segmental genomic duplications in human cancers. The larger amplification (>200 kb) and deletion observed after the overexpression of cyclin E may arise from nonallelic HR [59].
2. GC with pseudo-genes can result in the extinction of the functional allele (Fig. 20.6A).
3. The accumulation of HR intermediates is toxic and can generate genetic instability [60].
4. The DNA synthesis initiated by HR is error prone, at least in yeast [60a].

6. PROTECTION AGAINST EXCESSIVE HR

HR plays an essential role in genome stability maintenance but can also jeopardize it (see earlier). Particularly, excess HR initiation can lead to the accumulation of HR intermediates, thereby generating genomic instability and cell death [60]. Thus, HR is a double-edged sword; on the one hand, it protects against genetic instability, but on the other hand, it can trigger cell lethality, profound genomic rearrangements, and point mutations. Therefore, HR should be tightly controlled to

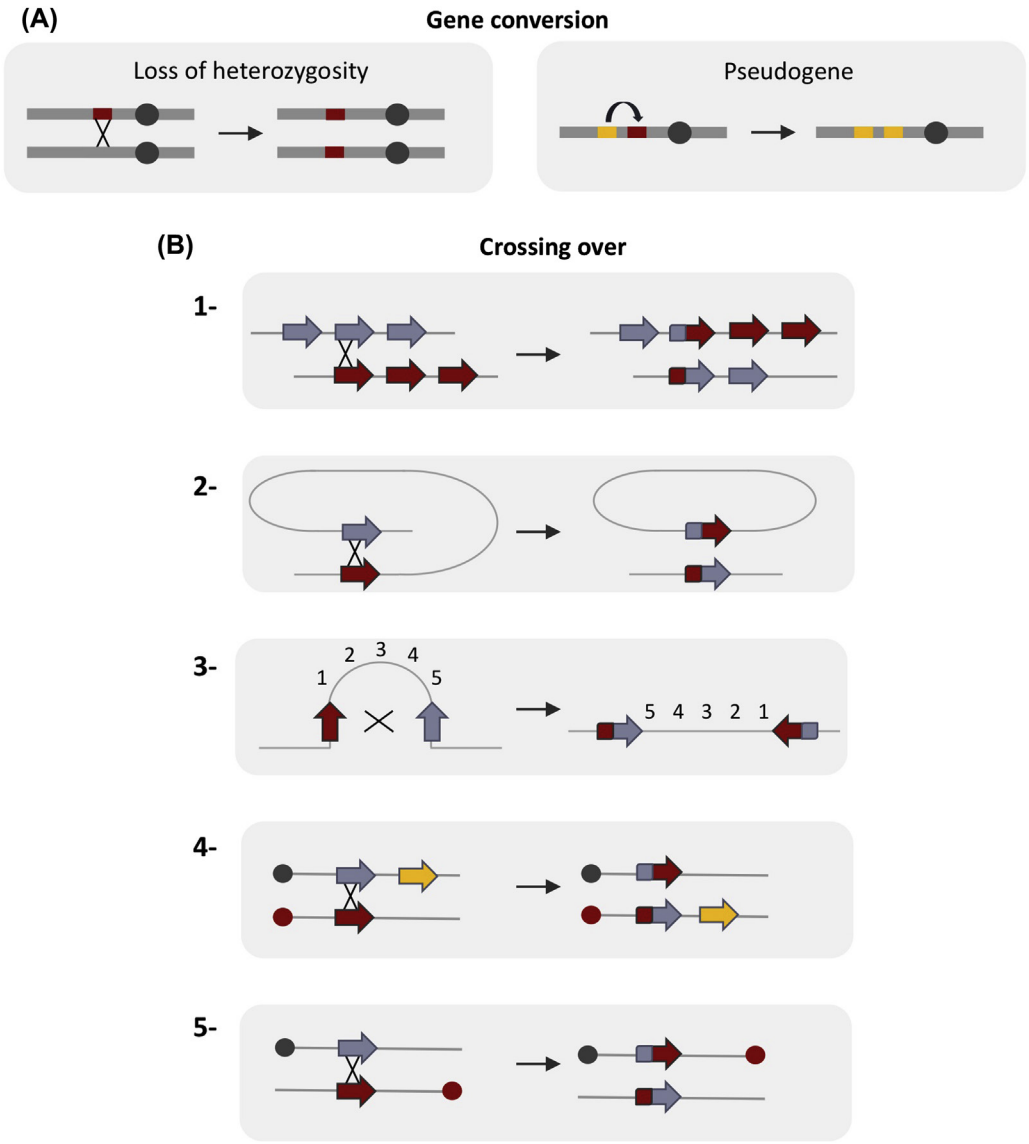

FIGURE 20.6 **Genetic instability induced by HR.** (A) By gene conversion. Nonreciprocal exchange of genetic information between two heteroalleles leads to a loss of heterozygosity (left panel). Gene conversion between a pseudogene (yellow), which often contains nonsense mutations, and a gene (red) transfers the stop codon, thereby inactivating gene expression (right panel). (B) By crossing over. Chromosomal rearrangements resulting from crossing over (CO) between repeat sequences. (1) Between repeat homologous sequences on two chromosomes or following unequal sister chromatid exchange on the same chromosome, resulting in the amplification of one molecule and the deletion of the other. (2) Intramolecular CO between two homologous sequences in a direct orientation, resulting in the excision of the intervening sequence. (3) Intramolecular CO between two homologous sequences in an inverted orientation, resulting in the inversion of the internal fragment. (4, 5) Interchromosomal CO. According to the orientation of the homologous sequences with respect to their centromeres (*gray or red circles*), this process generates translocation (4) or a dicentric and an acentric chromosome (5).

avoid unnecessary HR events. Excess HR can be controlled at several levels: initiation step, cell cycle, and destabilization of abortive HR intermediates through the action of helicases (reviewed in Refs. [61,62]). The fact that protective systems have evolved to counteract excess HR highlights the potential risks of this pathway.

6.1 Cell-Cycle Regulation

Prolonged blockage of replication forks leads to DSBs that can be addressed by HR or NHEJ [63]. However, unlike DSBs produced by enzymes, ionizing radiation, or endonucleases, breaks produced by replication stops have only one DSE. Ligation of two replication stress-induced DSEs involves distant DSEs, leading inexorably to a chromosomal rearrangement. During S phase, HR can take advantage of the intact sister chromatid to restore replication and avoid genetic instability.

Because the two chromatids have identical sequences, the genetic impact is minimal. The close proximity of the sister chromatids (particularly due to the cohesin complex) favors the use of sister chromatids for HR [64–66]. In addition, GC without crossover is favored in somatic cells to limit the risks associated with crossovers [64].

Sister chromatids are absent in G1. Therefore, should HR occur, the lack of sister chromatids would necessitate the use of sequences carried by other chromosomes, thereby jeopardizing genome stability. The maintenance of genome stability requires the restriction of HR to the S and G2 phases of the cell cycle when the sister chromatids are present. First, the CDK1/2-dependent phosphorylation of CtIP and EXO1 in S/G2 favors the initiation of resection and extension, respectively [67–69]. Second, resection is highly repressed by the loading of proteins on DSEs, such as 53BP1, RIF1, BLM, PTIP, the 2010 described REV7/MAD2L2 [70–75], and the C-NHEJ factors Ku70–80.

6.2 Protection Against HR Intermediate Accumulation

HR should be completed once initiated; otherwise, the accumulation of HR intermediates (RAD51 filaments or HJ) can generate genetic rearrangements and/or cell toxicity. The helicases from the RecQ family, which include *BLM*, *WRN*, *RECQ1*, and *RECQ5*, contribute to overall genome stability through the cleaning of abortive HR intermediates from the genome. RECQ5 can disrupt the RAD51 filament [76], and BLM and RECQ1 can melt D-loops. These helicases selectively dissociate recombination intermediates whose polarity can impair polymerase progression [77,78]. Additionally, two other helicases of the UvrD family (PARI and FBH1) can affect the stability of the RAD51 nucleoprotein filament. These helicases have been suggested to remove RAD51 from the ssDNA in a process that requires ATP hydrolysis by RAD51 [79–81]. Moreover, the ATP-dependent DNA helicase RTEL1 functions as an anti-recombinase that is dedicated to counteracting toxic recombination [82]. RTEL1 and FANCM also promote migration of the dHJ, thereby favoring SDSA and protecting against crossover events [83].

6.3 Repression of HR Initiation

Restricting the initiation of unscheduled HR has been proposed to prevent the accumulation of toxic HR intermediates. In mammalian cells, this protective role against excessive HR initiation has been proposed for Tp53, Bcl-2, and AKT1. Indeed, in these situations, essential HR components such as RAD51 or BRCA1 are trapped and mislocalized. Tp53 interacts with RAD51 and, in addition, may also affect the heteroduplexes resolution [84]; Bcl-2 interacts with BRCA1 and localizes it to the mitochondrial membrane [85]; and AKT1 activation BRCA1 and RAD51 are sequestrated into the cytoplasm leading to the inactivation of their nuclear functions [86]. In 2015, the POLQ polymerase was shown to play a role in the balance between HR and A-EJ after resection by inhibiting HR and triggering A-EJ at DSBs [87,88].

7. HOMOLOGOUS RECOMBINATION, GENOME STABILITY, AND CANCER

7.1 Misregulation of HR in Tumors

Both up- and down-regulation of HR have been described in oncogenic situations, highlighting the necessity for a precise equilibrium in HR regulation. Indeed, both decreased and increased HR can generate genetic instability (see earlier).

Defects in HR confer increased oncogenic risks. Most of the germ-line mutations involved in familial breast and ovary cancer affect genes directly involved in HR (the most frequently mutated genes BRCA1 and BRCA2, as well as Palb2, RAD51C, MRE11, NBS1, FANCJ, and FANCN), the HR/replication interface (claspin), or the regulation of HR (ATM, CHK2, and Tp53) [89]. A dominent negative form of RAD51 has been descibed in a subtype of Fanconi anemia, a syndrome associated with genetic instability and cancer predisposition [89a]. PTEN has also been shown to be mutated in familial breast cancer and to affect RAD51 expression [90]. Moreover, the oncogenic kinase AKT1 (which is antagonized by PTEN) has been demonstrated to be up-regulated in 40–60% of sporadic breast cancers and 40% of sporadic ovarian cancers. Importantly, AKT1 activation leads to HR repression through the cytoplasmic retention of BRCA1 and RAD51 [86]. Similarly, overexpression of the oncogene Bcl-2 results in HR down-regulation through the mitochondrial mislocalization of BRCA1 [85]. Finally, cells overexpressing a RAD51-dominant negative form that inhibits HR exhibit higher tumor development efficiency upon injection of nude mice [45].

Conversely, increased HR has also been described in oncogenic situation. Tp53 is the most frequently mutated gene in tumors. Importantly, Tp53 has been shown to inhibit HR, and *Tp53* cells exhibit increased HR activity. Bloom syndrome (BS) results from a mutation in BLM and is associated with high genetic instability and a cancer predisposition. Cells from BS patients exhibit high levels of SCE and hyper-recombination phenotypes (reviewed in ref. [24]). The fusion oncogene BCR/ABL from the translocation chromosome Philadelphia t(9:22) between BCR and ABL is present in chronic

myelogenous leukemia (CML) and a number of other forms of acute lymphocytic leukemia (ALL). BCR/ABL expression results in constitutive tyrosine kinase activity that is responsible for resistance to drugs that generate DNA damage. Particularly, BCR/ABL expression causes the overexpression of RAD51 and the paralogs RAD51B, RAD51D, and XRCC2, resulting in increased HR and conferring resistance to cisplatin and mitomycin C [91]. More generally, components of the HR pathway are aberrantly expressed in many tumors [92,93], and the radioresistance of tumors exhibiting increased HR activity has been correlated with a poor prognosis. Thus, HR stimulation should fuel genome instability toward carcinogenic development, and confer resistance to anticancer treatments.

7.2 Anticancer Strategies

Many anticancer therapies are based on the induction of DSBs (ie, ionizing radiation or topoisomerases inhibitors) or DNA interstrand cross-links (ie, cisplatin or mitomycin C) [94]. Due to its role in DSB repair and the reactivation of arrested replication forks, HR represents a pharmacological target for the optimization of chemo- and radiotherapy.

Inhibitors targeting components of the HR pathway (MRN, RPA, and Rad51) [95], mediators/transducers of DSB signaling, and cell cycle–checkpoint regulation (ATM/ATR kinases, Chk1 and Chk2, Tp53, Wee1, and Cdc25) are in development.

ssDNA gaps or nicks are transformed into DSBs when the replication fork reaches them. Due to the roles of HR in both the replication stress response and DSB repair, HR-defective cells are highly sensitive to these treatments. PARP1 is involved in ssDNA gap and nick repair. Inhibition of PARP1 results in the accumulation of DNA alterations. PARP1 is also involved in DSB signaling and the competing/alternative DSB-repair pathway A-EJ. HR deficient–tumor cells (ie, BRCA1- or BRCA2-deficient cells) are highly sensitive to PARP1 inhibitors [96,97]. This strategy that consists of the induction of the inhibition of two metabolic pathways to generate cell death is called *synthetic lethality*. Similar conclusions have also been drawn for other DSB-repair pathways [98].

8. HR IN GENOMIC MOLECULAR EVOLUTION

Because HR plays a pivotal role in the balance between genetic stability and diversity and requires sequence homology, it is involved in the evolution (divergence versus co-evolution) of homologous sequences such as multigene families.

Following duplication, the two resulting sequences diverge during the course of evolution. However, in some families of repeated genes the two duplicated units do not evolve independently but co-evolve similarly in a process called *concerted evolution* [6,7]. During concerted evolution, one mutation present in one duplicated unit is transferred to the second duplicated unit by GC, which is the driving force behind the homogenization of duplicated sequences, and therefore concerted evolution. Sequences heterologies between the interacting DNA molecules impair HR and thus, represent barriers to concerted evolution. Introns can accumulate mutations without affecting the expression of the protein encoded by the gene. Consequently, introns have been proposed to serve as protective barriers against HR between repeated sequences and favor the maintenance of the genome organization [99]. Therefore, one can speculate that introns are antagonistic evolutionary forces to concerted evolution, routing evolution toward the divergence of repeated sequences.

Concerted evolution can occur between repeated α globin, histone, ribosomal, ubiquitin, and even mitochondrial genes, as well as between noncoding sequences ranging from α satellite sequences to dispersed repeat sequences.

9. CONCLUDING REMARKS

HR is a double-edged sword that can play opposite roles in the maintenance of genomic stability and also favoring genetic diversity up to genetic instability. Therefore, unscheduled excess HR can jeopardize genome stability and cell fate. Depending on the structure of the DNA partners involved in HR, GC, and CO are intrinsically capable to generate genetic variability/instability. In addition to cell-cycle regulation, which restricts HR at the S–G2 phase (and the tight cohesion of the sister chromatids that orientate exchange to equal SCE), several additional mechanisms repress HR: mismatch repair, helicases, and p53. Defects in these systems are associated with genome instability and cancer predisposition. Collectively, these capacities have been used by cell to generate beneficial genetic diversity. However, accidental HR can account for many pathological genome rearrangements.

Because of its main roles in DSB repair and reactivation of arrested replication forks, HR can be advantageously used in several applications: it is involved in gene targeted replacement; it represents a promising pharmacological target for cancer therapy. Therefore methods aiming at precise modulations of HR (either stimulation or repression) represent exciting challenges for future research and medical applications. Finally, HR plays a pivotal role in fundamental processes such as meiosis and molecular evolution of multigene families. Any novel knowledge on HR should thus benefit both academic and applied research.

GLOSSARY

Break-induced replication (BIR) A nonreciprocal recombination–dependent replication process.
Concerted evolution A process in which the paralogous genes within one species are more closely related to each other than to members of the same gene family in another species, even though the gene duplication event preceded the speciation event.
Crossing-over Reciprocal exchange of the adjacent sequences.
Displacement loop (D-loop) The single-stranded DNA formed when two strands of dsDNA are separated by the invasion of a third strand that anneals by base-pair complementation.
DNA helicase An enzyme that unwinds complementary duplex DNA.
DNA topoisomerase Enzymes that alter DNA topology by catalyzing strand passage.
Double Holliday junction (dHJ) Two adjacent Holliday junctions formed between four strands of DNA.
Double-strand breaks DNA damage that results in a break of both strands of DNA.
Gene conversion HR product, nonreciprocal exchange of genetic material.
Holliday junctions (HJ) Cross-strand exchange between two DNA molecules that results in a four-way junction.
Loss of heterozygosity Deletion, or mutation or recombination events that result in loss of the wild-type allele in a heterozygote.
MRN Mre11–Rad50–Nbs1 complex, responsible for recognizing and processing DNA ends.
Resection Degradation of one of the complementary strands of DNA specialized.
Sister-chromatid exchange (SCE) Reciprocal recombination between two sister chromatids degradation.
Synthetic lethality When the association of mutations in two or genes leads to cell death, whereas a mutation in only one of these genes is viable.
Synthesis-dependent strand annealing (SDSA) A recombination process that occurs when an extended strand is displaced and base paired with a complementary single strand to create a duplex without a crossover.

LIST OF ABBREVIATIONS

53BP1 p53-binding protein 1
A-EJ Alternative end joining
BIR Break-induced replication
C-NHEJ Canonical nonhomologous end joining
CDK Cyclin-dependent kinase
CO Crossing over
CMM Congenital mirror movements
DDR DNA-damage response
dHJ Holliday junctions
D-loop Displacement loop
DSB Double-strand break
DSE Double-strand end
EJ End joining
FANC Fanconi
GC Gene conversion
HR Homologous recombination
IR Ionizing radiation
MRN MRE11/RAD50/NBS1 complex
SCE Sister chromatid exchange
SDSA Synthesis-dependent strand annealing
ssDNA Single-strand DNA
T-SCE Telomere sister chromatid exchange

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