= Homologous recombination =

Homologous recombination is a type of genetic recombination in which nucleotide sequences are exchanged between two similar or identical molecules of DNA . It is most widely used by cells to accurately repair harmful breaks that occur on both strands of DNA , known as double @-@ strand breaks . Homologous recombination also produces new combinations of DNA sequences during meiosis , the process by which eukaryotes make gamete cells , like sperm and egg cells in animals . These new combinations of DNA represent genetic variation in offspring , which in turn enables populations to adapt during the course of evolution . Homologous recombination is also used in horizontal gene transfer to exchange genetic material between different strains and species of bacteria and viruses .

Although homologous recombination varies widely among different organisms and cell types , most forms involve the same basic steps . After a double @-@ strand break occurs , sections of DNA around the 5 ' ends of the break are cut away in a process called resection . In the strand invasion step that follows , an overhanging 3 ' end of the broken DNA molecule then " invades " a similar or identical DNA molecule that is not broken . After strand invasion , the further sequence of events may follow either of two main pathways discussed below (see Models) ; the DSBR (double @-@ strand break repair) pathway or the SDSA (synthesis @-@ dependent strand annealing) pathway . Homologous recombination that occurs during DNA repair tends to result in non @-@ crossover products , in effect restoring the damaged DNA molecule as it existed before the double @-@ strand break .

Homologous recombination is conserved across all three domains of life as well as viruses , suggesting that it is a nearly universal biological mechanism . The discovery of genes for homologous recombination in protists ? a diverse group of eukaryotic microorganisms ? has been interpreted as evidence that meiosis emerged early in the evolution of eukaryotes . Since their dysfunction has been strongly associated with increased susceptibility to several types of cancer , the proteins that facilitate homologous recombination are topics of active research . Homologous recombination is also used in gene targeting , a technique for introducing genetic changes into target organisms . For their development of this technique , Mario Capecchi , Martin Evans and Oliver Smithies were awarded the 2007 Nobel Prize for Physiology or Medicine ; Capecchi and Smithies independently discovered applications to mouse embryonic stem cells , however the highly conserved mechanisms underlying the DSB repair model , including uniform homologous integration of transformed DNA (gene therapy) , were first shown in plasmid experiments by Orr @-@ Weaver , Szostack and Rothstein . Researching the plasmid @-@ induced DSB , using ? @-@ irradiation in the 1970 's @-@ 1980 's , led to later experiments using endonucleases (e.g. I @-@ SceI) to cut chromosomes for genetic engineering of mammalian cells , where nonhomologous recombination is more frequent than in yeast .

= = History and discovery = =

In the early 1900s , William Bateson and Reginald Punnett found an exception to one of the principles of inheritance originally described by Gregor Mendel in the 1860s . In contrast to Mendel 's notion that traits are independently assorted when passed from parent to child ? for example that a cat 's hair color and its tail length are inherited independent of each other ? Bateson and Punnett showed that certain genes associated with physical traits can be inherited together , or genetically linked . In 1911 , after observing that linked traits could on occasion be inherited separately , Thomas Hunt Morgan suggested that " crossovers " can occur between linked genes , where one of the linked genes physically crosses over to a different chromosome . Two decades later , Barbara McClintock and Harriet Creighton demonstrated that chromosomal crossover occurs during meiosis , the process of cell division by which sperm and egg cells are made . Within the same year as McClintock 's discovery , Curt Stern showed that crossing over ? later called " recombination " ? could also occur in somatic cells like white blood cells and skin cells that divide through mitosis .

In 1947 , the microbiologist Joshua Lederberg showed that bacteria ? which had been assumed to

reproduce only asexually through binary fission ? are capable of genetic recombination , which is more similar to sexual reproduction . This work established E. coli as a model organism in genetics , and helped Lederberg win the 1958 Nobel Prize in Physiology or Medicine . Building on studies in fungi , in 1964 Robin Holliday proposed a model for recombination in meiosis which introduced key details of how the process can work , including the exchange of material between chromosomes through Holliday junctions . In 1983 , Jack Szostak and colleagues presented a model now known as the DSBR pathway , which accounted for observations not explained by the Holliday model . During the next decade , experiments in Drosophila , budding yeast and mammalian cells led to the emergence of other models of homologous recombination , called SDSA pathways , which do not always rely on Holliday junctions .

= = In eukaryotes = =

Homologous recombination (HR) is essential to cell division in eukaryotes like plants , animals , fungi and protists . In cells that divide through mitosis , homologous recombination repairs double @-@ strand breaks in DNA caused by ionizing radiation or DNA @-@ damaging chemicals . Left unrepaired , these double @-@ strand breaks can cause large @-@ scale rearrangement of chromosomes in somatic cells , which can in turn lead to cancer .

In addition to repairing DNA , homologous recombination also helps produce genetic diversity when cells divide in meiosis to become specialized gamete cells ? sperm or egg cells in animals , pollen or ovules in plants , and spores in fungi . It does so by facilitating chromosomal crossover , in which regions of similar but not identical DNA are exchanged between homologous chromosomes . This creates new , possibly beneficial combinations of genes , which can give offspring an evolutionary advantage . Chromosomal crossover often begins when a protein called Spo11 makes a targeted double @-@ strand break in DNA . These sites are non @-@ randomly located on the chromosomes ; usually in intergenic promoter regions and preferentially in GC @-@ rich domains These double @-@ strand break sites often occur at recombination hotspots , regions in chromosomes that are about 1 @,@ 000 ? 2 @,@ 000 base pairs in length and have high rates of recombination . The absence of a recombination hotspot between two genes on the same chromosome often means that those genes will be inherited by future generations in equal proportion . This represents linkage between the two genes greater than would be expected from genes that independently assort during meiosis .

= = = Timing within the mitotic cell cycle = = =

Double @-@ strand breaks can be repaired through homologous recombination or through non @-@ homologous end joining (NHEJ) . NHEJ is a DNA repair mechanism which , unlike homologous recombination , does not require a long homologous sequence to guide repair . Whether homologous recombination or NHEJ is used to repair double @-@ strand breaks is largely determined by the phase of cell cycle . Homologous recombination repairs DNA before the cell enters mitosis (M phase) . It occurs during and shortly after DNA replication , in the S and G2 phases of the cell cycle , when sister chromatids are more easily available . Compared to homologous chromosomes , which are similar to another chromosome but often have different alleles , sister chromatids are an ideal template for homologous recombination because they are an identical copy of a given chromosome . In contrast to homologous recombination , NHEJ is predominant in the G1 phase of the cell cycle , when the cell is growing but not yet ready to divide . It occurs less frequently after the G1 phase , but maintains at least some activity throughout the cell cycle . The mechanisms that regulate homologous recombination and NHEJ throughout the cell cycle vary widely between species .

Cyclin @-@ dependent kinases (CDKs) , which modify the activity of other proteins by adding phosphate groups to (that is , phosphorylating) them , are important regulators of homologous recombination in eukaryotes . When DNA replication begins in budding yeast , the cyclin @-@ dependent kinase Cdc28 begins homologous recombination by phosphorylating the Sae2 protein .

After being so activated by the addition of a phosphate , Sae2 uses its endonuclease activity to make a clean cut near a double @-@ strand break in DNA . This allows a three @-@ part protein known as the MRX complex to bind to DNA , and begins a series of protein @-@ driven reactions that exchange material between two DNA molecules .

= = = Models = = =

Two primary models for how homologous recombination repairs double @-@ strand breaks in DNA are the double @-@ strand break repair (DSBR) pathway (sometimes called the double Holliday junction model) and the synthesis @-@ dependent strand annealing (SDSA) pathway . The two pathways are similar in their first several steps . After a double @-@ strand break occurs , the MRX complex (MRN complex in humans) binds to DNA on either side of the break . Next a resection , in which DNA around the 5 ' ends of the break is cut back , is carried out in two distinct steps . In the first step of resection , the MRX complex recruits the Sae2 protein . The two proteins then trim back the 5 ' ends on either side of the break to create short 3 ' overhangs of single @-@ strand DNA . In the second step , 5 ' ? 3 ' resection is continued by the Sgs1 helicase and the Exo1 and Dna2 nucleases . As a helicase , Sgs1 " unzips " the double @-@ strand DNA , while Exo1 and Dna2 's nuclease activity allows them to cut the single @-@ stranded DNA produced by Sgs1 .

The RPA protein , which has high affinity for single @-@ stranded DNA , then binds the 3 ' overhangs . With the help of several other proteins that mediate the process , the Rad51 protein (and Dmc1 , in meiosis) then forms a filament of nucleic acid and protein on the single strand of DNA coated with RPA . This nucleoprotein filament then begins searching for DNA sequences similar to that of the 3 ' overhang . After finding such a sequence , the single @-@ stranded nucleoprotein filament moves into (invades) the similar or identical recipient DNA duplex in a process called strand invasion . In cells that divide through mitosis , the recipient DNA duplex is generally a sister chromatid , which is identical to the damaged DNA molecule and provides a template for repair . In meiosis , however , the recipient DNA tends to be from a similar but not necessarily identical homologous chromosome . A displacement loop (D @-@ loop) is formed during strand invasion between the invading 3 ' overhang strand and the homologous chromosome . After strand invasion , a DNA polymerase extends the end of the invading 3 ' strand by synthesizing new DNA . This changes the D @-@ loop to a cross @-@ shaped structure known as a Holliday junction . Following this , more DNA synthesis occurs on the invading strand (i.e. , one of the original 3 ' overhangs) , effectively restoring the strand on the homologous chromosome that was displaced during strand invasion .

= = = = DSBR pathway = = = =

After the stages of resection , strand invasion and DNA synthesis , the DSBR and SDSA pathways become distinct . The DSBR pathway is unique in that the second 3 ' overhang (which was not involved in strand invasion) also forms a Holliday junction with the homologous chromosome . The double Holliday junctions are then converted into recombination products by nicking endonucleases , a type of restriction endonuclease which cuts only one DNA strand . The DSBR pathway commonly results in crossover , though it can sometimes result in non @-@ crossover products ; the ability of a broken DNA molecule to collect sequences from separated donor loci was shown in mitotic budding yeast using plasmids or endonuclease induction of chromosomal events . Because of this tendency for chromosomal crossover , the DSBR pathway is a likely model of how crossover homologous recombination occurs during meiosis .

Whether recombination in the DSBR pathway results in chromosomal crossover is determined by how the double Holliday junction is cut , or " resolved " . Chromosomal crossover will occur if one Holliday junction is cut on the crossing strand and the other Holliday junction is cut on the non @-@ crossing strand (in Figure 4 , along the horizontal purple arrowheads at one Holliday junction and along the vertical orange arrowheads at the other) . Alternatively , if the two Holliday junctions are cut on the crossing strands (along the horizontal purple arrowheads at both Holliday junctions in

Figure 4) , then chromosomes without crossover will be produced .

= = = = SDSA pathway = = = =

Homologous recombination via the SDSA pathway occurs in cells that divide through mitosis and meiosis and results in non @-@ crossover products . In this model , the invading 3 ' strand is extended along the recipient DNA duplex by a DNA polymerase , and is released as the Holliday junction between the donor and recipient DNA molecules slides in a process called branch migration . The newly synthesized 3 ' end of the invading strand is then able to anneal to the other 3 ' overhang in the damaged chromosome through complementary base pairing . After the strands anneal , a small flap of DNA can sometimes remain . Any such flaps are removed , and the SDSA pathway finishes with the resealing , also known as ligation , of any remaining single @-@ stranded gaps .

During mitosis , the major homologous recombination pathway for repairing DNA double @-@ strand breaks appears to be the SDSA pathway (rather than the DSBR pathway) . The SDSA pathway produces non @-@ crossover recombinants (Figure 4) . During meiosis non @-@ crossover recombinants also occur frequently and these appear to arise mainly by the SDSA pathway as well . Non @-@ crossover recombination events occurring during meiosis likely reflect instances of repair of DNA double @-@ strand damages or other types of DNA damages .

= = = = SSA pathway = = = =

The single @-@ strand annealing (SSA) pathway of homologous recombination repairs double @-@ strand breaks between two repeat sequences . The SSA pathway is unique in that it does not require a separate similar or identical molecule of DNA , like the DSBR or SDSA pathways of homologous recombination . Instead , the SSA pathway only requires a single DNA duplex , and uses the repeat sequences as the identical sequences that homologous recombination needs for repair . The pathway is relatively simple in concept : after two strands of the same DNA duplex are cut back around the site of the double @-@ strand break , the two resulting 3 ' overhangs then align and anneal to each other , restoring the DNA as a continuous duplex .

As DNA around the double @-@ strand break is cut back , the single @-@ stranded 3 ' overhangs being produced are coated with the RPA protein , which prevents the 3 ' overhangs from sticking to themselves . A protein called Rad52 then binds each of the repeat sequences on either side of the break , and aligns them to enable the two complementary repeat sequences to anneal . After annealing is complete , leftover non @-@ homologous flaps of the 3 ' overhangs are cut away by a set of nucleases , known as Rad1 / Rad10 , which are brought to the flaps by the Saw1 and Slx4 proteins . New DNA synthesis fills in any gaps , and ligation restores the DNA duplex as two continuous strands . The DNA sequence between the repeats is always lost , as is one of the two repeats . The SSA pathway is considered mutagenic since it results in such deletions of genetic material .

= = = = BIR pathway = = = =

During DNA replication , double @-@ strand breaks can sometimes be encountered at replication forks as DNA helicase unzips the template strand . These defects are repaired in the break @-@ induced replication (BIR) pathway of homologous recombination . The precise molecular mechanisms of the BIR pathway remain unclear . Three proposed mechanisms have strand invasion as an initial step , but they differ in how they model the migration of the D @-@ loop and later phases of recombination .

The BIR pathway can also help to maintain the length of telomeres (regions of DNA at the end of eukaryotic chromosomes) in the absence of (or in cooperation with) telomerase . Without working copies of the telomerase enzyme , telomeres typically shorten with each cycle of mitosis , which eventually blocks cell division and leads to senescence . In budding yeast cells where telomerase

has been inactivated through mutations , two types of " survivor " cells have been observed to avoid senescence longer than expected by elongating their telomeres through BIR pathways .

Maintaining telomere length is critical for cell immortalization , a key feature of cancer . Most cancers maintain telomeres by upregulating telomerase . However , in several types of human cancer , a BIR @-@ like pathway helps to sustain some tumors by acting as an alternative mechanism of telomere maintenance . This fact has led scientists to investigate whether such recombination @-@ based mechanisms of telomere maintenance could thwart anti @-@ cancer drugs like telomerase inhibitors .

= = In bacteria = =

Homologous recombination is a major DNA repair process in bacteria . It is also important for producing genetic diversity in bacterial populations , although the process differs substantially from meiotic recombination , which repairs DNA damages and brings about diversity in eukaryotic genomes . Homologous recombination has been most studied and is best understood for Escherichia coli . Double @-@ strand DNA breaks in bacteria are repaired by the RecBCD pathway of homologous recombination . Breaks that occur on only one of the two DNA strands , known as single @-@ strand gaps , are thought to be repaired by the RecF pathway . Both the RecBCD and RecF pathways include a series of reactions known as branch migration , in which single DNA strands are exchanged between two intercrossed molecules of duplex DNA , and resolution , in which those two intercrossed molecules of DNA are cut apart and restored to their normal double @-@ stranded state .

= = = RecBCD pathway = = =

The RecBCD pathway is the main recombination pathway used in many bacteria to repair double @-@ strand breaks in DNA , and the proteins are found in a broad array of bacteria . These double @-@ strand breaks can be caused by UV light and other radiation , as well as chemical mutagens . Double @-@ strand breaks may also arise by DNA replication through a single @-@ strand nick or gap . Such a situation causes what is known as a collapsed replication fork and is fixed by several pathways of homologous recombination including the RecBCD pathway .

In this pathway , a three @-@ subunit enzyme complex called RecBCD initiates recombination by binding to a blunt or nearly blunt end of a break in double @-@ strand DNA . After RecBCD binds the DNA end , the RecB and RecD subunits begin unzipping the DNA duplex through helicase activity . The RecB subunit also has a nuclease domain , which cuts the single strand of DNA that emerges from the unzipping process . This unzipping continues until RecBCD encounters a specific nucleotide sequence (5 ' -GCTGGTGG @-@ 3 ') known as a Chi site .

Upon encountering a Chi site , the activity of the RecBCD enzyme changes drastically . DNA unwinding pauses for a few seconds and then resumes at roughly half the initial speed . This is likely because the slower RecB helicase unwinds the DNA after Chi , rather than the faster RecD helicase , which unwinds the DNA before Chi . Recognition of the Chi site also changes the RecBCD enzyme so that it cuts the DNA strand with Chi and begins loading multiple RecA proteins onto the single @-@ stranded DNA with the newly generated 3 ' end . The resulting RecA @-@ coated nucleoprotein filament then searches out similar sequences of DNA on a homologous chromosome . The search process induces stretching of the DNA duplex , which enhances homology recognition (a mechanism termed conformational proofreading) . Upon finding such a sequence , the single @-@ stranded nucleoprotein filament moves into the homologous recipient DNA duplex in a process called strand invasion . The invading 3 ' overhang causes one of the strands of the recipient DNA duplex to be displaced , to form a D @-@ loop . If the D @-@ loop is cut , another swapping of strands forms a cross @-@ shaped structure called a Holliday junction . Resolution of the Holliday junction by some combination of RuvABC or RecG can produce two recombinant DNA molecules with reciprocal genetic types , if the two interacting DNA molecules differ genetically . Alternatively , the invading 3 ? end near Chi can prime DNA synthesis and form a replication fork . This type of

resolution produces only one type of recombinant (non @-@ reciprocal) .

= = = RecF pathway = = =

Bacteria appear to use the RecF pathway of homologous recombination to repair single @-@ strand gaps in DNA . When the RecBCD pathway is inactivated by mutations and additional mutations inactivate the SbcCD and ExoI nucleases , the RecF pathway can also repair DNA double @-@ strand breaks . In the RecF pathway the RecQ helicase unwinds the DNA and the RecJ nuclease degrades the strand with a 5 ? end , leaving the strand with the 3 ? end intact . RecA protein binds to this strand and is either aided by the RecF , RecO , and RecR proteins or stabilized by them . The RecA nucleoprotein filament then searches for a homologous DNA and exchanges places with the identical or nearly identical strand in the homologous DNA .

Although the proteins and specific mechanisms involved in their initial phases differ , the two pathways are similar in that they both require single @-@ stranded DNA with a 3 ? end and the RecA protein for strand invasion . The pathways are also similar in their phases of branch migration , in which the Holliday junction slides in one direction , and resolution , in which the Holliday junctions are cleaved apart by enzymes . The alternative , non @-@ reciprocal type of resolution may also occur by either pathway .

= = = Branch migration = = =

Immediately after strand invasion , the Holliday junction moves along the linked DNA during the branch migration process . It is in this movement of the Holliday junction that base pairs between the two homologous DNA duplexes are exchanged . To catalyze branch migration , the RuvA protein first recognizes and binds to the Holliday junction and recruits the RuvB protein to form the RuvAB complex . Two sets of the RuvB protein , which each form a ring @-@ shaped ATPase , are loaded onto opposite sides of the Holliday junction , where they act as twin pumps that provide the force for branch migration . Between those two rings of RuvB , two sets of the RuvA protein assemble in the center of the Holliday junction such that the DNA at the junction is sandwiched between each set of RuvA . The strands of both DNA duplexes ? the " donor " and the " recipient " duplexes ? are unwound on the surface of RuvA as they are guided by the protein from one duplex to the other .

= = = Resolution = = =

In the resolution phase of recombination , any Holliday junctions formed by the strand invasion process are cut , thereby restoring two separate DNA molecules . This cleavage is done by RuvAB complex interacting with RuvC , which together form the RuvABC complex . RuvC is an endonuclease that cuts the degenerate sequence 5 ' - (A / T) TT (G / C) -3 ' . The sequence is found frequently in DNA , about once every 64 nucleotides . Before cutting , RuvC likely gains access to the Holliday junction by displacing one of the two RuvA tetramers covering the DNA there . Recombination results in either " splice " or " patch " products , depending on how RuvC cleaves the Holliday junction . Splice products are crossover products , in which there is a rearrangement of genetic material around the site of recombination . Patch products , on the other hand , are non @-@ crossover products in which there is no such rearrangement and there is only a " patch " of hybrid DNA in the recombination product .

= = = Facilitating genetic transfer = = =

Homologous recombination is an important method of integrating donor DNA into a recipient organism 's genome in horizontal gene transfer , the process by which an organism incorporates foreign DNA from another organism without being the offspring of that organism . Homologous recombination requires incoming DNA to be highly similar to the recipient genome , and so horizontal gene transfer is usually limited to similar bacteria . Studies in several species of bacteria

have established that there is a log @-@ linear decrease in recombination frequency with increasing difference in sequence between host and recipient DNA .

In bacterial conjugation , where DNA is transferred between bacteria through direct cell @-@ to @-@ cell contact , homologous recombination helps integrate foreign DNA into the host genome via the RecBCD pathway . The RecBCD enzyme promotes recombination after DNA is converted from single @-@ strand DNA ? in which form it originally enters the bacterium ? to double @-@ strand DNA during replication . The RecBCD pathway is also essential for the final phase of transduction , a type of horizontal gene transfer in which DNA is transferred from one bacterium to another by a virus . Foreign , bacterial DNA is sometimes misincorporated in the capsid head of bacteriophage virus particles as DNA is packaged into new bacteriophages during viral replication . When these new bacteriophages infect other bacteria , DNA from the previous host bacterium is injected into the new bacterial host as double @-@ strand DNA . The RecBCD enzyme then incorporates this double @-@ strand DNA into the genome of the new bacterial host .

= = = Bacterial transformation = = =

Natural bacterial transformation involves the transfer of DNA from a donor bacterium to a recipient bacterium , where both donor and recipient are ordinarily of the same species . Transformation , unlike bacterial conjugation and transduction , depends on numerous bacterial gene products that specifically interact to perform this process . Thus transformation is clearly a bacterial adaptation for DNA transfer . In order for a bacterium to bind , take up and integrate donor DNA into its resident chromosome by homologous recombination , it must first enter a special physiological state termed competence . The RecA / Rad51 / DMC1 gene family plays a central role in homologous recombination during bacterial transformation as it does during eukaryotic meiosis and mitosis . For instance , the RecA protein is essential for transformation in Bacillus subtilis and Streptococcus pneumoniae , and expression of the RecA gene is induced during the development of competence for transformation in these organisms .

As part of the transformation process , the RecA protein interacts with entering single @-@ stranded DNA (ssDNA) to form RecA / ssDNA nucleofilaments that scan the resident chromosome for regions of homology and bring the entering ssDNA to the corresponding region , where strand exchange and homologous recombination occur . Thus the process of homologous recombination during bacterial transformation has fundamental similarities to homologous recombination during meiosis .

= = In viruses = =

Homologous recombination occurs in several groups of viruses . In DNA viruses such as herpesvirus , recombination occurs through a break @-@ and @-@ rejoin mechanism like in bacteria and eukaryotes . There is also evidence for recombination in some RNA viruses , specifically positive @-@ sense ssRNA viruses like retroviruses , picornaviruses , and coronaviruses . There is controversy over whether homologous recombination occurs in negative @-@ sense ssRNA viruses like influenza .

In RNA viruses , homologous recombination can be either precise or imprecise . In the precise type of RNA @-@ RNA recombination , there is no difference between the two parental RNA sequences and the resulting crossover RNA region . Because of this , it is often difficult to determine the location of crossover events between two recombining RNA sequences . In imprecise RNA homologous recombination , the crossover region has some difference with the parental RNA sequences ? caused by either addition , deletion , or other modification of nucleotides . The level of precision in crossover is controlled by the sequence context of the two recombining strands of RNA : sequences rich in adenine and uracil decrease crossover precision .

Homologous recombination is important in facilitating viral evolution . For example , if the genomes of two viruses with different disadvantageous mutations undergo recombination , then they may be able to regenerate a fully functional genome . Alternatively , if two similar viruses have infected the

same host cell , homologous recombination can allow those two viruses to swap genes and thereby evolve more potent variations of themselves .

Homologous recombination is the proposed mechanism whereby the DNA virus human herpesvirus @-@ 6 integrates into human telomeres .

When two or more viruses , each containing lethal genomic damage , infect the same host cell , the virus genomes can often pair with each other and undergo homologous recombinational repair to produce viable progeny . This process , known as multiplicity reactivation , has been studied in several bacteriophages , including phage T4 . Enzymes employed in recombinational repair in phage T4 are functionally homologous to enzymes employed in bacterial and eukaryotic recombinational repair . In particular , with regard to a gene necessary for the strand exchange reaction , a key step in homologous recombinational repair , there is functional homology from viruses to humans (i. e. uvsX in phage T4 ; recA in E. coli and other bacteria , and rad51 and dmc1 in yeast and other eukaryotes , including humans) . Multiplicity reactivation has also been demonstrated in numerous pathogenic viruses .

= = Effects of dysfunction = =

Without proper homologous recombination , chromosomes often incorrectly align for the first phase of cell division in meiosis . This causes chromosomes to fail to properly segregate in a process called nondisjunction . In turn , nondisjunction can cause sperm and ova to have too few or too many chromosomes . Down 's syndrome , which is caused by an extra copy of chromosome 21 , is one of many abnormalities that result from such a failure of homologous recombination in meiosis .

Deficiencies in homologous recombination have been strongly linked to cancer formation in humans . For example , each of the cancer @-@ related diseases Bloom 's syndrome , Werner 's syndrome and Rothmund @-@ Thomson syndrome are caused by malfunctioning copies of RecQ helicase genes involved in the regulation of homologous recombination : BLM , WRN and RECQ4 , respectively . In the cells of Bloom 's syndrome patients , who lack a working copy of the BLM protein , there is an elevated rate of homologous recombination . Experiments in mice deficient in BLM have suggested that the mutation gives rise to cancer through a loss of heterozygosity caused by increased homologous recombination . A loss in heterozygosity refers to the loss of one of two versions ? or alleles ? of a gene . If one of the lost alleles helps to suppress tumors , like the gene for the retinoblastoma protein for example , then the loss of heterozygosity can lead to cancer .

Decreased rates of homologous recombination cause inefficient DNA repair , which can also lead to cancer . This is the case with BRCA1 and BRCA2 , two similar tumor suppressor genes whose malfunctioning has been linked with considerably increased risk for breast and ovarian cancer . Cells missing BRCA1 and BRCA2 have a decreased rate of homologous recombination and increased sensitivity to ionizing radiation , suggesting that decreased homologous recombination leads to increased susceptibility to cancer . Because the only known function of BRCA2 is to help initiate homologous recombination , researchers have speculated that more detailed knowledge of BRCA2 's role in homologous recombination may be the key to understanding the causes of breast and ovarian cancer .

= = Evolutionary Conservation = =

While the pathways can mechanistically vary , the ability of organisms to perform homologous recombination is universally conserved across all domains of life . Based on the similarity of their amino acid sequences , homologs of a number of proteins can be found in multiple domains of life indicating that they evolved a long time ago , and have since diverged from common ancestral proteins .

RecA recombinase family members are found in almost all organisms with RecA in bacteria , Rad51 and DMC1 in eukaryotes , RadA in archaea , and UvsX in T4 phage .

Related single stranded binding proteins that are important for homologous recombination , and many other processes , are also found in all domains of life .

Rad54 , Mre11 , Rad50 , and a number of other proteins are also found in both archaea and eukaryotes .

= = = The RecA Recombinase Family = = =

The proteins of the RecA recombinase family of proteins are thought to be descended from a common ancestral recombinase . The RecA recombinase family contains RecA protein from bacteria , the Rad51 and Dmc1 proteins from eukaryotes , and RadA from archaea , and the recombinase paralog proteins . Studies modeling the evolutionary relationships between the Rad51 , Dmc1 and RadA proteins indicate that they are monophyletic , or that they share a common molecular ancestor . Within this protein family , Rad51 and Dmc1 are grouped together in a separate clade from RadA . One of the reasons for grouping these three proteins together is that they all possess a modified helix @-@ turn @-@ helix motif , which helps the proteins bind to DNA , toward their N @-@ terminal ends . An ancient gene duplication event of a eukaryotic RecA gene and subsequent mutation has been proposed as a likely origin of the modern RAD51 and DMC1 genes .
The proteins generally share a long conserved region known as the RecA / Rad51 domain . Within this protein domain are two sequence motifs , Walker A motif and Walker B motif . The Walker A and B motifs allow members of the RecA / Rad51 protein family to engage in ATP binding and ATP hydrolysis .

= = = Meiosis specific proteins = = =

The discovery of Dmc1 in several species of Giardia , one of the earliest protists to diverge as a eukaryote , suggests that meiotic homologous recombination ? and thus meiosis itself ? emerged very early in eukaryotic evolution . In addition to research on Dmc1 , studies on the Spo11 protein have provided information on the origins of meiotic recombination . Spo11 , a type II topoisomerase , can initiate homologous recombination in meiosis by making targeted double @-@ strand breaks in DNA . Phylogenetic trees based on the sequence of genes similar to SPO11 in animals , fungi , plants , protists and archaea have led scientists to believe that the version Spo11 currently in eukaryotes emerged in the last common ancestor of eukaryotes and archaea .

= = Technological applications = =

= = = Gene targeting = = =

Many methods for introducing DNA sequences into organisms to create recombinant DNA and genetically modified organisms use the process of homologous recombination . Also called gene targeting , the method is especially common in yeast and mouse genetics . The gene targeting method in knockout mice uses mouse embryonic stem cells to deliver artificial genetic material (mostly of therapeutic interest) , which represses the target gene of the mouse by the principle of homologous recombination . The mouse thereby acts as a working model to understand the effects of a specific mammalian gene . In recognition of their discovery of how homologous recombination can be used to introduce genetic modifications in mice through embryonic stem cells , Mario Capecchi , Martin Evans and Oliver Smithies were awarded the 2007 Nobel Prize for Physiology or Medicine .
Advances in gene targeting technologies which hijack the homologous recombination mechanics of cells are now leading to the development of a new wave of more accurate , isogenic human disease models . These engineered human cell models are thought to more accurately reflect the genetics of human diseases than their mouse model predecessors . This is largely because mutations of interest are introduced into endogenous genes , just as they occur in the real patients , and because they are based on human genomes rather than rat genomes . Furthermore , certain technologies enable the knock @-@ in of a particular mutation rather than just knock @-@ outs associated with

older gene targeting technologies .

= = = Protein engineering = = =

Protein engineering with homologous recombination develops chimeric proteins by swapping fragments between two parental proteins . These techniques exploit the fact that recombination can introduce a high degree of sequence diversity while preserving a protein 's ability to fold into its tertiary structure , or three @-@ dimensional shape . This stands in contrast to other protein engineering techniques , like random point mutagenesis , in which the probability of maintaining protein function declines exponentially with increasing amino acid substitutions . The chimeras produced by recombination techniques are able to maintain their ability to fold because their swapped parental fragments are structurally and evolutionarily conserved . These recombinable " building blocks " preserve structurally important interactions like points of physical contact between different amino acids in the protein 's structure . Computational methods like SCHEMA and statistical coupling analysis can be used to identify structural subunits suitable for recombination .

Techniques that rely on homologous recombination have been used to engineer new proteins . In a study published in 2007 , researchers were able to create chimeras of two enzymes involved in the biosynthesis of isoprenoids , a diverse class of compounds including hormones , visual pigments and certain pheromones . The chimeric proteins acquired an ability to catalyze an essential reaction in isoprenoid biosynthesis ? one of the most diverse pathways of biosynthesis found in nature ? that was absent in the parent proteins . Protein engineering through recombination has also produced chimeric enzymes with new function in members of a group of proteins known as the cytochrome P450 family , which in humans is involved in detoxifying foreign compounds like drugs , food additives and preservatives .

= = = Cancer therapy = = =

Cancer cells with BRCA mutations have deficiencies in homologous recombination , and drugs to exploit those deficiencies have been developed and used successfully in clinical trials . Olaparib , a PARP1 inhibitor , shrunk or stopped the growth of tumors from breast , ovarian and prostate cancers caused by mutations in the BRCA1 or BRCA2 genes , which are necessary for HR . When BRCA1 or BRCA2 is absent , other types of DNA repair mechanisms must compensate for the deficiency of HR , such as base @-@ excision repair (BER) for stalled replication forks or non @-@ homologous end joining (NHEJ) for double strand breaks . By inhibiting BER in an HR @-@ deficient cell , olaparib applies the concept of synthetic lethality to specifically target cancer cells . While PARP1 inhibitors represent a novel approach to cancer therapy , researchers have cautioned that they may prove insufficient for treating late @-@ stage metastatic cancers . Cancer cells can become resistant to a PARP1 inhibitor if they undergo deletions of mutations in BRCA2 , undermining the drug 's synthetic lethality by restoring cancer cells ' ability to repair DNA by HR .