Recombination and Genome Rearrangements

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Encyclopedia of Molecular Cell Biology and Molecular Medicine, 2nd Edition. Volume 12
Edited by Robert A. Meyers.

ISBN: 3-527-30649-8

Keywords

Crossing-over
A recombination that involves physical exchange of DNA between nonsister strands of homologous chromosomes, resulting in a nonparental combination of linked markers.

Gene Conversion
Nonreciprocal recombination between homologous chromosomes with one allele being the donor of information to convert the recipient allele to the donor sequence.

Heteroduplex
DNA hybrid formed from single strands of two nonsister chromatids; an intermediate in recombination.

Holliday Junction
Crossed-strand structure consisting of two DNA duplexes joined by a bridge as an intermediate in recombination.

Mismatch Repair
Repair of mismatched bases in heteroduplex DNA by a mismatch repair system, resulting in homoduplex DNA.

Nonhomologous End Joining
Joining of blunt ends or ends with microhomologies of usually 2 to 4 bases.

Resolution
Cutting of the Holliday junction by a resolvase to disconnect the recombining DNA duplexes and restoring the parental configuration of markers or resulting in a nonparental configuration of markers termed crossovers.

Strand Exchange
One of the processes to form heteroduplex DNA during recombination in which a strand from a homoduplex is displaced and a strand from a nonsister duplex is transferred to form a hybrid region on the chromatid.

Transformation
Addition of exogenous DNA to a cell to change the genotype of the recipient cell.

Homologous recombination (HR) is an essential activity of all cells as it is one of two major pathways used to repair potentially lethal double-strand breaks (DSBs). DSBs arise from many sources. They may be induced by exogenous agents such as ionizing radiation or chemicals that modify the DNA bases of the DNA backbone, such as methylmethane sulfonate, bleomycin, or mitomycin C. Agents that inhibit DNA topoisomerases, such as cisplatin, also cause DSBs. Endogenous DSBs may occur from programmed breaks, such as those involved in immune system V(D)J recombination of vertebrates, faulty topoisomerases, or oxygen free radicals. When the DNA replication apparatus encounters bulky lesions or a nicked template strand, DSBs may form, collapsing the replication fork. Alternatively, the replication fork may stall with single-strand DNA at the fork. This is a substrate for HR, or further processing to form a DSB. These DSBs are repaired by HR or nonhomologous DSB rejoining events. Therefore, although mitotic HR occurs at significantly reduced rates compared to meiotic HR, it is nonetheless an essential aspect of vegetative growth.

HR is essential for maintaining integrity of the genome, for preventing chromosome rearrangements, for preventing changes in chromosome number, for telomere maintenance when the normal telomere replication pathway is defective, and for preventing the occurrence of alternate double-strand break repair pathways that may result in loss of heterozygosity (LOH) events. Since spontaneous DSBs occur in every replication cycle in vertebrate cells, HR is an essential function of a normal replication cycle. Meiotic HR may also be linked to repairing premeiotic DNA replication errors, but its main purpose lies in ensuring genetic variation and proper chromosome segregation. Meiotic HR gives genetic variation to future generations by providing new combinations of alleles in the gametes. The most important function of meiotic HR is to provide a mechanism for proper segregation of homologous chromosomes at the first meiotic division. In the absence of crossing-over, chromosomes do not disjoin at the first meiotic division and give rise to aneuploid meiotic products. In this chapter, the current models for double-strand break repair by HR are reviewed, differences between mitotic and meiotic HR are discussed, and the consequences of defective HR are considered.

1 Homologous Recombination Events

1.1 Homologous Recombination (HR) Mechanisms

Homologous recombination (HR) uses information from a homologous DNA sequence, usually the sister chromatid or the homolog chromosome, to accurately repair a double-strand break (DSB), and in the process HR can transfer information from the donating DNA duplex to the recipient DNA duplex. Traditionally, HR has been thought of as reciprocal exchange of nonsister chromatids. Crossing-over, often referred to as *reciprocal recombination*, involves the physical joining of two parental molecules of homologous chromosomes. Genetically this is detected as recombinant progeny. If one parent has the linked markers AB and the other

parent has the alternate alleles, ab, the crossover products have the genotypes Ab and aB (Fig. 1a). Gene conversions, often referred to as *nonreciprocal recombination*, involve the transfer of information from one parental molecule to another parental molecule. The genotype of the donor molecule remains unchanged while the genotype of the recipient is converted to the donor genotype (Fig. 1b).

Nonreciprocal recombination or gene conversion is best studied in the fungi where all four products of a single meiosis can be recovered. Gene conversion does not result from mutation. Genetic and molecular studies have shown that the gene conversion product has the same DNA sequence as the donor sequence. Occasionally, a meiotic product is recovered that has genetic information from both parents. This mosaic product is called a *postmeiotic segregation event*. In wild-type strains, gene conversion events occur at a much higher frequency than postmeiotic segregation events. Postmeiotic segregation events result from the failure to repair a DNA mismatch in heteroduplex DNA. Mutants that are defective for mismatch repair show higher PMS frequencies and reduced gene conversion frequencies, indicating that heteroduplex formation is an intermediate in gene conversion.

1.1.1 Double-strand Break Repair

The first clues that HR is initiated by DSBs came from studies showing that

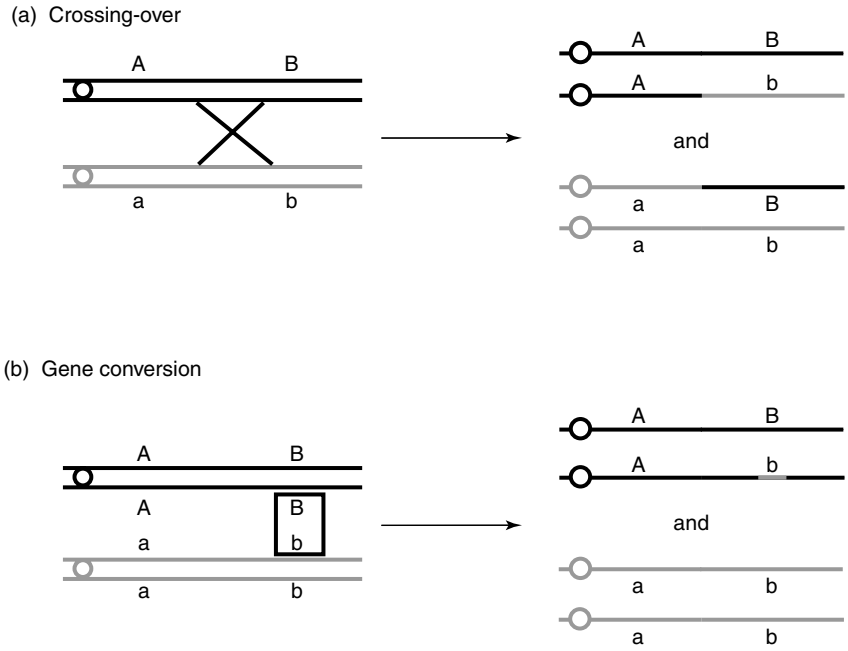


Fig. 1 Types of homologous recombination events. (a) Crossing-over, indicated by the X, generates two recombinant chromosomes and two nonrecombinant chromosomes. (b) Gene conversion, indicated by the box, yields only one recombinant chromosome. This chromosome has only a small region of substitution, at the B gene, from the donor chromosome. The donor chromosome remains unchanged, and is of the parental genotype.

HR was stimulated by ionizing radiation. This idea was further strengthened by experiments of transformation in the yeast *Saccharomyces cerevisiae*. When intact plasmid molecules that carry a yeast gene are applied to yeast cells, the cells take up the plasmid and express the yeast gene or marker located on the plasmid after it is integrated into the yeast genome. The site of integration is not random; instead, it occurs by homologous crossing-over. Thus, transformation in yeast was viewed as a model for HR. Although transformation using intact plasmid molecules is relatively efficient, the frequency could be greatly increased if the plasmid molecules were first treated with a restriction enzyme that cuts within the yeast gene located on the plasmid.

When yeast cells are treated with linear plasmid molecules, transformation still occurs by homologous crossing-over. If the plasmid molecule contains two unlinked yeast genes A and B, the plasmid molecule will integrate into either the A gene or the B gene with approximately equal efficiency in transformation experiments. However, if the plasmid is first treated with a restriction enzyme that cuts only in the A gene, then most transformation events will be integrations at the A gene, demonstrating that DSBs are highly recombinogenic.

When the linear transforming plasmid contained a gap in a yeast gene, it was repaired using the chromosomal information. This is a gene conversion event and shows that DSBs are recombinogenic for both gene conversion and crossing-over events. Transformation using gapped molecules has also been performed using molecules that contain a sequence that acts as an origin of DNA replication. As these molecules can replicate autonomously, they are not obliged to integrate, although the gap must be repaired. Transformation with such molecules was associated with repair (gene conversion) and homologous crossing-over (integration) approximately 50% of the time and with simple repair (gene conversion without crossing-over) for the remainder 50% of the time. This result gave further support for the double-strand break repair model as a general model for meiotic recombination.

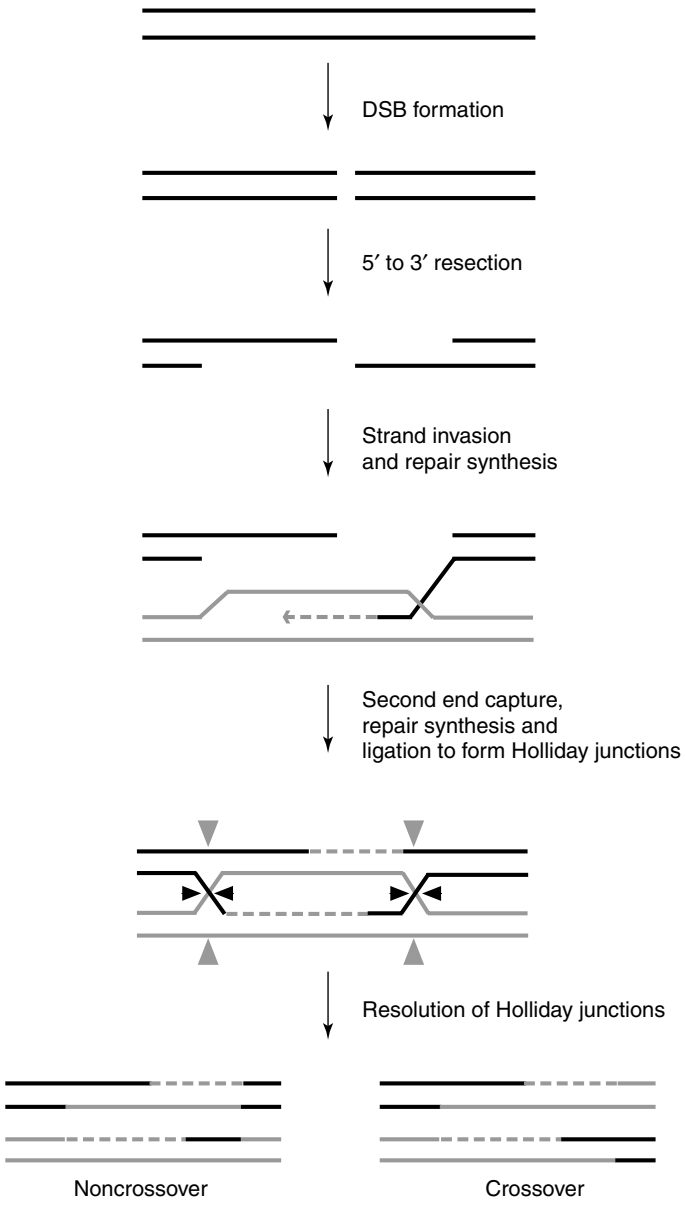
The general features of the double-strand break repair (DSBR) model for HR are shown in Fig. 2. After DSB formation, the ends of the break are resected to give 3′ single-strand tails of up to 600 nucleotides in length. The single-strand tails invade a homologous DNA sequence, and then primer synthesis from the 3′ end until synthesis fills in the gap on both strands. Ligation to the 5′ ends of the break creates a joint molecule with two Holliday Junctions (HJs). The joint molecule contains heteroduplex DNA, which is a target for the mismatch repair system. Resolution of the HJs results in crossover or non-crossover molecules. Gap repair gives rise to the gene conversion events.

1.1.2 Synthesis-dependent Strand Annealing

Although the DSBR model nicely explained why DSBs promoted HR, how a DSB could be repaired to give a gene conversion, and how crossing-over could be mechanistically linked to gene conversion via formation and resolution of the HJs, the model did not satisfactorily explain some aspects of mitotic HR. First, in other experimental systems, gap repair of a plasmid molecule was not often associated with a crossing-over event. Second, in yeast, mitotic gene conversion was not frequently associated with crossing-over. Third, mating type switching in yeast, which is DSB-promoted via the HO endonuclease,

was rarely associated with crossing-over in both haploid and diploid cells.

These observations promoted a revision of the DSBR model to form a new model that retained the features of DSB repair and gene conversion through gap filling, but did not result in intermediates with HJs. Hence, the gene conversion events were not mechanistically linked to crossing-over. The key feature of this model, called



the *synthesis-dependent strand annealing* or *SDSA* model, is a migrating D-loop that does not capture the second DSB end (Fig. 3). As in the DSBR model, the DSB is resected to give 3′ tails, one of which invades into homologous sequences on paired DNA molecule. The 3′ end primes DNA synthesis, and as synthesis proceeds, the D-loop migrates. However, the second 3′ end never is paired with the D-loop. Instead, the invading 3′ end eventually is displaced from the template duplex, and pairs with the single-strand region from the other end of the DSB. Repair synthesis fills in the gaps, resulting in DSB repair to give a gene conversion not associated with crossing-over.

SDSA has also been proposed to operate in meiosis to form all of the noncrossover gene conversion events. This will be discussed further in Sect. 3.1.

1.1.3 **Break-induced Replication**

The DSBR model is a two-ended strand invasion, where both 3′ ends of the DSB are involved in strand invasion into a homologous DNA duplex. In contrast, the SDSA model is a one-ended invasion, where only one of the two 3′ ends is involved in a strand invasion, and the other end participates in the annealing reaction. Both of these repair models are initiated from a DSB that is flanked by sequence on both sides. However, there are also DSBs that come from essentially terminal deletions, where only one side of the DBS can pair with homologous sequence (Fig. 4). Repair of this DSB by HR of necessity uses a one-ended invasion pathway. Repair is proposed to occur by processing of the DSB to give a 3′ end and invasion into homologous sequence. Repair is completed by synthesis to the end of the chromosome. This type of repair is called *break-induced replication* or *BIR*.

Evidence for BIR first came from studies in bacteria, where replication of the *Escherichia coli* chromosome was dependent on both recombination factors and replication factors. BIR differs from DSBR and SDSA in that very long tracts of DNA are replicated in a semiconservative manner. BIR was first observed in yeast from transformation with broken chromosomes. The repaired DNA fragments were linear, and contained a centromere. One chromosome arm ended in a normal telomere sequence, while the other chromosome arm was truncated internally without a telomere. This truncated arm was repaired by strand invasion into an intact homologous chromosome followed by synthesis to the end of the chromosome. This regenerated the deleted telomere and chromosome arm. The acquired sequences were identical to those from the intact chromosome arm used as template for semiconservative replication. Thus, BIR results in

Fig. 2 Double-strand break repair (DSBR) model of homologous recombination. After induction of a double-strand break (DSB), both ends are resected to yield 3′ ends. The 3′ end invades a homologous sequence and primes repair DNA synthesis using the 3′ end and the invaded strand as template. As synthesis proceeds, the second 3′ end of the DSB can be captured by either strand invasion or annealing to the displaced homologous DNA sequence. Ligation of the ends results in two-crossed strand Holliday Junctions. Resolution of the Holliday Junctions can yield noncrossover products (resolution of both Holliday Junctions by cutting at the black arrow heads) or crossover products (resolution of one Holliday Junction by cutting at the black arrow head and the second Holliday Junction by cutting at the gray arrow head).

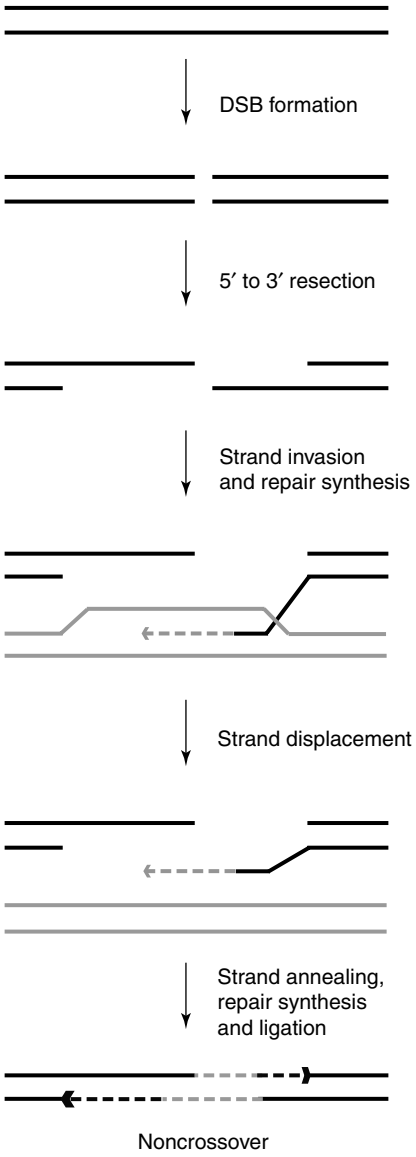


Fig. 3 Synthesis-dependent strand annealing (SDSA) model of homologous recombination. After induction of a double-strand break (DSB), both ends are resected to yield 3′ ends. The 3′ end invades a homologous sequence and primes repair DNA synthesis using the 3′ end and the invaded strand as template. After synthesis, the invading strand becomes displaced and anneals with the other 3′ single-strand tail of the processed DSB. This process differs from the DSBR in that the second end never is captured into the donor homologous duplex. The gaps in the annealed strand structure are filled in by repair synthesis, using the original strand as template. The reaction is completed by ligation of the nicks.

LOH events, and genetically are identical to products from mitotic crossing-over. The BIR process in yeast was initially called *break copy duplication*, based on the results from transformation with chromosome fragments. BIR may function in cells to maintain telomeres when the telomere replicating enzyme telomerase is absent. BIR can also result in nonreciprocal translocations if the broken end invades into homologous repeated sequences located on a different chromosome.

1.1.4 **Single-strand Annealing**

The last DSB repair process that uses homology to be discussed is single-strand annealing (SSA). SSA is thought to occur between directly repeated sequences (Fig. 5), but could also occur between sequences located on different chromosomes. In the SSA model, the ends of the DSB are resected to give 3′ tails, but these are not engaged in strand invasion of homologous sequences. Rather, the 3′ ends anneal to each other at the complementary single-stranded regions that have been revealed by the resection. The 3′ tails emanating from the annealed duplex are removed by endonucleases, and the nicks are then sealed by DNA ligase. SSA events are considered error-prone as they result in deletions between direct repeats, or potentially translocations and other rearrangements if sequences

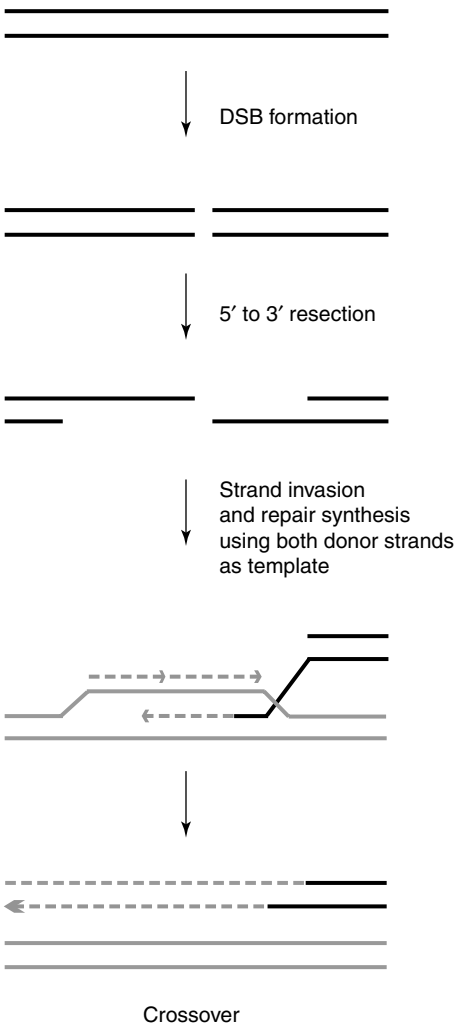


Fig. 4 Break-induced replication (BIR) model of homologous recombination. After induction of a double-strand break (DSB), both ends are resected to yield 3′ ends. The 3′ end invades a homologous sequence and primes repair DNA synthesis using the 3′ end and the invaded strand as template. The second end of the DSB is never engaged in the DSB repair reaction. DNA synthesis is established in the opposite direction, using the displaced D-loop as template. Synthesis continues to the end of the donor chromosome, copying both strands to repair the lost DNA sequence. The resultant product looks like half of a crossover reaction (see Fig. 1).

on different chromosomes anneal. Since SSA cannot occur without some type of chromosome rearrangement, it most likely is rare and usually involves direct repeats.

1.2 Nonhomologous End Joining

In addition to DSB repair using homology, cells also possess pathways to repair DSBs by nonhomologous methods. This type of repair is called *nonhomologous end joining* (*NHEJ*). NHEJ may use microhomologies of 1 to 2 nucleotides at the DSB ends, or there may be no homology at all. Ends are brought together by proteins specific for NHEJ, called *Ku70 and Ku80*. Additional factors essential for NHEJ include the protein kinase DNA-PKcs. Binding of these proteins to the DSB ends helps to bring the ends together. The ends are then ligated by a special ligase called *ligase IV* and its partner *XRCC4*. NHEJ is often error-prone and small deletions or additions occur at the ligated ends.

Although NHEJ is frequently error-prone, it is the major DSB end joining process used in mitotic cells in the G1 phase of the cell cycle. In G1, the only available homologous partner is the homolog chromosome, and pairing by a search for homology may be rate limiting when chromosomes are not naturally paired. Since only about 5% of the mammalian genome sequence encodes ORFs, error-prone end joining often may not be deleterious. During S phase and G2, the sister chromatid is available for repair

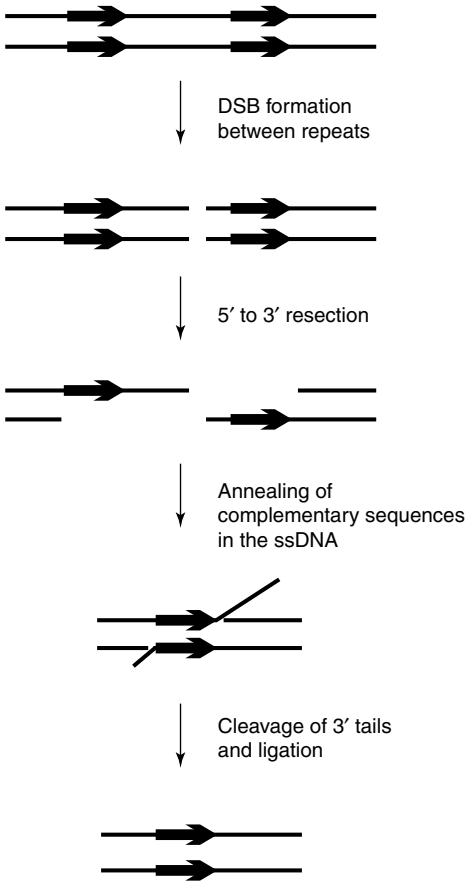


Fig. 5 Single-strand annealing (SSA) model of DSB repair. After induction of a double-strand break (DSB) between direct repeats, both ends are resected past the repeats to yield 3′ ends that have the repeat sequence in single-strand configuration. The complementary strand anneal at the repeat sequences, generating 3′ single-strand tails. The tails are cleaved by the structure specific Rad1/10 endonuclease (XPF/ERCC1 in humans), and the nicks are sealed by ligation. The SSA reaction results in a deletion of one copy of the repeat and the DNA sequence located between the repeats.

through HR and is held in close proximity through the cohesion complexes, which hold the sister chromatids together until mitosis.

NHEJ appears to be the preferred methods for repair of DSBs in vertebrate somatic cells. However, DSBs generated by sequence-specific endonucleases are often repaired by HR. Most HR proteins are essential and knockout mice for these proteins die as early embryos. This shows that HR is essential for repair of spontaneous damage during embryogenesis. The damage most likely occurs during replication, resulting in stalled or collapsed replication forks. Vertebrate cells that have nonlethal mutations in HR genes, such as Rad54, some of the Rad51 paralogs, or BRCA1, are sensitive to irradiation, showing that HR is also used to repair damage resulting from irradiation. NHEJ appears to be avoided in meiotic cells by downregulating the Ku proteins.

In yeast, NHEJ is used in the G1 phase of the cell cycle to repair DSBs while HR is used during late S and G2. NHEJ is also regulated through sequestration of the yeast XRCC4-like protein Lif1 to the cytoplasm in diploid cells that are able to undergo meiosis.

2
Steps in Homologous Recombination

In this section, the molecular mechanisms of the DSBR and SDSA models will be discussed, as these have been the most extensively studied of the HR mechanisms and are the most common DSB repair processes by HR. The proteins discussed are those used in eukaryotic systems. Most of these proteins are conserved from yeast to human. SSA usually occurs between direct repeats and appears to involve nucleolytic processing of the DSB, annealing of complementary strand promoted by the annealing activity of the Rad52 protein and RPA protein, processing of any nonannealed DNA tails by the ERCC1/XPF endonuclease, and sealing of the nicks by DNA ligase.

2.1
Initiation

Once a DSB is formed, to prepare the ends for HR, they must be resected to reveal 3′ single-strand tails. The resection most likely occurs through the action of the MRN complex and additional nuclease and DNA helicase activities. Once single-strand tails with 3′ ends are formed, these become coated with RPA protein to protect the single-stranded DNA and to remove any secondary structure. Next, RPA is displaced and Rad51 protein coats the single-strand DNA to form a DNA-protein filament. Rad51 loading is facilitated by a set of proteins related to Rad51 called the *Rad51 paralogs*, plus Rad52, and Rad54 proteins. Additional proteins related to Rad52 and Rad54 may also be involved. There are also a number of proteins that interact with Rad51 and may aid in the initiation stage of loading Rad51 or in the search for homology with a DNA partner. One important interactor is the Brca2 protein. Brca2 is mutated in some heritable human breast cancers and is clearly involved in HR. The DNA helicases WRN and BLM also are required in HR. Some models place these early in the initiation step, while other models propose that these helicases act during the later stage of resolution. The helicases could also act in the extension step, to open up the D-loop for extension of the invading 3′ end by replication.

The second step in initiation involves invasion of the intact donor DNA duplex by the 3′ Rad51 filament tail of the recipient DNA at a point of DNA homology, signaling the end of the search for homology and the beginning of strand invasion. Strand invasion is promoted by Rad54 protein and may also require Rad52 protein at the 3′ ends of the DSB. During strand invasion, the Rad51 protein is displaced from the 3′ single-strand region as this sequence enters into duplex-paired DNA. In the DSBR model, the displaced D-loop sequence is enlarged by extension of the 3′ end of the invading strand by replication. This requires leading strand DNA polymerases. Extension continues until the 3′ end completes the gap repair and can be ligated to the recessed 5′ end of the second DSB end. The second DSB end can also engage in a strand invasion of the D-loop, or be captured by extension of the D-loop until the displaced single strand of the D-loop can anneal with the other 3′ end of the DSB. In the SDSA model, the displaced D-loop sequence from the strand invasion is small and migrates, propagated by DNA synthesis.

2.2
Propagation

Propagation can be considered in two parts, extension of the 3′ end by replication,

and branch migration of the entire joint molecule. DNA synthesis from the invading 3′ strand appears to require the replicative DNA polymerases δ and ε (POL2 and POL3). It is not clear whether any of the error-prone translesion synthesis polymerases are also involved. HR is considered to be error-free as the DNA synthesis in replication uses the replicative DNA polymerases. However, some recent studies in yeast suggest that DSB repair by HR is more error-prone than replicative DNA synthesis. As described above, in the DSBR model, synthesis extends from the 3′ ends of the DSB until the gap is repaired and the ends can be ligated to the resected 5′ ends of the DSB, forming two HJs. In the SDSA model, synthesis proceeds only from the invading 3′ end, and continues until it is displaced from the template donor duplex. The newly replicated portion of the DSB is now able to base pair with the 3′ end from the other tail of the DSB, thus providing a template for synthesis from this 3′ end. When synthesis reaches the resected 5′ ends of the DSB, ligation can occur.

There is genetic evidence that a cross-strand DNA molecule with HJs can branch migrate. *In vitro*, the *E. coli* RecG DNA helicase and the *E. coli* RuvB helicase in association with RuvA can branch migrate a HJ. Both activities are required for normal HR in *E. coli*. Similar proteins have not been found in eukaryotes, although genetic evidence suggests that symmetric heteroduplex tracts can form during HR, and their formation is best explained by branch migration of HJs.

2.3 Resolution

Resolution in HR refers to resolution of the HJs. In the SDSA model, no HJs are formed and the repair products are always noncrossover. The DSBR model proposes the formation of double HJs. The search for proteins with endonuclease activity specific for HJ structures, producing products that can be ligated without errors has been ongoing for many years. In *E. coli*, the proteins that fulfill these criteria are the RuvC and RusA proteins. *In vitro*, RuvC cleaves HJs and *in vivo* it is required for HR. In eukaryotes, an endonucleolytic activity that is specific for HJs has been found to act on mitochondrial DNA. Loss of this activity has no effect on mitotic HR in chromosomal DNA or on meiotic HR. Recent studies point to a role for some of the Rad51 paralogs in HJ resolution activity. Alternatively, resolution of HJs could occur through the combined action of DNA topoisomerases and DNA helicases.

However resolution occurs, it must be able to resolve the HJs in a manner that allows the ends to be ligated together after cutting and the interlinked DNA strand to be untwined. Resolution may give crossover or noncrossover products, depending on which strands are cut, as shown in Fig. 2.

3 Regulation of Recombination

HR and NHEJ are the major DSB repair pathways. NHEJ is preferred in mammalian cells for DSB repair while HR is preferred in yeast. The consequences of deletion of NHEJ-specific factors in yeast are minimal, but in mammalian cells, loss of NHEJ results in increased HR. NHEJ and HR repair DSBs by different mechanisms, but have compensatory roles in maintenance of genomic integrity. Vertebrate cells defective in both NHEJ

and HR have high rates of genome instability and cannot be maintained as stable cell lines. There is greatly increased chromosomal aberrations and cell death. These observations show that spontaneous DSBs occur frequently enough to be lethal if unrepaired and that NHEJ and HR have overlapping roles in genome stability maintenance. Nonetheless, the use of NHEJ or HR to repair DSBs is regulated on several levels, including cell cycle regulation. NHEJ acts primarily in G1-early S for repair of both induced and spontaneous damage, while HR acts in late S-G2. This compartmentalization of the mode DSB repair helps to ensure that most HR DSB repair involves sister chromatid HR instead of interhomolog HR.

3.1 Meiosis versus Mitosis

Meiotic HR differs from mitotic HR in several aspects. These include the nature of the HR initiation events, the frequency of HR, meiotic-specific proteins involved in HR, the presence of the synaptonemal complex, the requirement for crossover HR events, preferential use of the homolog for HR, and the phenomenon of interference, which limits the number of crossovers per chromosome arm in meiotic HR.

While mitotic HR is essential for replication restart after fork stalling or collapse, meiotic HR is essential for proper chromosome segregation in meiosis I, through the occurrence of crossovers between homologous chromosomes. Thus, the ability of most organisms to produce euploid and viable meiotic products is dependent on HR. Most mitotic HR is initiated from spontaneous or exogenously induced DSBs. There are a few instances of programmed DSBs, including mating type switching in the fungi and V(D)J recombination in the immune system of vertebrates. Repair of these breaks occurs though a special program for each DSB system. In meiosis, HR occurs at induced DSBs, induced by action of the type II topoisomerase-like protein called *Spo11*. Spo11 DSBs occur at preferred chromosomal regions, which often have transcription initiation sites, but there is no specific DNA sequence that is recognized by any of the Spo11 proteins. Spo11-induced DSBs occur frequently enough such that the meiotic HR rate is at least 100-fold higher than the mitotic HR rate, and often is 1000-fold higher.

Mitotic HR occurs in the G2 phase of the cell cycle. Although there does not seem to be a specific structure required for mitotic HR, some chromosome scaffold or structure is required. Recent experiments show that DSB repair in G2 requires that chromosome cohesion, cohesion between sister chromatids, be established during S phase and be present in G2. Whether the cohesion complex helps direct most DSB repair by HR to interactions with the sister chromatid instead of the homolog is not known. In meiosis, in most eukaryotic organisms, the chromosomes become associated with a protein structure called the *synaptonemal complex* (*SC*). SC forms between homologous chromosomes. In yeast, DSB formation by Spo11 occurs prior to SC and the SC is thought to aid in homolog alignment. Recombination by the DSBR model occurs in the zygotene and early pachytene stages of meiosis. By pachytene, chromosomes are condensed and paired along their entire lengths, with SC. The SC dissolves in diplotene as chromosomes come apart in preparation for the first meiotic chromosome division.

The link between SC and HR is complicated as in some organisms SC formation is dependent on meiotic DSB formation, while in other organisms SC can form in the absence of DSBs. Nonetheless, mutations in SC components reduce meiotic HR, with resulting chromosome missegregation.

In addition to the meiotic-specific Spo11 protein and SC components, there are proteins that are required for meiotic HR that are expressed only in meiosis. Some of these include the homolog pairing proteins Hop1, Hop2, and Red1, a meiosis-specific Rad51 paralog called *Dmc1*, mismatch repair-like proteins called *Msh4* and *Msh5*, and a meiotic-specific checkpoint kinase called *Mek1*. These proteins are essential for meiotic HR, but have no detectable function in mitosis. Dmc1 does not substitute for Rad51 in the HR step of strand exchange, but does have strand exchange activity *in vitro*. Loss of Dmc1 results in arrest in meiosis during pachytene, without completion of HR. The arrest suggests that there is a checkpoint signal for incomplete HR. Some of the mitotic DNA damage checkpoint factors function in meiosis to ensure efficient repair of the meiotic DSBs by HR and arrest cells when DSBs are not repaired by HR. The Mek1 kinase appears to regulate DSB repair through Dmc1. Msh4 and Msh5 are related to the mismatch repair proteins MutS of *E. coli* and Msh2 of yeast. However, Msh4 and Msh5 do not function in mismatch repair. Rather, they are essential for crossing-over in meiosis and loss of Msh4 or Msh5 in mice results in aberrant meioses and sterility. Msh4 and Msh5 act with the mismatch repair protein Mlh1 in meiosis in this pathway.

Most mitotic DSB repair that produces genetic recombinant products is of the gene conversion type without any associated crossing-over. This observation led to the development of the SDSA model. In contrast, about half of the meiotic genes conversions are associated with crossing-over. This observation was crucial in the development of the DSBR model, with resolution of the HJs as crossover or noncrossover. However, recent studies on the timing of molecular intermediates in meiotic HR in yeast have shown that noncrossovers occur before crossovers. This has led to the suggestion that there are two types of meiotic HR pathways. The first is dedicated to noncrossovers and occurs by the SDSA model for gene conversions. The second pathway occurs slightly later through the DSBR model. Resolution of the HJs results in crossovers. The suggestion is that all noncrossovers come from SDSA HR while crossovers come from DSBR, which has HJs. Moreover, the HJs are always resolved to give crossovers. Both the SDSA and DSBR models involve formation of heteroduplex DNA, which is a substrate for the mismatch repair proteins. Mismatch repair occurs whenever DNA mismatches arise. In mitotic growth, most mismatches occur from errors during DNA replication where an incorrect nucleotide is used, resulting in a base-pair mismatch. If the mismatch is not corrected by the editing function of the DNA polymerase, it becomes a target for the mismatch repair proteins. Mismatch repair is an essential step in recombination, correcting the heteroduplex that has formed between two recombining DNA strands. In the absence of mismatch repair, mutations will accumulate and the strains will have a mutator phenotype. The strains are able to undergo recombination, but maturation of the heteroduplex is defective.

3.2
Sister Chromatid Recombination versus Homolog Recombination

Sister chromatid recombination can only occur in late S or G2, after chromosomes are replicated. As the recombination occurs between identical DNA duplexes, there is no genetic signal for its occurrence. Sister chromatid recombination can be detected genetically when it occurs out of register within a duplication or a multiple

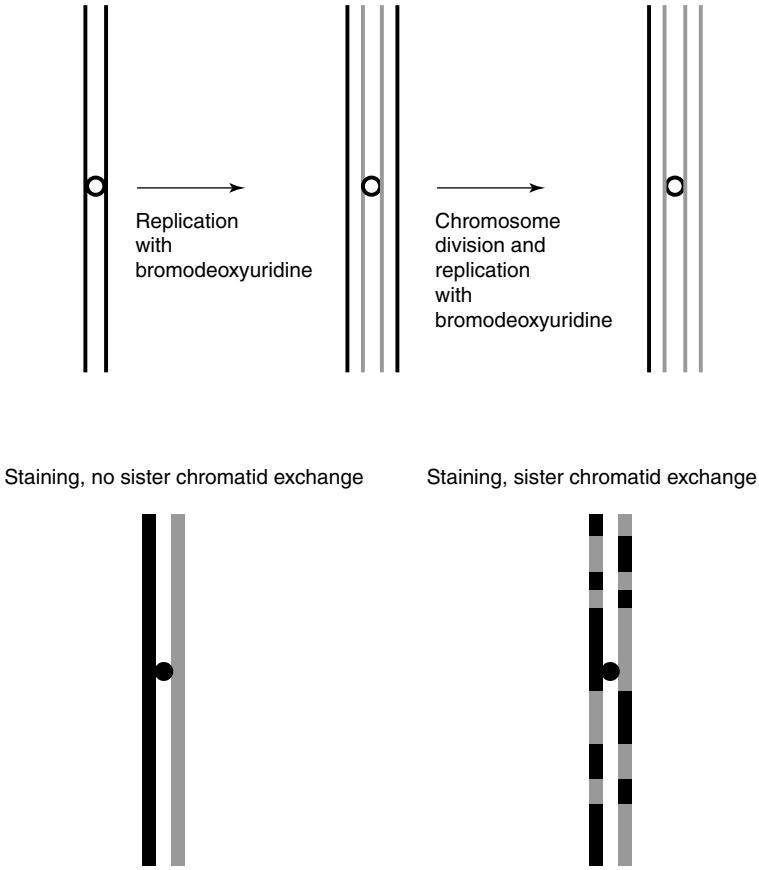


Fig. 6 Cytological detection of sister chromatid exchange. Cell is grown for two rounds of replication in the presence of the nucleotide analog bromodeoxyuridine. After one round of replication, the chromatids are hybrid, with one substituted and one unsubstituted DNA strand. After the second round of replication, cells are arrested in mitosis. The chromosomes have one fully substituted chromatid and one hemi-substituted chromatid. Differential staining of the fully substituted and hemi-substituted chromatids results in a microscopic light/dark pattern, with the fully substituted chromatid being light colored. If no sister chromatid exchange has occurred, then one chromatid is fully dark while the attached sister chromatid is fully light. If sister chromatid exchange has occurred, each chromatid will show an alternating pattern of light and dark regions, and the two attached chromatids are mirror images of each other.

tandem repeat. Assays using duplications have been devised to detect unequal sister chromatid exchange (Fig. 6). Alternatively, sister chromatid recombination can be detected visually in vertebrate cells using chromosome staining methods. Cells are allowed to go through two rounds of replication with a nucleotide substitute of bromodeoxyuridine for thymidine. Cells are then arrested in mitosis with a microtubule inhibitor, so that cells will have replicated chromosomes with both sister chromatids still attached at the centromere. The chromosomes are stained with a fluorescent dye, which reveals the bromodeoxyuridine incorporation, and Giemsa stain for the chromosome regions that do no have bromodeoxyuridine. If no sister chromatid exchange has occurred, the chromatid with both strand containing bromodeoxyuridine will be light colored while the chromatid with one substituted strand will be dark colored. Where sister chromatid exchange has occurred, a single chromatid will contain both light and dark sections, and this alternates with the light/dark staining pattern of the sister chromatid. Such chromosomes are called *harlequin chromosomes*.

Using the harlequin assay, it has been found that spontaneous sister chromatid exchange requires HR proteins, but not NHEJ proteins. DNA damage induced sister chromatid exchanges also required HR proteins, showing that sister chromatid HR is important for DSB repair and damage avoidance. In fact, a hallmark of some human diseases that are defective in the correct response to DNA damage is increased sister chromatid exchange. The defect probably lies in an inability to regulate repair by nonrecombinogenic pathways at stalled replication forks.

Since most mitotic HR between homologs is gene conversion that is not associated with crossing-over, one might expect the same to be true for sister chromatid recombination. Using a recombination substrate consisting of a marked duplication with a site-specific DSB to induce the HR, it has been found that most DSB repair uses the sister chromatid as a template for repair, and that most sister chromatid recombination is in fact gene conversion, not exchange. This is consistent with an SDSA model for sister chromatid recombination and consistent with the observation that crossing-over, either between homolog chromosomes or sister chromatids is infrequent in mitotic cells, even in the presence of DSB damage.

If most DSB repair occurs between sister chromatids, how is the meiotic HR program modified to promote interhomolog HR? Part of the answer must lie in meiotic-specific proteins that promote interhomolog HR. Some of these include the meiotic-specific chromosome cohesion protein Rec8, the SC structure itself, and a newly recognized protein called *Mnd1*, which is proposed to promote close homolog juxtaposition in preparation for strand exchange.

3.3 Regulation by Cell Cycle DNA Damage Checkpoint Factors

DNA damage is sensed by cell cycle checkpoint factors that recognize unrepaired DSBs. One important signal in the checkpoint response is single-strand DNA coated with the single-strand DNA binding protein RPA. DNA damage checkpoint factors bind to unrepaired DSBs and set off a cascade of signal transduction through protein kinases that modify components of the replication and recombination repair machinery and arrest cells in the G2 phase of the cell cycle until damage has

been repaired. At this point, the arrest is reversed and cells resume growth and proceed through mitosis to the next cell cycle. In mammalian cells, apoptosis is also a response to unrepaired DSBs. Key proteins in the checkpoint response are the ATM (ataxia-telangiectasia mutated) and ATR (ATM and RAD3-related) protein kinases, which transduce the damage signal and phosphorylate many proteins involved in DNA repair, recombination, replication transcription, and cell cycle progression.

3.4 Alternative Repair Pathways

As has been discussed in Sects. 1.1 and 1.2, there are multiple DSB repair pathways in eukaryotic cells, some of which do not involve HR. However, not all spontaneous damage originates as DSBs. DSBs may arise from processing of stalled replication forks, but the initial damage may be single-strand DNA gaps at the fork. There is overlap in the pathways used for repair of spontaneous damage. Evidence for this comes from several sources. First, genetic studies in yeast have revealed several genes that function in alternative repair pathways for damage associated with stalled replication forks. One of these pathways involving the yeast *MPH1* gene also requires HR gene functions. However, loss of Mph1 function does not affect HR. Mph1 functions in error-free bypass of DNA lesions, in a manner that also requires HR. This most likely involves sister chromatid interactions, possibly some type of template switching, which requires strand invasion of the sister chromatid to synthesize past DNA lesions.

The second set of observations on repair pathways that overlap with HR comes from studies of chicken DT40 cells. The HR mutant RAD54 (−/−) is viable, but sensitive to ionizing radiation and defective in DSB-promoted HR assays. RAD18 is a key gene in the translesion synthesis repair, to fill in gaps on the daughter strands caused by lesions in the template strands during replication. The mutant RAD18 (−/−) is viable, but sensitive to UV radiation. The mutant has a great increase in sister chromatid exchange events, suggesting that damage normally repaired by RAD18 is now repaired by the HR pathway. The double mutant Rad54 (−/−) RAD18 (−/−) is lethal. These types of experiments highlight the cross talk between different repair modes of the cell.

4 Consequences of Defects in Homologous Recombination

4.1 Rearrangements Associated with Mutations in HR Genes

In yeast, the HR gene mutants are viable. Haploid HR mutants, in addition to reduced HR rates, are associated with increased genomic instability and deletions, translocations, and telomere fusions. Many of the rearrangements have breakpoints with no homology or microhomology. These rearrangements are similar to those observed in cancer cells, suggesting that defective HR results in the types of chromosome aberrations seen in tumor cells. Diploid HR mutants have chromosome loss as the most frequent type of chromosome aberration, although the aberrations listed above also arise, but at much lower frequencies than chromosome loss. These findings show that HR is essential for genome stability maintenance.

In vertebrate cells, many HR mutants are not viable. However, examination of

early embryonic cells has revealed massive chromosome rearrangements. Mutants in the RAD51 paralog genes are associated with chromosome and chromatid breaks. In the chick DT40 system, it has been possible to make conditional RAD51 mutant cells. These mutants have very high levels of spontaneous chromosome breaks and have increased sensitivity to DNA damaging agents.

BRCA2-deficient cells accumulate several different types of chromosome rearrangements and aberrations. Most prevalent are chromosome breaks, chromatid breaks, end-to-end fusions of chromosomes, translocations, and deletions, and chromosomes with multiple arms that appear to result from multiple aberrant recombination reactions. Similar chromosome aberrations have been seen in RAD51-deficient cells. The overlap in phenotypes is expected given that BRCA2 and RAD51 function together at an early stage in HR. The finding that the rearrangements occur spontaneously underscores the importance of HR in maintaining genomic stability in growing cells, repairing lesions that occur during DNA replication.

4.2 Meiotic Defects Associated with Mutations in HR Genes

Meiotic phenotypes associated with defects in HR genes have been seen only in mutants of genes that are either only expressed in meiosis or genes that have milder HR consequences in mitosis. In yeast, the HR mutants are mitotically viable, although they are associated with decreased HR and increased genomic instability and chromosome rearrangements. In meiosis, the same mutants fail to complete meiosis and give no viable meiotic products. Thus, these mutants are meiotic-inviable, but mitotic-viable. There exists another set of mutants in meiotic-specific genes such as *SPO11*, *DMC1*, *MSH4*, and *MSH5* plus genes encoding subunits of the synaptonemal complex. Mutants in these genes give no viable meiotic products, or a reduced number of viable meiotic products with aneuploid karyotypes. In the mouse, *SPO11*−/− mutants are infertile, have synapsis defects cells undergoing meiosis, and apoptosis is increased in the spermatocytes and ooctyes cells.

In vertebrates, most HR mutants are not mitotically viable. Most work has focused on the meiotic-specific genes. Mutants in these genes are associated with aberrant chromosome structures in meiosis and infertility, consistent with the essential role of HR in meiotic chromosome segregation.

5 Concluding Remarks

In the past few years, our understanding of HR models, proteins, and regulation has been elevated by quantum leaps. It is now appreciated that HR occurs by distinct mechanisms, even with the same initiating substrate of a DSB. Most mitotic HR occurs by SDSA, and in fact most mitotic HR probably occurs between sister chromatids, not between homologs. Meiotic HR occurs by SDSA and DSBR, with the two types of HR occurring with different timing in a meiotic program that carefully regulates the number and spacing of crossovers. Most meiotic HR occurs between homologs, in contrast to mitotic HR.

Although some of the HR proteins are related, such as RAD51 and DMC1, and RAD54 and RAD54B, each protein has a distinct role in the HR process. The

number of accessory proteins seems to be increasing to include DNA damage checkpoint factors. One aspect of HR that remains controversial is the eukaryotic resolvase. While resolvase activity has been identified in cell extracts and found to be missing from mutant cell extracts, the resolvase protein has not yet been definitively identified.

The importance of HR is underscored by the finding that HR is essential for all dividing cells. Given its essential role, it is not surprising that there are few human diseases associated with mutations in the genes required for the HR process itself, such as the *RAD51* gene. However, there are rare cases of mutations in genes that encode proteins that are mediators of HR or are checkpoint proteins for the DNA damage response pathway. These include ATM, leading to ataxia-telangiectasia, NBS1, leading to Nijmegen breakage syndrome, ATR, leading to Sekel syndrome, BLM, leading to Bloom syndrome, WRN, leading to Werner syndrome, MRE11, leading to AT-like disease, the FA genes, leading to Fanconi anemia, BRCA1 and BRCA2, leading to breast cancer. Most of these diseases are associated with increased cancer risk, and on the cellular level, there is increased genomic instability and defective repair of DNA damage, including DSBs. Understanding how these HR defects result in genomic instability is the first step in being able to limit the damage caused by genomic instability.

Acknowledgment

Work in the Klein laboratory is supported by grants from the National Institutes of Health (GM53738 and GM070711).

See also Genetics, Molecular Basis of; RecA Superfamily Proteins; Repair and Mutagenesis of DNA.

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