Chapter 27A. DNA double-strand break repair by homologous recombination 220727cv3

Drugs Against Cancer: Stories of Discovery and the Quest for a Cure

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CHAPTER 27A

DNA double-strand break repair by homologous recombination

What is a DNA double-strand break (DSB)?

Although DNA single-strand breaks (SSB) production and repair were actively studied in the 1970's, studies of double-strand breaks (DSB) lagged. One reason was confusion about what qualified as a DSB as opposed to two SSB's near each other, one on each DNA stand. If both DNA strands were cleaved at precisely the same place along the double helix, that would clearly be a DSB. But how far apart on opposite strands could SSB's be to qualify as a DSB? If there were a sufficient number of base-pairs between SSB's, the DNA would only need the simpler SSB repair mechanism to restore continuity. If, on the other hand, there were insufficient base-pairs between the breaks, then the DNA would separate at the breaks (Figure 27A.1), and the more complicated DSB-repair would be needed to restore continuity. How far apart the SSB's had to be to maintain DNA continuity depended also on the conditions used in the DSB measurement.

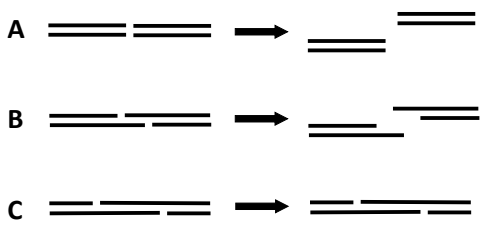

Figure 27A.1. What is a double-strand break (DSB)? **A** and **B** qualify as DSB's, but **C** does not. In **B**, the single-strand breaks (SSB's) are so close that the number of base-pairs between them is insufficient to hold the DNA together. In **C**, there are sufficient base-pairs between the SSB's to hold the DNA together.

What causes DNA double-strand breaks?

We are all subject to background radiation and cosmic rays, which damage DNA by producing both single- and double-strand breaks (SSB and DSB), as well as DNA base damage. In cells that are ongoing DNA replication, SSB are sometimes converted to DSB. That occurs when a replication fork, as it moves along the DNA, happens to collide with an SSB -- as shown and explained in Figure 27A.2.

The ratio of DSB relative to SSB produced by radiation depends on the type of radiation. The most effective DSB-producing radiations are those that consist of atomic nuclei, such as a-particles. Although they have a short range of travel and do not penetrate the skin, a-particles are apt to cause DSB if they pass through or very close to DNA, as shown in Figure 27A.3. Ordinary x-rays may produce some DSB, but an equal dose of a-particle radiation would produce a much higher frequency of DSB, which are harder than SSB to repair and are likely to produce chromosome breaks.

Therefore, a-particle radiation is very dangerous if it is produced within the body. From the 1930s and into the early 1950s, radiologists commonly administered a suspension of thorium dioxide, known as Thorotrast, to produce good contrast in x-ray images. Several million people received Thorotrast. Although it produced no immediate ill-effects, the thorium was slightly radioactive, emitting a-particles within the body. Years later, many who had received Thorotrast eventually developed cancers. Occasional DSB, as well as SSB, also result from reactive oxygen molecules escaping from the energy-producing electron-transfer chain in mitochondria.

DNA double-strand breaks produced by chemicals and anticancer drugs.

While studying DNA damage produced by various alkylating agents, Bradley and Dysart (Bradley and Dysart, 1985) obtained some surprising results. They used the alkaline and neutral filter elution methods to quantify the production of both single- and double-strand breaks (SSB and DSB) in the DNA of cells and animals (Chapter 9). The surprise was that alkylating agents that simply added methyl or ethyl groups to guanines in DNA produced DSB as well as SSB. Those methylated or ethylated guanines would have been repaired by base excision repair (BER; Chapter 24), which could transiently produce SSB at an intermediate step in the repair, but not DSB.

Here is how DSB may result during the repair of methylated or ethylated guanines or adenines. In the first step, a DNA glycosylase removes the alkylated base and leaves behind a base-free sites in the DNA (Chapter 24). A base-free site in DNA is a nucleotide that has no base attached to it. During the repair of a base-free site, a transient single-strand break (SSB) appears and is soon resealed. However, if a SSB is encountered by a progressing DNA

replication machine, the molecular collision is apt to produce a DSB, as suggested by (Vriend and Krawczyk, 2017) (Figure 27A.2).

Other sources of DSB are DNA-binding drugs, such as bleomycin, that produce reactive molecular species close to where the drug is bound (see Chapter 13). In addition, topoisomerase II inhibitors, such as doxorubicin and etoposide, produce DSBs by blocking or perturbing the normal function of the enzyme (see Chapter 10). Moreover, topoisomerase I inhibitors, such as camptothecin, produce DNA double-strand ends when a DNA replication fork collides with a blockage where the drug is bound to the enzyme (see Chapter 11).

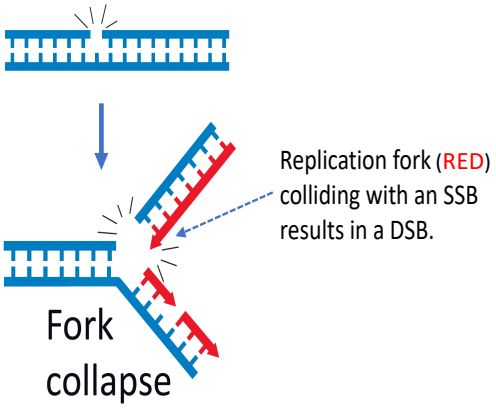

Figure 27A.2. Disaster when a replication fork collides with a single-strand break (SSB). The newly replicated strands are shown in red. The newly replicated double-helix falls off and the replication of that part of the chromosome has to start over again – which prolongs the duration of S-phase. (Modified from (Vriend and Krawczyk, 2017)).

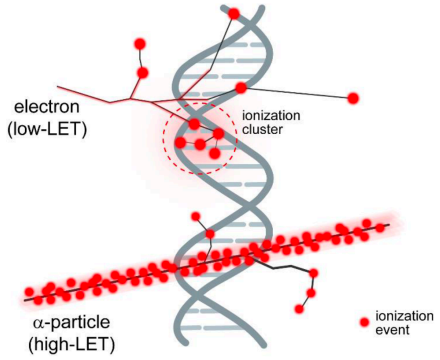

Figure 27A.3. The ratio of DSB relative to SSB produced by ionizing radiation depends on the kind of radiation used. Low energy x-rays produce slow electrons that scatter widely, because they produce low linear energy transfer (LET) in the medium through which they travel. Therefore, they produce mainly SSB, as shown in the upper part of the diagram. a-particles, on the other hand, are heavy and don't scatter as much. When they pass through or very close to DNA, they mostly damage both strands in a small neighborhood, resulting in DSB whose strand ends are staggered as shown in Figure 27A.1, and there may also be base damage at the DSB sites (from (Iliakis et al., 2019)).

Overview of how DNA double-strand breaks (DSB) are repaired.

Two remarkable mechanisms for DSB repair were discovered: homologous recombination repair (HRR) and non-homologous end joining (NHEJ) (Figure 27A.4). The story of how homologous recombination came to be discovered was told in the previous chapter (Chapter 26), including the discovery of RAD51, BRCA1, and BRCA2.

Repair by NHEJ almost always loses or occasionally gains sequences when it rejoins the ends of the DNA breaks, whereas repair by HR is usually error-free; it is nevertheless NHEJ that cells most frequently to use to repair DSB (Iliakis et al., 2019). The likely reason is that HR requires a nearby undamaged sister DNA helix from which to copy normal sequence information to replace damaged regions. A nearby sister DNA is available only late in DNA replication (S-phase), when there are DNA sister chromosomes held together at a centromere. NHEJ, on the other hand, removes the damaged region without replacing it with normal sequence and therefore does not need a sister chromosome. DSB repair by NHEJ therefore can occur during almost any phase of the cell cycle – and can occur even in quiescent cells that are not multiplying (Critchlow and Jackson, 1998). Indeed, DNA repair by NHEJ may have evolved very early in the history of life in haploid organisms that never passed through a stage where sister chromosomes were present to permit repair by HR.

Figure 27A.4 presents an overview of how the two major DSB repair processes work. The diagram and legend of the Figure is a prelude for going on to the history and mechanisms of those processes.

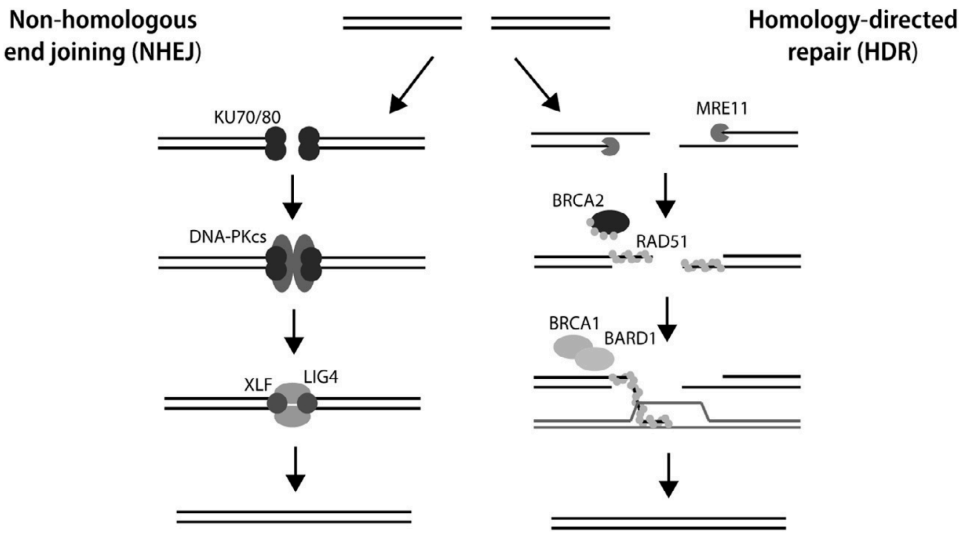

Figure 27A.4. Outline of the two major double-strand break repair alternatives (from (Kaplan and Glazer, 2020)).

The left side of the diagram shows the essentials of the non-homologous end joining (NHEJ) pathway, in which the first step involves binding of the Ku70-Ku80 dimer to each end of the break. In the next step, DNAPKcs is recruited to each end, which binds the two ends together. Finally, XLF and LIG4 ligate the ends together.

The right side of the diagram shows the essentials of the homologous recombination repair (HRR) pathway, which was called "homology-directed repair (HDR)" in the diagram. In the first step, MRE11 chews away part of one strand at each end, so as to produce a single-strand region long enough to invade and base-pair with a region of a sister DNA. The projecting single-strand at each end of the break becomes coated with RAD51 molecules with the aid of BRCA2. The RAD51-coated single-strand at one or both ends of the break then invade a homologous region of a nearby newly replicated sister DNA. Further actions by a polymerase and ligase then restore an accurately repaired DNA.

The homologous recombination DNA repair (HRR) story.

In 1979, Charles Radding (Figures 27A.5A and B) wrote:

"Nothing is more intriguing about homologous recombination than its beginning. How, for example, do homologous double-stranded molecules recognize each other, and what enzymic events, overcoming the energetic barrier posed by the stability of duplex DNA, begin an exchange of parts?" (Shibata et al., 1979).

A diagram of homologous recombination that Radding and his colleagues came up with in 1979 is depicted in Figure 27A.5C. It was based on experiments they had done using purified RecA, the bacterial version of mammalian Rad51. They showed that a DNA single-strand, with RecA bound to it, was able to unwind a region of DNA helix and associate with one of its strands to form the D-shaped loop in their diagram. They also showed how ATP provided the energy to drive the reaction.

The Rad51 gene was first isolated from yeast mutants that were unable to carry out recombination between sister chromatids. Since recombination in bacteria required RecA, it seemed that the two genes might be related to each other. Also, the Rad51 and RecA proteins had similar DNA binding properties. Indeed, the two proteins had similar amino acid sequences (Figure 27A.6) (Shinohara et al., 1992). Further research confirmed that the two proteins carried out similar homologous recombination functions in their respective organisms. But what exactly did these proteins do to help the homologous recombination process? That will be part of the story of homologous recombination DNA repair that will be related in the next section.

Figure 27A.5A. Charles M. Radding (1930-2020) at a laboratory celebration. *Image credit: David Keith Gonda (West and Kowalczykowski, 2021).* (Radding, by the way, was a friend and classmate of mine at Harvard College and then at NIH.)

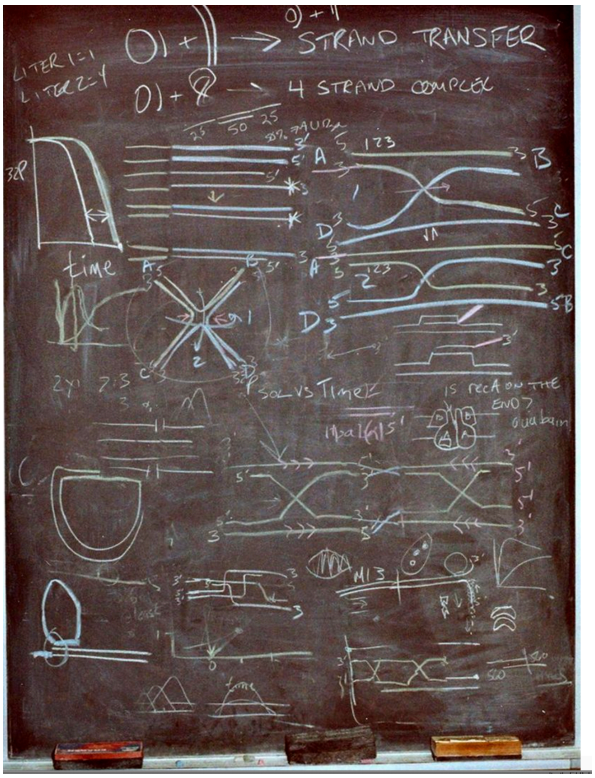

Figure 27A.5B. The blackboard in the Radding laboratory with new ideas about recombination models and mechanisms for how RecA might drive recombination. *Image credit: David Keith Gonda (West and Kowalczykowski, 2021).*

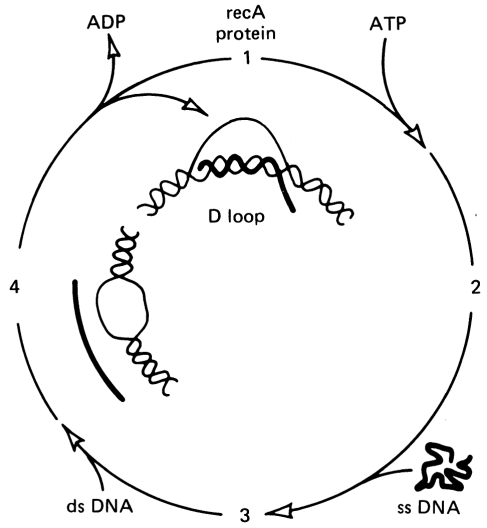

Figure 27A.5C. A model of homologous recombination, proposed by Charles Radding and colleagues in 1979 (Shibata et al., 1979). The model was based on experiments with purified components using the bacterial RecA, which corresponds to the

mammalian RAD51. In step 1, RecA binds ATP. In step 2, RecA-ATP coats a segment of single-strand DNA (ssDNA), which starts as random coil but becomes linear when coated with RecA. In steps 3 and 4, the RecA-coated DNA single-strand binds to the recipient double-strand DNA, unwinding a section of helix as it invades and base-pairs with one of the strands, thereby forming a D-loop. This process is driven by ATP release as ADP. The process can continue with the next section of the ssDNA.

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Rad51 154 RS-ELICLTTGSKNLDTLLG-GGVETGSITELFGEFRTGKSQLCHTLAVTCQIPLDIGGGEGK-CLYIDTEGTFRPVRLVSIAQR
RecA   33 RSMDVETISTGSLSLDIALGAGGLPMGRIVEIYGPESSGKTTLTLQVIAAAQR-------EGKTCAFIDAEHALDPI----YARK

Rad51 236 FGLDPDDALNNVAYARAYNADHQLRLL-DAAAQMMSESRFSLIVVDSVMALY-RTDFSGRGELSARQMHLA-KFMRALQR-LADQ
RecA  107 LGVDIDNLLCSQPDTGE-----QALEICDALA---RSGAVDVIVVDSVAALTPKAEIE--GEIGDSHMGLAARMMSQAMRKLAGN

Rad51 317 FG---VAVVVTNQVVAQVDGGMAF-NPDPKKPIGGNIMAHSSTTRLGFKKGKGCQRLC-KVVDS
RecA  182 LKQSNTLLIFINQIRMKI--GVMFGNPETTT--GGNALKFYASVRLDIRRI-GAVKEGENVVGS
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Figure 27A.6. Amino acid sequence homology between bacterial RecA and yeast Rad51 (Shinohara et al., 1992).

How homologous recombination repairs DNA double-strand breaks (DSB).

As already told in the previous chapter, the story DNA repair by homologous recombination (HRR) can be traced back to 1866, when Paul Broca noted a high incidence of breast cancer among his relatives. Other families were discovered that eventually led to the discovery the BRCA1 and BRCA2 genes; mutation of either of those genes was associated with the high breast cancer-incidence families. As explained in the previous chapter, the two BRCA genes were found to bind to each other and to another protein, the above-mentioned Rad51 that was implicated in HRR. For HRR to work, a single-strand extension from at least one end of the break must become coated with Rad51 protein, which enables the strand to find and invade another double-stranded DNA with the same nucleotide sequence (Figure 27A.7A). But how could the broken strand find another DNA of the same sequence? The answer is that, when DNA replicates, it produces two identical DNA molecules that are held together at the centromere, as explained in Figure 26.8 of the previous chapter. The resulting two identical DNA molecules become “sister chromatids” of nearly identical DNA sequence, and recombination can occur between them.

Unless efficiently repaired, double-strand breaks (DSB) are among the most lethal kinds of DNA damage. The repair based on Radding’s model uses homologous recombination between the DNA of sister chromatids. First, one strand from each end of the DNA break is resected to produce a single-strand extension from each end of the break (top of Figure 27A.7A). The single-strand extension then becomes coated with Rad51 proteins, which

occurs with the aid of BRCA1 and BRCA2 (the three proteins bind together, as explained in the previous chapter). We get a picture of what a Rad51-coated DNA single-strand looks like from the molecular structure shown in Figure 27A.7B: the relatively much greater size of the protein overwhelms the thin DNA strand. The invaded structures at the bottom of Figure 27A.7A can then resolve in several ways, some of which result in recombination between the two sister-chromatid DNA's (Figure 27A.8).

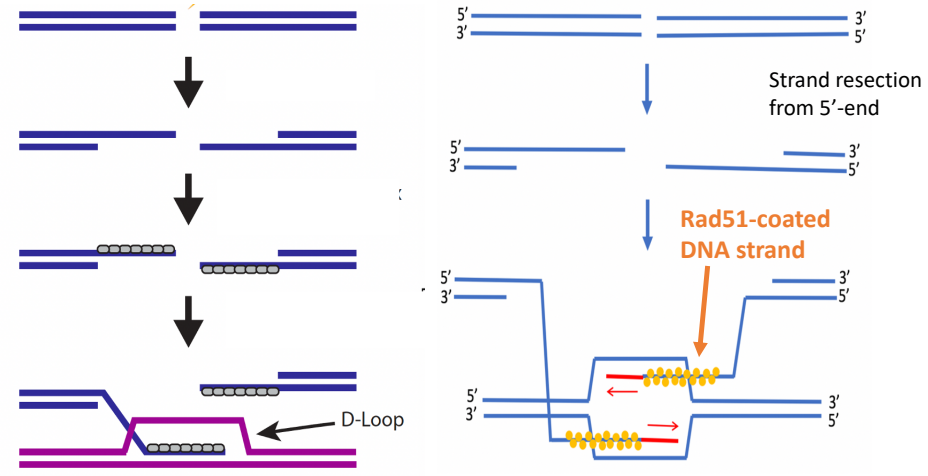


Figure 27A.7A. Two simplified versions of the early stages of homologous recombination in the repair of a DNA double-strand break. In the first step, one of the strands on each side of the break are resected to form single-strand extensions that then become coated with Rad51 protein. The Rad51-coated DNA strand can then invade an undamaged DNA and set the stage for homologous recombination. The diagram on the left shows a single-strand from the damaged DNA invading an undamaged DNA, forming a D-loop (Greene, 2016). The diagram on the right shows another possibility, where both single-strand segments of the damaged DNA invade the undamaged DNA.

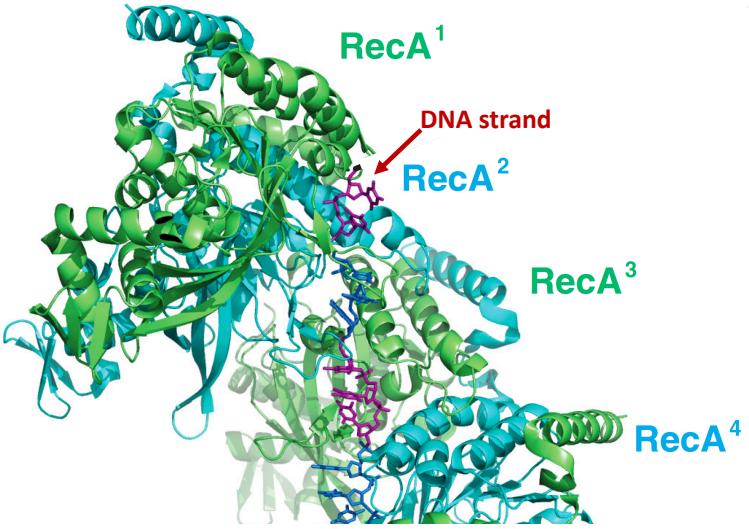


Figure 27A.7B. Part of a molecular structure of a RecA-coated DNA single-strand. The structure shows a section of a DNA single-strand with four RecA protein molecules wrapped around it. RecA is the bacterial version of mammalian Rad51. The RecA molecules (alternating blue and green) fit into each other and wrap around the relatively much thinner DNA strand. (*(Greene, 2016)* with label in red added).

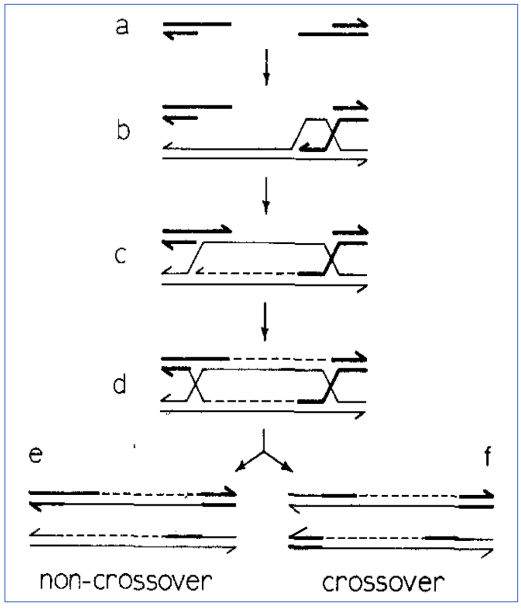


Figure 27A.8. How the final repair products form, as portrayed by (Szostak et al., 1983). The process shown begins after one strand at each end of the double-strand break (DSB) has been resected, leaving a single-strand extension at each end (a). The arrowheads show the

5'→3' direction of each DNA strand. In (b), the single-strand extension of the *right* end has invaded an undamaged sister DNA and paired with one of the strands of the sister. In (c), repair synthesis, shown by the dashed line, has extended the invaded strand. In (d), the single-strand extension of the left end has paired with the other strand of the sister DNA and has been extended by repair synthesis (dashed line). (e) shows two possible outcomes, depending on whether the junction on the left has crossed over.

Repair of double-strand breaks (DSB) was intensively studied over the years and new information gradually accumulated. By 2007, new information allowed Thorslund and West of the London Cancer Research Institute to diagram DSB repair by homologous recombination, as shown in Figure 27A.9 (Thorslund and West, 2007). They portrayed repair of DSB by homologous recombination (HR) beginning with step **a**, which determines whether a DSB will be repaired by HR or by non-homologous end joining (NHEJ). HR repair occurs almost only when DNA replication has produced a sister chromatid that is nearby and from which homologous sequences could be copied. HR can occur during S or G2 of the cell cycle but rarely happen during G0 or G1, because it would then be less likely for a homologous target DNA to be located nearby. Accordingly, DSB repair during G0 and G1 happens predominantly via NHEJ. The 2007 diagram shows repair via HR beginning in step **a**, where the MRN complex resects one of the strands at each end of the DSB -- which allows RPA molecules to associate with the exposed single-strand segments.

Steps **b** and **c** in Figure 27A.9 bring in Rad52 and BRCA2, leading to Rad51-coating of the single-strand segments. In 2016, more details about this complicated process were clarified in the later diagram by George Illiakis and colleagues (Figure 27A.10).

Step **d** in Figure 27A.9 shows a Rad51-coated strand (blue) invading the DNA of a sister chromatid (red). In step **e**, a DNA-repair polymerase copies sequences in the sister chromatid, leading in step **f** to two crossovers called Holliday junctions, which can be resolved in several ways, one of which is shown in step **g**. The final product is an error-free reconstruction of the DNA.

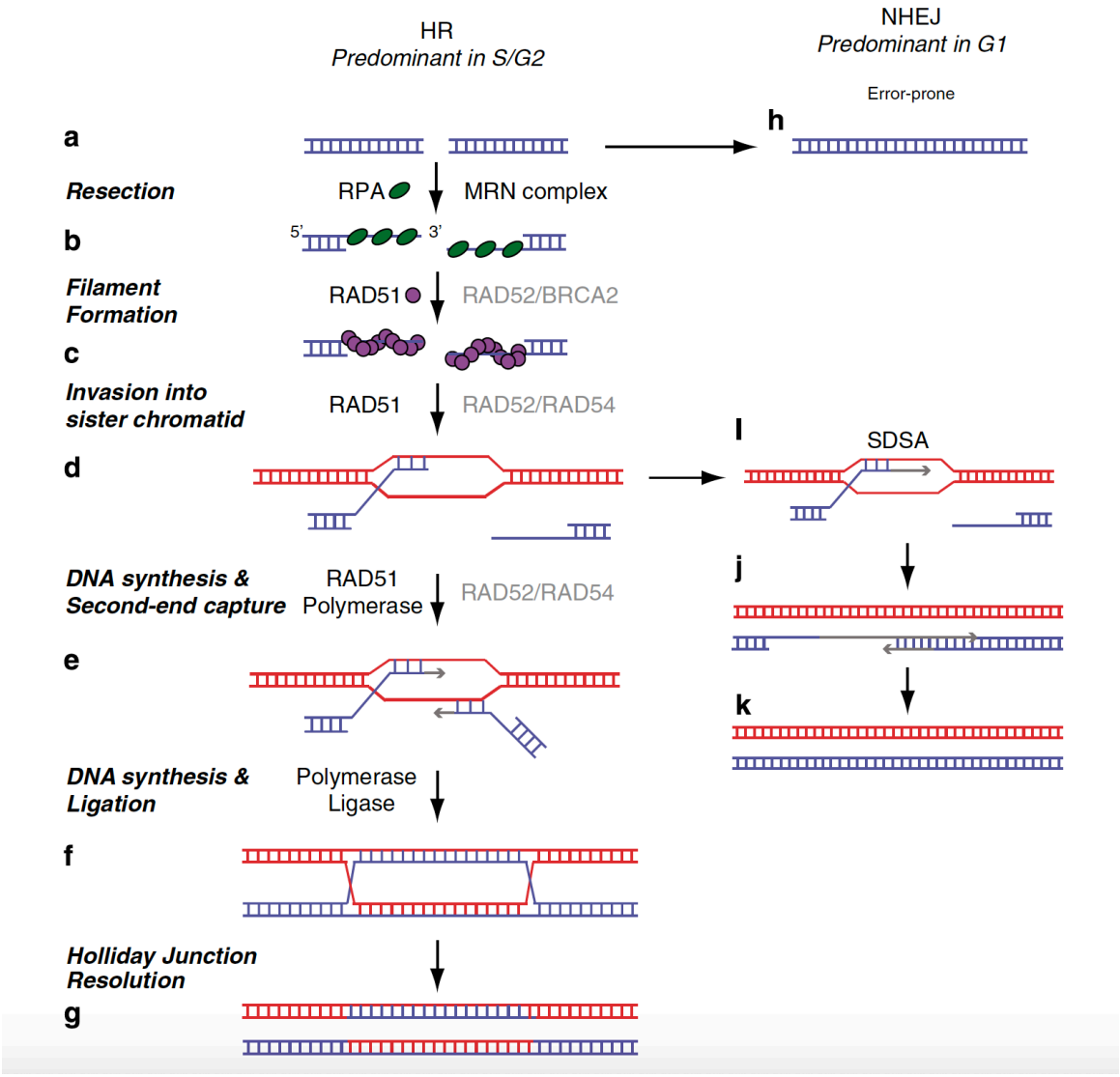


Figure 27A.9. Steps in the repair of double-strand breaks (DSB) by homologous recombination (HR) as diagrammed in 2007 by Thorslund and West (Thorslund and West, 2007). The steps are explained in the text above the Figure.

Then, in 2016, George Iliakis and his colleagues at the University of Duisburg-Essen Medical School, Germany, summarized further details of the steps from strand resection to the coating of the strand with Rad51 (Mladenov et al., 2016) (Figure 27A.10). They began by pointing out that repair of DSB by homologous recombination may have to overcome complications such as damaged nucleotides and staggering of the breaks on the two DNA strands. Such complicated DSB are produced particularly by the high-energy radiation used in modern radiotherapy (see Figure 27A.3).

The first step in repair of DSB by homologous recombination repair (HRR) therefore is to cut away any nucleotide damage and then to resect one of the strands from each end, as shown in Figure 27A.7A. This is accomplished by a combination of three proteins: Mre11, Rad50 and Nbs1 (step A in Figure 27A.10). The complex of those three proteins is often abbreviated MRN. The action of MRN in resecting the strands from the 5'-ends is crucial for the initiation of repair by HRR, and it is what determines that repair will go by HRR, rather than by non-homologous end joining (NHEJ). MRN (with the aid of another protein, CtIP) however only begins the resection. The story of how the MRN genes were discovered and what happens when one of those genes is defective will be told later in this chapter.

Step B in Figure 27A.10 unwinds the DNA to allow the Exo1 exonuclease to nibble away more of the other strand, so as to produce the long single-strand extension needed to probe, reach and invade the sister DNA. The helix unwinding is carried out by the helicase BLM together with another protein, Dna2, to which it is bound. A genetic defect in BLM causes Bloom syndrome, which will be discussed later in this chapter.

In step C, the long single-strand extensions become bound by a line-up of RPA protein molecules that have a high affinity for such binding. That stabilizes the strand and prevents untoward binding events. Next, the RPA has to be removed before the required coating by Rad51 can take place (see Figure 27A.7). The problem here is that RPA binds rather tightly to single-stranded DNA, so some special effort is required, which is carried out by a triplex consisting of BRCA1, BRCA2, and BALB2 (step D). The stories of BRCA1 and BRCA2 were told in Chapter 26. Then, in step E, the BRCA1-BALB2-BRCA2 trimer, with the additional aid of Rad52, manage to remove the RPA molecules and replace them with Rad51 – at last! Step F then allows strand invasion to take place as shown in Figures 27A.7.

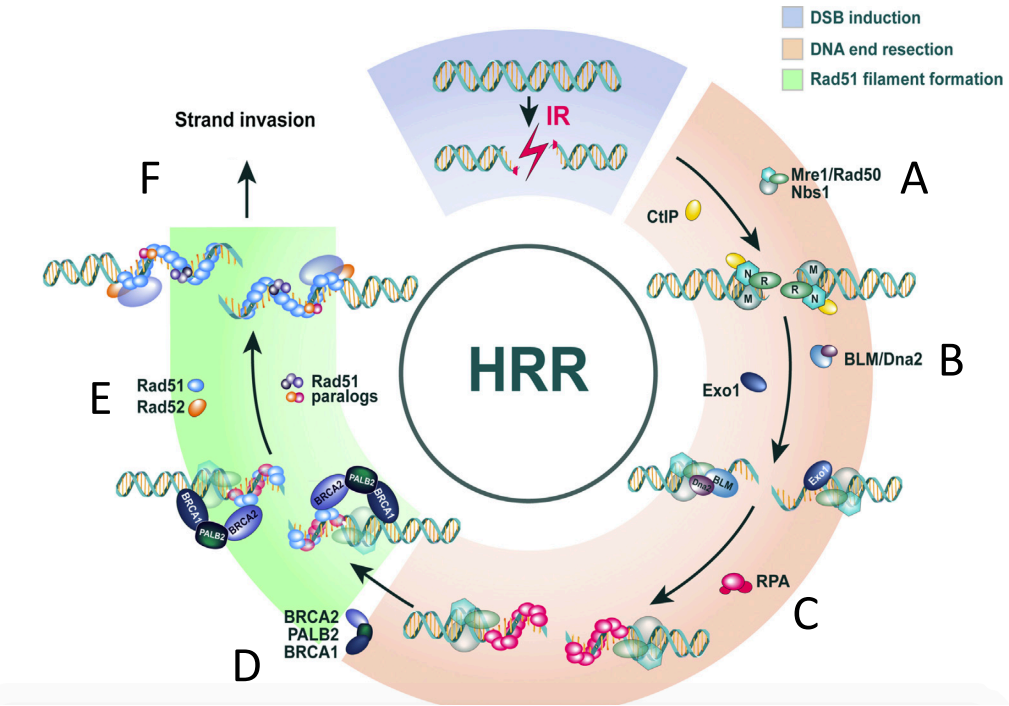


Figure 27A.10. The steps in homologous recombination repair (HRR) from DNA double-strand breaks (DSB) to stand invasion, according to George Illiakis and colleagues in 2016 (Mladenov et al., 2016). The steps are explained in the text.

Some of the essentials of DSB repair by homologous recombination, as understood at the time of this writing, were presented by Eli Rothenberg, Michael R. Lieber and colleagues (Zhao et al., 2019), as shown in Figures 27A.11A and B. The process is explained in the captions in the two parts of the Figure.

After this survey of how our understanding of the events in the repair of DNA damage by homologous recombination developed, we turn next to some of the relevant stories of discovery and clinical implications.

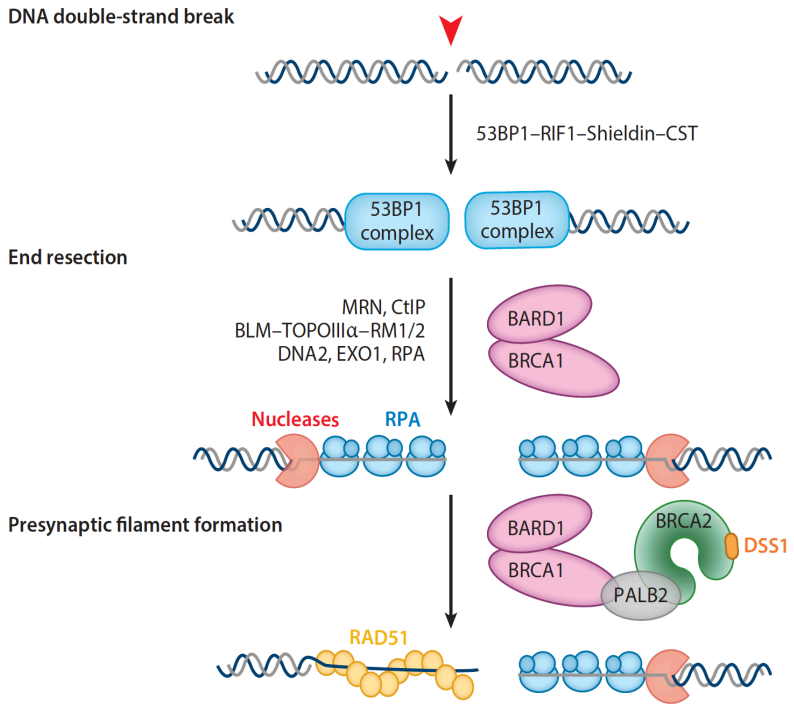


Figure 27A.11A. The first part of the scheme for repair of a DNA double-strand break by homologous recombination. This first part of the process cuts back one strand of DNA double-helix to reveal a single-strand section projecting from the end of the break. The cutting back is done by the nuclease, EXO1. BRCA1, together with its partner, BARD1, as well as several other proteins, then coats the projecting single-strand with RPA, which consists of 3 parts (RPA1, RPA2, and RPA3). The BRCA1-BARD1 pair then bring in BRCA2 together with two other proteins (PALB2 and DSS1). The complex of those 5 proteins, all bound together, then manage to replace the RPA molecules arrayed along the single-strand with an array RAD51 molecules. RAD51 confers a helical structure to the strand filament. The DNA single-strand coated with RAD51, aided by its helical structure, has the capability to invade another DNA double-helix and to initiate the recombination process, as shown in the next part of the scheme in Figure 27A.11B. (From (Zhao et al., 2019).)

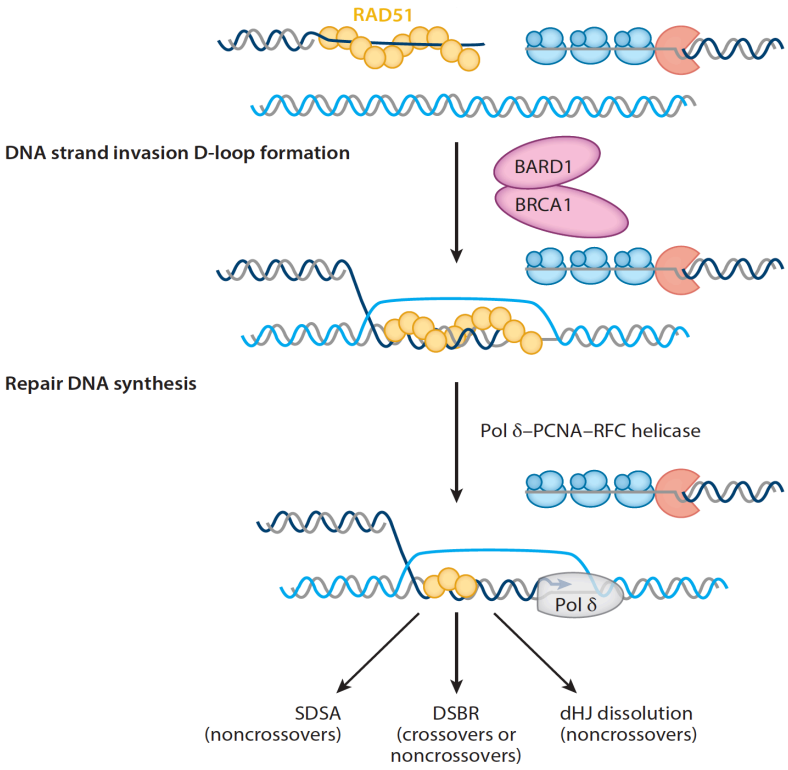


Figure 27A.11B. The second part of the scheme for repair of a DNA double-strand break by homologous recombination. The RAD51-coated DNA single-strand filament is now ready to invade an undamaged homologous DNA helix located nearby. The BRCA1-BARD1 pair also functions here to bring about the invasion. From here, several pathways can complete the error-free repair of the damaged DNA. (From (Zhao et al., 2019).)

The MRN story.

The MRE11-RAD50-NBS1 (MRN) complex is one of the first sensors and responders to DNA damage and initiates the repair of DNA double-strand breaks (DSB). It has a key role in deciding between the two major DSB repair pathways: whether the repair will go by way of homologous recombination (HRR) or by way of non-homologous end joining (NHEJ). The current chapter focusses on the former, the next chapter on the latter. This section of the chapter will take a closer look at how the remarkable MRN molecular machine does its job. But first a brief review of how the three parts of the MRN complex were discovered.

Of the three genes whose protein products make up the Mre11-Rad50-Nbs1 (MRN) complex, the first to be discovered were found in yeast strains that needed those genes to initiate homologous recombination during meiosis. Genetic recombination during meiosis begins by creating a double-strand break (DSB) and resecting one of the strands so as to produce a single-strand extension. Looking for yeast mutants that were unable to carry out this first step of meiosis, Hideuki Ogawa and colleagues at Osaka University isolated two

genes whose mutation destroyed the ability to produce the DSB (Ogawa et al., 1995). One of the genes was new and was dubbed Mre11 (for meiotic recombination); the other was the previously known yeast gene Rad50. They then went on to show that the two genes, Mre11 and Rad50, bind to each other in a complex required for DSB repair (Johzuka and Ogawa, 1995). However, where did the Nbs1 part of the MRN complex come from?

The Nijmegen Breakage Syndrome.

The third gene of the MRN complex came from a clinical and cytological description of a 10-year-old boy in 1981 by clinicians at the University of Nijmegen, The Netherlands. An older brother with similar clinical features had died at age 6 of severe infections. The main clinical features were reduced head size (microcephaly), stunted growth, mental retardation, and respiratory tract infections. The overall picture suggested a relationship with the previously known familial chromosome abnormality diseases: xeroderma pigmentosum, ataxia telangiectasia, Fanconi's anemia, and Bloom's syndrome, but there were distinguishing features that suggested a new genetic disease. As in those other DNA repair defect syndromes, there were immunoglobulin abnormalities and frequent chromosome breaks and translocations. The new syndrome came to be known as Nijmegen Breakage Syndrome (NBS) and the responsible gene *Nbs1*. In addition to random chromosome breaks, NBS patients often had specific translocations between the immunoglobulin and T-cell receptor genes on chromosomes 7 and 14 (Figure 27A.12.) (Digweed and Sperling, 2004; Weemaes et al., 1981).

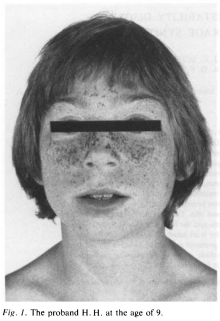


Fig. 1. The proband H. H. at the age of 9.

Proband at age 9.

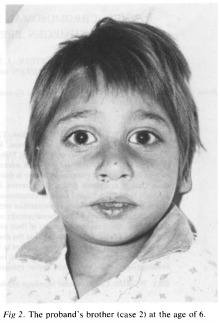
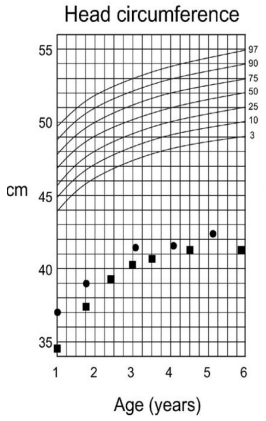
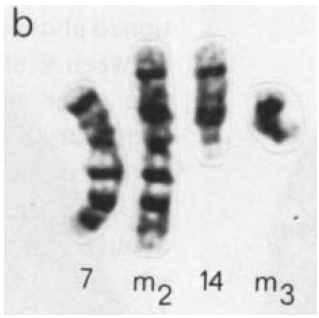


Fig 2. The proband's brother (case 2) at the age of 6.

Proband's brother at age 6.





Translocation of chromosomes 7 and 14 in proband's lymphocytes.

Figure 27A.12. The first described cases of Nijmegen breakage syndrome. The proband (defining case), born in 1969, had repeated upper respiratory infections; in addition to the freckles, he had several café-au-lait spots on the trunk. His brother, born in 1964, had multiple severe infections and died at age 6 of respiratory failure. Both had reduced head circumference and mental retardation. They had chromosome abnormalities, such as translocation between chromosomes 7 and 14 to produce abnormal recombined products, such as m2 and m3 shown on the right (Weemaes et al., 1981).

The Mre11-Rad50-Nbs1 (MRN) molecular machine.

A DNA double-strand break (DSB) presents a challenging problem for any repair machinery, because the break completely separates the DNA into two parts. A general repair device would have to grab onto both ends of the separated DNA ends, bring them back into alignment, and process the ends in a manner that allows enzymes to reconnect the two strand breaks. To make this possible, the process has to digest away a few bases, which entails loss of some information and makes the repair error-prone. This repair process is known as non-homologous end joining (NHEJ) and is the subject of the next chapter. The current chapter focusses on repair by homologous recombination (HRR), which is an even more daunting process and more restricted in when it can occur. But it has the merit of being error-free. The MRN machinery is needed for both of those repair pathways.

MRN was thought of as having three actions: (1) binding to the ends of DSBs and processing the ends chemically to allow further repair steps, (2) bridging between the ends of a DSB in repair by NHEJ or between homologous DNA regions of two sister chromatids in repair by HRR, and (3) signaling control networks to pause cell cycle events in order to give more time for DNA repair (Bian et al., 2019). The importance of MRN function for the cell was indicated by its widespread conservation among species in evolution; Mre11 and Rad50 are conserved from bacteria to mammals, and Nbs1 is conserved among eukaryotes. Figure 27A.13 shows a structure of the MRN complex as deduced by (Hopfner et al., 2002) and depicted by (Bian et al., 2019). Figure 27A.14 shows how a pair of MRN complexes may link a DSB with a homologous sequence in a sister chromatid in HRR (*left*) or link together the ends of a DSB in NHEJ (*right*).

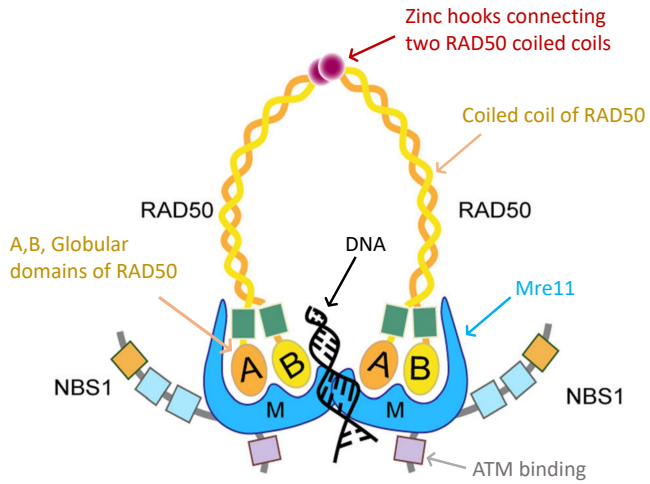


Figure 27A.13. The MRN complex consists of two molecules, each of the Mre11, Rad50, and Nbs1 triplet and could form a structure such as shown in the figure. Rad50 is made up of a long coiled-coil, each end of which has a globular domain (A and B) that fits into a pocket in the Mre11 protein. The midpoint of the coiled coil, where it loops back, has a pair of

cysteines that bind a Zn atom. The Zn regions of the coiled coils of the two Rad50 molecules form hooks that link them together. DNA can fit between the two Mre11 molecules. Nbs1 can bind ATM (ataxia telangiectasia mutated protein) to signal cell cycle delay while DNA repair is in progress and could link to other control molecules. ((Bian et al., 2019) with added labels.)

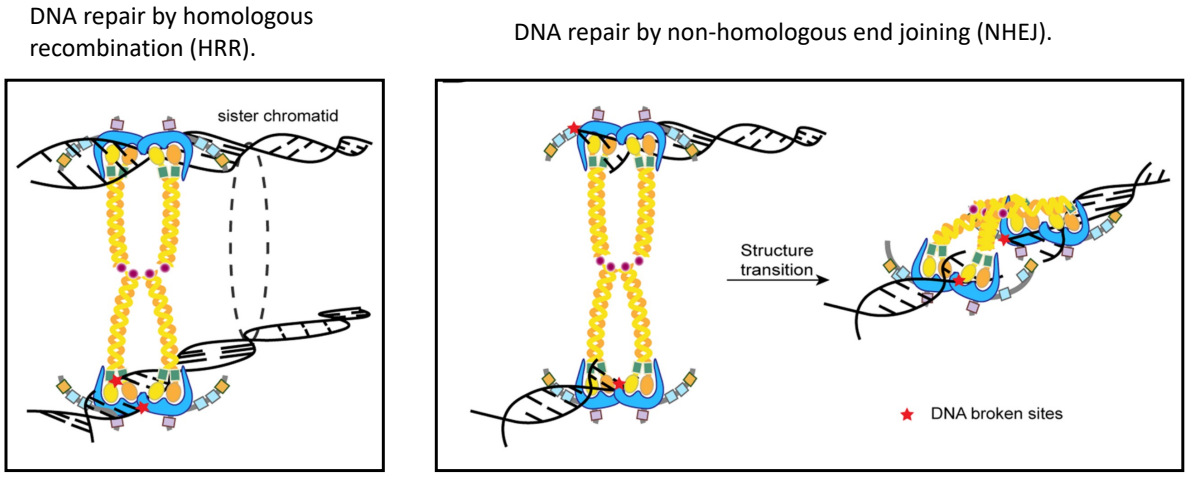


Figure 27A.14. How a pair of NRM complexes may function in DNA repair by homologous recombination (*left*) or by non-homologous end joining (*right)* (Modified from (Bian et al., 2019).)

Homologous recombination repair (*left*): The DNA with the double-strand break is at the bottom. The breaks in its two strands are indicated by red stars. A pair of MRN complexes (see Figure 27A.13) holds together the broken DNA and the DNA of a homologous chromatid.

Non-homologous end joining (*right*): In this case, the DSB separates the DNA into two parts, the ends of which are held together by a pair of MRN complexes. A structural change in the MRN complexes may bring the broken ends into alignment.

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