

## Deep Insight Section

# Tying up Loose Ends: Generation and Repair of DNA Double-Strand Breaks

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DNA is continually subject to the threat of damage by a wide variety of external agents as well as by spontaneous endogenous processes (Friedberg et al., 1995). A given agent or process can produce a complex variety of DNA lesions: for example, ionizing radiation generates free radicals that make single and double strand breaks, destroy deoxyribose residues and induce numerous base alterations (Frimmer et al., 1976). DNA damage can be classified in several ways. One broad category consists of base modifications, including chemical alterations, covalent joining between adjacent bases (the predominant lesion is the UV-induced pyrimidine-pyrimidine dimer (see nucleotide excision repair) and base loss. These damages to DNA templates can result in misincorporation during replication and can lead to heritable changes in DNA sequence. Another type of damage is intrastrand cross-linking, which can prevent DNA replication and transcription. This review focuses on the repair of a third type of DNA damage, DNA strand breaks, and more particularly, DNA double-strand breaks (DSBs). DSBs may occur as a result of exposure to such exogenous factors such as radiation or chemical DNA-damaging agents. DSBs can also arise as programmed developmental modifications, such as immunoglobulin rearrangement or in the initiation of meiotic recombination. There is also growing evidence that DSBs can form when DNA polymerases stall at natural replication pause sites or when they encounter other types of DNA damage (reviewed in Rothstein et al.,

2000). Since DSBs may be lethal for a cell, organisms have developed several repair pathways.

An important DSB repair pathway is Homologous Recombination (HR), which is potentially a very precise mode of repair. When a homologous sequence at the equivalent position on a sister chromatid is used as a template for repair, the original sequence of the broken chromosome can be restored (Jablonovich et al., 1999) (Figure 1a). Recombinational repair may also utilize allelic sequences (i.e., at equivalent positions on homologous chromosomes) in the resolution of DSBs (Figure 1b). Although recombination between allelic sequences is not mutagenic per se, this mode of repair can be associated with another hazard: loss of heterozygosity (LOH). Recessive mutations may be uncovered in a heterozygote, if a damaged wild type allele is lost and a second copy of a mutant allele is concomitantly gained (Lasko et al., 1991). Another unfavorable outcome of HR is chromosomal rearrangement, which can happen if recombination occurs between homologous sequences at non allelic loci. This possibility arises because a large fraction of mammalian genomes consists of numerous classes of repetitive DNA sequences distributed among numerous loci. (For example, in human cells there are approximately 106 Alu elements.) If an exchange takes place between repeats at non equivalent positions on sister chromatids, deletions or expansions can result (Figure 1c). Exchanges between repeats on different chromosomes can give rise to translocations (Figure 1d).

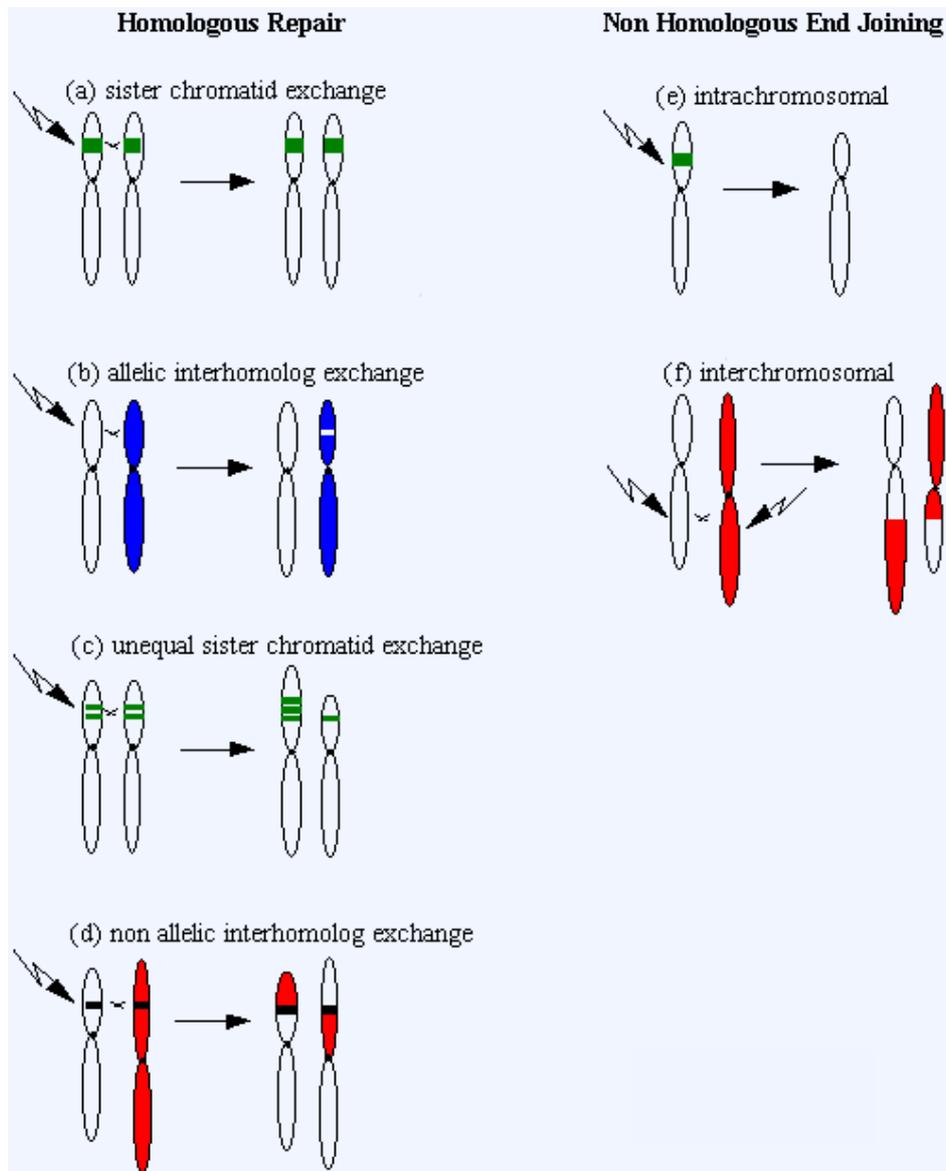


Figure 1: Modes of DSB repair and some chromosomal consequences of faulty repair repair (see also " An introduction to chromosomal aberrations).

**Homologous Repair:** (a) Precise homologous recombination with the sister chromatid restores the original sequence. (b) Recombination between allelic sequences on homologous chromosomes can lead to Loss Of Heterozygosity if a damaged wild type sequence is repaired using a mutant allele as a template. (c) A recombination event between two homologous sequences located in non equivalent positions on sister chromatids can give rise to an unequal exchange. In this example, one chromatid has lost one of two repeated sequences and the other has gained a repeat. (d) When a DSB is repaired by an exchange between two homologous sequences located on different chromosomes, a reciprocal translocation can result.

**Non Homologous End Joining:** (e) Following processing of a DSB, a rejoining event can lead to an intra-chromosomal deletion. (f) Ligation of two DNA ends located on different chromosomes results in a non reciprocal translocation.

The principal DSB repair mechanism in higher eukaryotes is Non Homologous End Joining (NHEJ), which entails the simple ligation of two DNA ends with little or no homology to one another (Karran, 2000; Lewis and Resnick, 2000). Ligation can precisely rejoin two DNA ends so as introduce no changes in sequence. However, NHEJ is most often accompanied by remodeling or processing of the ends before the rejoining event. (Figure 1e). The occurrence of several DSBs in the cell can lead to the joining of DNA ends

that are located on two different chromosomes (Figure 1f). Thus, NHEJ is relatively error-prone and is frequently associated with genomic rearrangements such as deletions and translocations.

In this review, we focus on the pathways and enzymology of DSB main mechanisms that cells use to repair DSBs. Many insights concerning DSB repair originated in studies of meiotic and mitotic recombination in the yeast *Saccharomyces cerevisiae*. Therefore, we begin with a discussion of the DSB

repair model as it applies to meiotic HR as a means to introduce many of the key proteins implicated in DSB repair in higher organisms. We then describe mitotic HR and NHEJ in yeast and mammalian cells, emphasizing both parallels and differences between the two systems. We also outline some of the cellular responses to DNA damage. And finally, we describe several human diseases for which DSB repair defects are responsible.

### Meiotic DSB repair in yeast provides a model to illustrate HR

Much of what is known about the mechanism of the repair of DSBs by HR is derived from genetic and biochemical studies of meiotic recombination in the yeast *Saccharomyces cerevisiae*. Yeast offers many advantages for the study of DSB induction and repair, including the ease of synchronization of cells for simultaneous entry into the meiotic cell cycle and the relative facility of designing screens to detect mutations that affect a given point of the process. Also, DNA intermediates in the repair process can be isolated and

characterized. In yeast, meiotic recombination is initiated by programmed DSBs at numerous sites throughout the genome (Baudat and Nicolas, 1997; Gerton et al., 2000; Kleckner, 1996; Roeder, 1997; Smith and Nicolas, 1998). The repair of these breaks establishes connections (crossovers) between homologous chromosomes. Crossovers are critical to the proper disjunction of homologs from one another in the first of the two meiotic divisions. They are also responsible for the reciprocal exchange of sequences between homologous chromosomes, thereby contributing to genetic variation. In yeast and in higher organisms, failures to create DSBs, or defects in the repair of DSBs, lead to random disjunction of chromosomes, hence aneuploidy and gamete inviability (Bascom-Slack et al., 1997). Mammalian homologs have been identified for many of the components of the yeast meiotic DSB repair pathway, which underscores the likelihood that observations concerning the mechanism of DSB-initiated meiotic recombination in yeast are applicable to human cells (Table 1).

Yeast	Human	Disruption in mice	Molecular Function
RAD51	RAD51	lethal (Tsuzuki et al., 1996)	RecA homolog, pairing and strand exchange
RAD55, RAD57	RAD51 B XRCC2 XRCC3 RAD51 C RAD51 D	homologous recombination deficiency (Brenneman et al., 2000; Johnson et al., 1999; Pierce et al., 1999; Takata et al., 2000) midgestation lethality (Pittman and Schimenti, 2000; Shu et al., 1999)	RecA homolog, stimulates pairing and strand exchange
DMC1	DMC1	sterility (Pittman et al., 1998; Yoshida et al., 1998)	RecA homolog, pairing
RAD54	RAD54L RAD54B	radiation sensitivity (Essers et al., 1997)	DNA dependent ATPase, involved in pairing and recombination between sister chromatids
RDH54			
RAD52	RAD52	homologous recombination deficiency (Rijkers et al., 1998)	ssDNA annealing, stimulates pairing and strand exchange
RAD59			
RAD50	RAD50	lethal (Luo et al., 1999)	DNA-binding
MRE11	MRE11	lethal (Xiao and Weaver, 1997)	ssDNA endonuclease, 3' to 5' exonuclease
XRS2	NBS1	lethal (Zhu et al., 2001)	
HDF1	Ku70	growth retardation radiation sensitivity non-homologous end joining deficiency (Gu et al., 1997a; Gu et al., 1997b)	DNA end binding protein
HDF2	Ku80	early senescence radiation sensitivity non-homologous end joining deficiency (Nussenzweig et al., 1996; Nussenzweig et al., 1997; Zhu et al., 1996)	DNA end binding protein
	DNA-PK	radiation sensitivity non-homologous end joining deficiency (Gao et al., 1998; Jhappan et al., 1997; Taccioli et al., 1998)	kinase
DNL4	Ligase4	late embryonic lethality (Barnes et al., 1998; Frank et al., 1998)	DNA ligase
LIF1	XRCC4	lethal (Gao et al., 2000)	DNA ligase co-factor
	BRCA1	lethal (Hakem et al., 1998)	
	BRCA2	lethal (Hakem et al., 1998; Suzuki et al., 1997)	
	ATM	growth retardation, neurologic dysfunction, male and female infertility defects in T lymphocyte maturation, extreme sensitivity to gamma-irradiation, malignant thymic lymphomas between 2 and 4 months of age (Barlow et al., 1996; Xu et al., 1996)	protein kinase

Table 1: The DSB repair factors in yeast and their human homologs.

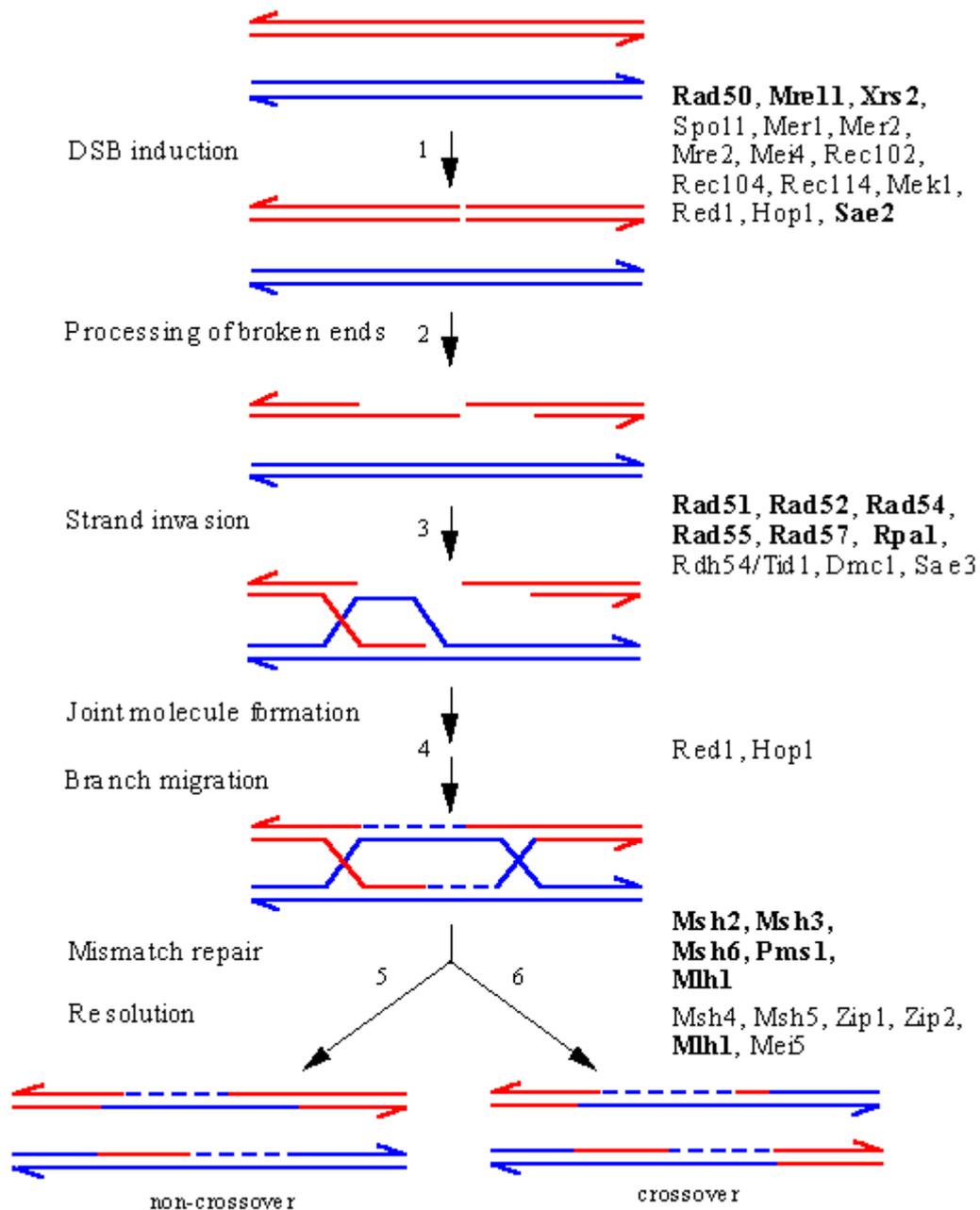


Figure 2: Meiotic DSB repair in yeast.

An overview of meiotic recombination between two homologous chromosomes, based on the DSB repair model, showing intermediates and the proteins implicated in their formation by genetic and/or molecular criteria. The points at which several proteins act is still under investigation, and some may be required at several steps. Proteins that function in both mitotic and meiotic HR are indicated in bold; all others are unique to meiosis. A DSB is introduced in a DNA duplex by the Spo11 nuclease, likely acting in conjunction with several other proteins that are known to be required for DSB induction. The 5' ends of the break undergo 5' to 3' resection to yield 3'-OH single-stranded tails. 3) One of these single-stranded tails invades a homologous duplex, displacing a D-loop. 4) Several steps resulting in the formation of a bimolecular intermediate with double Holliday junctions follow. At this point, correction of mismatches in heteroduplex DNA can result in gene conversion. 5) Resolution of the intermediate to yield a product with a parental configuration of flanking sequences (non-crossover). 6) Resolution to yield a crossover product.

Similarly, as exemplified by the homology of the yeast DSB repair protein Rad51 (and with Rad51 homologs found in humans and other higher organisms) with the bacterial strand exchange protein RecA, the yeast meiotic DSB repair pathway represents an eukaryotic adaptation of primordial bacterial and archaeobacterial

repair systems (Ogawa et al., 1993a; Ogawa et al., 1993b; Sandler et al., 1996).

The mechanism of meiotic DSB repair by HR can be described by a model that was initially proposed to account for the resolution of broken DNA molecules in mitotic cells (Szostak et al., 1983). Many of the molecular events predicted by this model have been

demonstrated to take place during yeast meiosis (Figure 2). Shortly after the synchronous entry of cells into meiosis, DSBs can be detected by Southern analysis at numerous loci, particularly those which are recombinational "hotspots" - they exhibit high frequencies of gene conversion and crossing over (Baudat and Nicolas, 1997). At least twelve genes are required for meiotic DSB formation (reviewed in Smith and Nicolas, 1998). Although biochemical functions have not been established for most of them, DNA cleavage is likely mediated by the Spo11 protein in a sequence-independent manner (Bergerat et al., 1997; Keeney et al., 1997). Spo11 resembles archaeobacterial type II topoisomerases, which create transient DSBs by a trans-esterification reaction that involves a covalent link between the protein and the DNA substrate. Spo11 homologs are phylogenetically ubiquitous, having been found in all kingdoms examined (Celerin et al., 2000; Dernburg et al., 1998; Grelon et al., 2001; Keeney et al., 1999; McKim and HayashiHagihara, 1998; Metzler-Guillemain and de Massy, 2000; Romanienko and CameriniOtero, 1999; Shannon et al., 1999; Tokuyama and Tokuyama, 2000). The meiotic failure of the mouse Spo11 mutant suggests that Spo11 proteins have a similarly critical role in initiating meiotic recombination in higher eukaryotes, including in the human germline (Baudat et al., 2000; Romanienko and Camerini-Otero, 2000). The Rad50/Mre11/Xrs2 complex, which is involved in mitotic DSB repair (see below), is also required for DSB formation (reviewed in Smith and Nicolas, 1998). This complex is also implicated in the next molecularly detectable event of DSB repair, removal of the Spo11 protein and resection of the two DSB ends to varying extents by a 5' - 3' exonucleolytic activity. DSB processing results in the production of 3'-OH single-stranded DNA tails, which are highly recombinogenic and can invade a homologous duplex. Strand invasion requires the participation of several proteins related to bacterial RecA, including Rad51, Rad55, Rad57, and Dmc1 (see below). The Rad52 protein is also involved at this stage. Of these proteins, only Dmc1 is specific for meiotic recombination; the others are also required for mitotic DSB repair. Human (and other mammalian) homologs of Rad51, Dmc1, and Rad52 have been found (see below).

An important intermediate predicted by the DSB repair model is a joint molecule containing two Holliday junctions, and its existence has been confirmed by two-dimensional electrophoresis (Schwacha and Kleckner, 1994). This structure is produced by the Rad51-mediated invasion of a homologous DNA duplex by a single-stranded DNA end, resulting in the displacement of a so-called "D-loop" which can anneal to the non-invading strand of the processed DSB. DNA synthesis primed by the invading end drives further annealing of the D-loop to the initiating duplex. Migration of Holliday junctions (branch migration) can extend the

tract of heteroduplex DNA (i.e., that which contains one single strand from each of the two recombining duplexes). Sequence differences in heteroduplex DNA created by strand invasion or by branch migration can be corrected by the mismatch repair (MMR) system, which also functions in the repair of mismatches that arise in mitotic cells. The principle components that resolve meiotic mismatches are Msh2, Msh3 and Msh6 (which share homology with the bacterial mismatch repair protein MutS), along with Mlh1 and Pms1 (homologs of bacterial MutL) (Borts et al., 2000). MutS-like proteins recognise and bind to DNA distortions caused by mismatches, and Mlh1 and Pms1 subsequently bind to the same sites but the details of later stages of repair are unclear. MutS and MutL homologs have been found in higher eukaryotes, including mice and humans, and in both, derangements of MMR function have been associated with cancer development. MMR proteins may also influence the extension and/or maturation of the heteroduplex tract. Holliday junctions must be cleaved to produce recombinant molecules. Depending on the direction of cleavage, the resulting molecules will or will not have exchanged sequences flanking the region of heteroduplex DNA (crossing over). This step could be performed either by a Holliday junction-specific resolvase or by a topoisomerase. Resolvase activity has been identified in yeast meiotic and in mammalian testis extracts (Constantinou et al., 2001) but the corresponding protein(s) have not yet been identified. Throughout the genome, the distribution of crossovers and resulting chiasmata (the cytologically detectable manifestation of crossovers) is non-random, but each pair of homologous chromosomes must experience at least one crossover, which is probably sufficient to ensure proper segregation during the first meiotic division (Kaback, 1996). Moreover, crossovers are non-randomly distributed along chromosomes, such that a crossover at a given locus decreases the possibility of a nearby event. Little is known about the molecular basis of this phenomenon, termed "interference". Two other MutS homologs, Msh4 and Msh5 (which have no role in mismatch repair), Mlh1 and an exonuclease, Exo1, are required for normal levels of crossing over. The Msh4 homolog has been cloned in mammals but its role in chiasma formation has not yet been determined. The murine Mlh1 protein is essential for both male and female meiosis; mlh1 meiotic cells arrest before the first division and are deficient in chiasmata. Since most of the proteins that participate in meiotic DSB repair in yeast have homologs in higher eukaryotes, it is likely that the meiotic recombination pathway described for yeast is conserved throughout evolution. In addition, most of these proteins also have important roles during DSB repair in vegetatively growing cells, suggesting that there is much mechanistic overlap between meiotic and mitotic DSB repair.

## Mitotic DSB repair by homologous recombination

In yeast, DSBs are repaired predominantly by HR. Although it was previously assumed that DSBs in mammalian cells were repaired mainly by non-homologous mechanisms, there is growing evidence that HR is significant for higher organisms as well (Jasin, 2000). In addition, recent results suggest that mutations in genes encoding proteins that function in HR can potentially confer a hypermutable phenotype conducive to tumorigenesis. There are several types of homologous repair: single-strand annealing, break-induced replication and gene conversion, which differ with respect to the recombinant products they yield and with respect to their genetic control (for review see (Haber, 2000)). In post-replicative diploid cells, DSBs can be repaired by using either the sister chromatid or the homologous chromosome as a template, and the choice between the two is strongly regulated. During meiotic DSB repair in yeast, genetical and physical studies have shown that most repair events involve homologous chromosomes, rather than sister chromatids. In contrast, in mitosis, when the sister chromatid is available (after S-phase), cells are likely to choose it as the repair partner (Haber, 2000).

### The Rad52 epistasis group

As has been shown for meiotic recombination, HR in mitotic cells [349] requires the RAD52 epistasis group of proteins, which includes Rad51, Rad55, Rad57, Rad52, Rad54 and Rad59 (Sung et al., 2000). Rad50, Mre11 and Xrs2 also have an important role in non-homologous recombination as well. In yeast, the *rad51*, *rad52* and *rad54* mutations confer X-ray hypersensitivity and severe defects in meiotic and mitotic recombination (Ivanov and Haber, 1997). In vertebrates, Rad51 is a key player in cell proliferation: *rad51*<sup>-/-</sup> knock out mice die early in development (Lim and Hasty, 1996) and *rad51*<sup>-/-</sup> cell lines cannot be established (Tsuzuki et al., 1996). The depletion of Rad51 from chicken DT40 cells leads to an accumulation of chromosome breaks, cell cycle arrest and death (Sonoda et al., 1998). Human Rad51 can be seen as discrete foci in nuclei exposed to ionizing radiation or radiomimetic chemicals but not to UV irradiation. Although the yeast RAD55 and RAD57 share similarities with RAD51, mutations in these two genes confer a less pronounced phenotype. Furthermore, overexpression of RAD51 can suppress defects seen in the *rad55* and *rad57* null mutants (Hays et al., 1995). Mammalian cells have six Rad51 homologs- Rad51B, Rad51C, Rad51D, Xrcc2, Xrcc3, and Dmc1 (Liu et al., 1998; Pittman and Schimenti, 2000; Shu et al., 1999; Takata et al., 2000). Of these, Dmc1 is expressed only meiotically and likely has no mitotic function (Dosanjh et al., 1998; Pittman et al., 1998; Yoshida et al., 1998). All of these Rad51-like

proteins, however, have important roles in HR but, unlike Rad51, are not essential for cell viability. The human and yeast Rad51 proteins initiate pairing between single-stranded DNA and duplex DNA and catalyse strand exchange (reviewed in Sung et al., 2000). The *in vitro* reaction mediated by yeast Rad51 is stimulated by yeast Rad52, Rad54, Rad55, Rad57 and the single stranded binding protein RPA.

In yeast, RAD52 is the most essential mitotic DSB repair gene, because it is required for all three types of homologous DSB repair described above (single-strand annealing, break-induced repair and gene conversion) (Ivanov and Haber, 1997). RAD59 shares some homology with RAD52 but mutant *rad59* strains are less X-ray sensitive (Bai and Symington, 1996). RAD59-mediated repair events still require the RAD52 gene product. *Rad52*<sup>-/-</sup> knockout mice are viable and fertile. Murine mutant stem cells are not hypersensitive to radiation although they exhibit in HR, as measured by gene targeting (Rijkers et al., 1998). This absence of a strong phenotype in mammalian cells could be due to a functional redundancy with Rad52 homologs not yet identified. Biochemical studies have shown that the yeast and human Rad52 proteins facilitate the annealing of complementary single-stranded DNAs and stimulate Rad51-mediated strand exchange, presumably by targeting Rad51 to single-stranded DNAs. In addition, Rad52 appears to bind selectively to DNA ends (Sung et al., 2000). Curiously, in *Drosophila melanogaster*, *Caenorhabditis elegans*, and *Arabidopsis thaliana*, the complete genome sequence for these organisms provides no evidence of a Rad52 homolog.

The Rad54 protein is a member of the SWI2/SNF2 family of DNA dependent-ATPases which is involved in several aspects of DNA metabolism, including transcription, nucleotide excision repair, and post-replicative repair (Sung et al., 2000). Yeast *rad54* mutants are more impaired in sister chromatid recombination than in interhomolog gene conversion. One possible explanation is that the RAD54 related gene, RDH54/TID1, could be specialised for interhomolog recombination (Klein, 1997). Consistent with this proposal is the observation that animals that are homologous for a knockout of the RAD54 gene are viable and fertile but their cells are hypersensitive to ionizing radiation and DSB- inducing agents (Dronkert et al., 2000; Essers et al., 1997). The efficiency of gene targeting is reduced 5- to 10-fold in *rad54* cells. The Rad54 protein stimulates RAD51-mediated strand exchange (Sung et al., 2000). Based on the functions of other members of the SWI2/SNF2 family, it has been suggested that RAD54 could also be involved in remodelling chromatin for repair and recombination.

### BRCA1 and BRCA2

The recent connection between HR and tumorigenesis comes from observations that Rad51 interacts with the

tumor suppressor protein p53 and the two breast cancer susceptibility gene products, BRCA1 and BRCA2 (reviewed in Dasika et al., 1999). [20026] p53 plays a crucial role in linking cell cycle progression to the presence of DNA lesions. Embryos that lack both Rad51 and p53 develop further than do embryos deficient for Rad51 alone, and some double knockout animals can survive. This suggests that the failure to repair DSBs leads to death in a p53-dependent pathway. However, the nature of this interaction at the molecular level is not known.

More attention has been given to the link between Rad51 and the two BRCA proteins. Mouse BRCA1 or BRCA2 knockouts show early embryonic lethality, similar to what is seen for RAD51 knockout mice (Hakem et al., 1998; Suzuki et al., 1997). A truncated BRCA2 gene can allow embryo survival but these animals are prone to tumor development. Their cells are radiation sensitive and exhibit spontaneous chromosomal aberrations. Loss of functional Brca1 results in a slight sensitivity to radiation and DNA-damaging chemicals and a decrease in homologous gene targeting. A fraction of Rad51 colocalises with BRCA1 and BRCA2 in mitotic S-phase cells. After UV irradiation or exposure to DNA damaging agents, these three proteins relocalize to potentially active repair sites. BRCA1 is a phosphorylated target of the ATM protein, which is a sensor of DNA damaging agents (see below). BRCA1 also interacts with the Rad50/Mre11/Nbs1 complex, which is involved in both HR, mentioned above, and in NHEJ (see below). Little is known about the functions of BRCA1 and BRCA2: although they could have a direct role in DSB repair, they are more likely involved in the regulation of DNA repair processes (see below).

Numerous other proteins that participate in mitotic HR have been described. Among these, Rad50, Mre11 and Xrs2 (described below in conjunction with NHEJ) have multiple functions: HR, telomere length maintenance, meiotic DSB induction, and checkpoint regulation. HR events also involve nearly all the components of the replication machinery. Mismatch repair proteins correct some of the mismatches created during repair and are involved in DSB processing in the single-strand annealing pathway. The maturation of recombination intermediates is performed by Holliday junction migration and resolution proteins.

Beyond its role in DSB repair, HR is relevant to other chromosomal maintenance processes. In yeast, in the absence of telomerase activity, the HR machinery can restore telomere length (Le et al., 1999; Nugent et al., 1998). In addition, there is growing evidence that HR has a critical role in the reinitiation of DNA synthesis at broken replication forks (Rothstein et al., 2000).

## Non Homologous End Joining

NHEJ is a repair mechanism that is utilized in both lower and higher eukaryotes, as evidenced by the conservation of many of the protein components involved in the process. In mammalian cells, it is considered to be the major repair pathway since cells mutated in NHEJ components display significantly increased sensitivity to ionizing radiation. In contrast, in lower eukaryotes, such as in yeast, NHEJ is less prominent in DSB repair. Indeed, *S. cerevisiae* mutants with defects in NHEJ proteins display increased sensitivity to ionizing radiation only in the absence of HR. In mammalian cells, much of our recent knowledge concerning the function of NHEJ components comes from V(D)J recombination studies (for review see (Fugmann et al., 2000).

V(D)J recombination is a programmed mechanism which assembles the genes encoding immunoglobulin and T-cell receptors by fusing two or more gene segments (termed V, D and J) in developing lymphocytes. The lymphoid-specific recombination activating proteins Rag1 and Rag2 initiate this reaction by creating a DSB at recombination signal sequences, which are adjacent to the V, D and J segments. This cleavage creates two types of recombination intermediate, covalently sealed (hairpin) coding ends and blunt signal ends. Signal ends are generally joined without processing, producing precise signal joints. In contrast, when hairpin coding ends are joined, terminal nucleotides are frequently lost or added, and imprecise coding joints of great variety are thereby generated. Through the analysis of DNA repair mutants it has been shown that many of the repair steps involved in the V(D)J reaction are dependent on NHEJ components.

## Factors involved in NHEJ

### Ku protein and DNA-dependent protein kinase (DNA-PKcs)

The Ku protein was originally identified as an autoantigen recognized by sera from autoimmune patients. It was subsequently shown to be a DNA end-binding complex composed of a 70 kDa (Ku70) and an 80 kDa (Ku80) subunit (for review see Featherstone and Jackson, 1999). The corresponding genes in mammalian cells are XRCC5 (Ku80) and XRCC6 (Ku70). Mice containing disruptions of both of these genes have been generated and they display significant growth retardation as well as premature senescence (Gu et al., 1997a; Gu et al., 1997b; Nussenzweig et al., 1996; Zhu et al., 1996). Additionally, they are extremely sensitive to ionizing radiation and are immunodeficient, due to their inability to carry out

V(D)J recombination. The *S. cerevisiae* Ku homolog consists of 70 and 85 kDa subunits (Hdf1 and Hdf2) that, like the mammalian heterodimer, exhibit DNA end-binding activity (Boulton and Jackson, 1996; Feldmann et al., 1996; Feldmann and Winnacker, 1993; Milne et al., 1996). Yeast Ku mutants are viable but are defective in NHEJ. However, they exhibit sensitivity to ionizing radiation only if HR is not functional (i.e., in a rad52 background).

DNA-PKcs is a 460 kDa protein with a serine/threonine protein kinase activity that can be stimulated through an interaction with DNA ends (for review see Smith and Jackson, 1999). Studies *in vitro* have demonstrated that potential targets for the kinase activity of DNA-PKcs include p53, c-myc, the transcription factor Sp1, RNA polymerase II and the Ku protein. The corresponding mammalian gene is XRCC7, and similar to what is observed for Ku disruptions in mice, XRCC7 mutations confer severe immunodeficiency and radiation hypersensitivity (Gao et al., 1998; Jhappan et al., 1997; Taccioli et al., 1998). However, in contrast to Ku mutants, DNA-PKcs mutants are not completely defective in V(D)J recombination. While they are unable to carry out coding joint formation, they are capable of forming signal joints at levels similar to those formed in normal mice. This result suggests that there may be some differences between the roles of DNA-PKcs and Ku in the process of NHEJ. Interestingly, in *S. cerevisiae* there are at present no known homologs of DNA-PKcs. Several proteins that share limited homology with DNA-PKcs (Tcl1, Mec1) have been identified; however, cells containing mutations of these related proteins do not display any NHEJ defects.

In mammalian cells, the Ku heterodimer has been shown to interact with DNA-PKcs, the resulting complex being collectively referred to as DNA-PK. The Ku heterodimer is considered to be the regulatory subunit while the kinase is the catalytic component of this complex. Extensive biochemical analysis of DNA-PK suggests that, following the formation of a DSB, the Ku heterodimer binds and recruits DNA-PKcs (Yaneva et al., 1997; Yoo and Dynan, 1999) (Figure 3). This enzymatically active complex subsequently promotes the translocation of Ku away from the break and the recruitment of additional protein factors necessary for the completion of the end joining process.

#### **Xrcc4 and DNA ligase IV**

Another factor required for NHEJ is encoded by the gene XRCC4. In mammalian cells, this gene was cloned by complementation of a rodent cell line (XR-1) mutation that confers hypersensitivity to ionizing radiation, as well as defects in V(D)J recombination (Li et al., 1995).

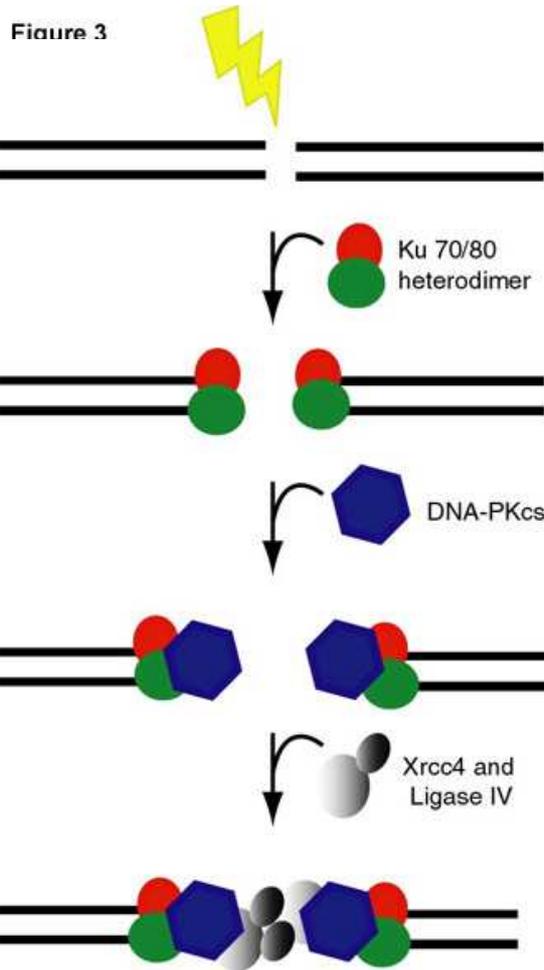


Figure 3: DSB repair by Non Homologous End Joining.

A single Ku heterodimer binds to each free DNA end of a DSB and recruits DNA-PKcs, resulting in the formation of the DNA-PK complex. Subsequently, Xrcc4 /Ligase IV complex binds to each of the DNA ends and interacts to form a tetramer that may serve to bridge the DNA ends. Other repair proteins are likely involved in this pathway, including the Mre11/Rad50/Nbs1 (Xrs2) complex.

Interestingly, disruption of the gene in mice leads to embryonic lethality due to massive apoptotic death in the nervous system (Gao et al., 2000). Analysis of fibroblast cell lines derived from these embryos indicate that, similar to what is observed for Ku-deficient cell lines, these cells display premature senescence, increased radiation sensitivity, and defects in V(D)J recombination. Similarly, in yeast, mutants of the XRCC4 homolog (LIF1), also have a phenotype that closely resembles that of Ku mutants: decreased levels of NHEJ as well as radiation sensitivity in the absence of HR (Herrmann et al., 1998). These results strongly suggest that XRCC4 and Ku function in the same DNA repair pathway.

XRCC4 is a ubiquitously expressed protein that appears to homodimerize. Although the amino acid sequence provides no clues as to its function, the finding that XRCC4 interacts with DNA ligase IV suggests that it may recruit or activate ligase IV to complete the end joining reaction (Critchlow et al., 1997; Grawunder et al., 1997). Indeed, evidence of a functional interaction comes from studies showing that, similar to the effect of XRCC4 inactivation, disruption of the DNA ligase IV gene in mice leads to embryonic lethality (Barnes et al., 1998; Frank et al., 1998). However, disruption of ligase IV in an established mammalian cell line results in radiation sensitivity and a defect in V(D)J recombination (Grawunder et al., 1998). Overexpression of other DNA ligases in this cell line (DNA ligase I or III) is unable to compensate for these defects, suggesting that DNA ligase IV is the major if not only DNA ligase involved in end joining. A homolog has also been identified in *S. cerevisiae* (DNL4), and *dnl4* mutants have been shown to have significant defects in NHEJ (Schar et al., 1997; Wilson et al., 1997).

#### **Rad50, Mre11 and Xrs2 (Nbs1)**

The Rad50/Mre11/Xrs2 complex was first identified in *S. cerevisiae* as a factor involved in DSB repair (for review see Haber, 1998) It has been more explicitly demonstrated subsequently that this complex is involved in both HR as well as in NHEJ. Interestingly, in *S. cerevisiae*, the only end joining events recovered in *mre11* mutants are those that do not exploit terminal homology, thus suggesting that the Rad50/Mre11/Xrs2 complex is specifically required for the use of microhomology. Mammalian Mre11 and Rad50 homologs associate with the Nbs1 protein, which is likely a functional homolog of Xrs2 in higher eukaryotes (Carney et al., 1998). A truncated form of the NBS1 gene is responsible for the human autosomal recessive disorder Nijmegen Breakage Syndrome (NBS) (Matsuura et al., 1998; Varon et al., 1998). Additionally, a truncating mutation of the MRE11 gene has been demonstrated to be at the origin of an ataxia-telangiectasia (A-T, see below) related syndrome (Stewart et al., 1999). Both of these disorders are associated with genomic instability, cancer predisposition and immunodeficiency. In contrast, studies in mice indicate that a complete disruption of MRE11, RAD50 or NBS1 results in early embryonic lethality (Luo et al., 1999; Xiao and Weaver, 1997; Zhu et al., 2001). Rad50 has been shown to be an ATP-dependent DNA binding protein (Raymond and Kleckner, 1993). Mre11 possesses several biochemical activities, the most relevant being a 3'-5' double-stranded DNA exonuclease activity that is postulated to play a role in removing damaged or mismatched DNA

termini and exposing short lengths of single-stranded DNA (Paull and Gellert, 1998; Trujillo et al., 1998).

## **The cellular response to DNA damage**

The cellular response to DSBs is a complex process that involves a network of interacting signal transduction pathways (for review see Dasika et al., 1999; Zhou and Elledge, 2000). This process is initiated by as yet unidentified proteins that detect or sense DNA damage and subsequently transmit a signal by activating a cascade of phosphorylation events. This ultimately results in the initiation of a number of cellular responses, which help to ensure the maintenance of genomic stability, including cell cycle arrest, transcriptional activation, recruitment and activation of DNA repair proteins and, in some cases, induction of cell death by apoptosis (Figure 4). The importance of this response is evidenced by the fact that mutations that alter any aspect of the process have significant effects on DSB repair. Indeed, several syndromes associated with genomic instability and cancer predisposition, such as A-T and Nijmegen breakage syndrome (NBS), involve mutations in genes that are involved in the cellular response to DNA damage.

#### **Ataxia telangiectasia**

Ataxia telangiectasia (A-T) is a disease clinically characterized by progressive neurodegeneration, facial telangiectasia, immune deficiency, gonadal dysgenesis and cancer predisposition (for review see (Rotman and Shiloh, 1999). A-T cells display increased sensitivity to killing by ionizing radiation and are defective in several DNA damage-induced checkpoint controls. The product of the ATM gene (A-T mutated) belongs to a family of protein kinases that are structurally related to phosphatidylinositol 3 (PI-3)-kinases (Savitsky et al., 1995). However, the ability of ATM to function as a protein and not a lipid kinase suggests that it is involved in the signal transduction cascade that is initiated in response to DNA damage. Indeed, Atm has an important role in the response to ionizing radiation, by phosphorylating several key proteins such as p53, Mdm2, Chk1, Nbs1 and Brcal in response to DNA damage. These phosphorylation events are, in part, responsible for the cell cycle arrest that is necessary for DSB repair. Cells in which ATM is mutated are defective in the arrest at both the G1 and G2 phases of the cell cycle. In addition, while normal cells exhibit a dose dependent inhibition of DNA synthesis following exposure to ionizing radiation, A-T cells display almost no alteration in their rates of replication.

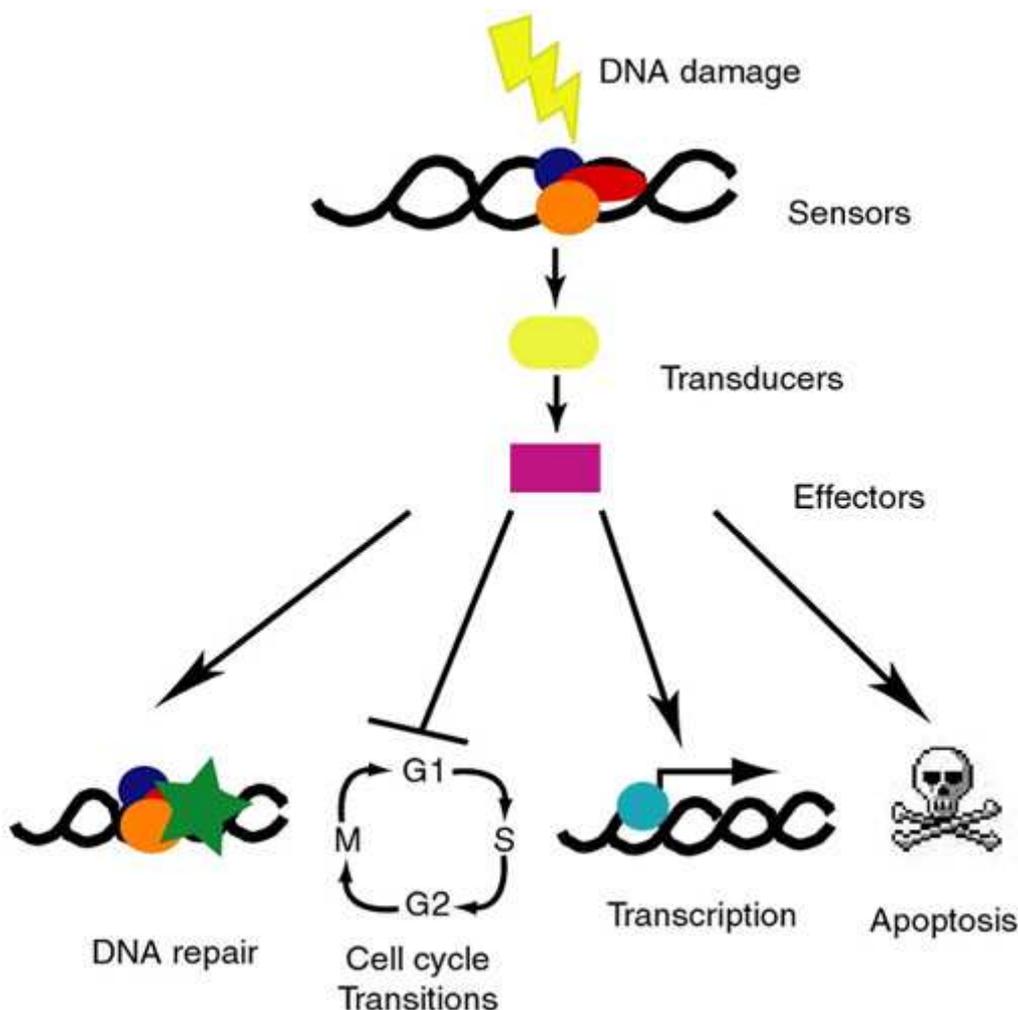


Figure 4: DNA damage response (after Zhou and Elledge, 2000).

DNA damage is recognized by sensor proteins that then initiate a network of signal transduction pathways. This ultimately results in the activation of effector proteins that execute the functions of the DNA damage response, including recruitment of DNA repair proteins, cell cycle arrest, damage induced transcription, or the induction of apoptosis.

**Nijmegen breakage syndrome**

NBS is characterized by clinical and cellular features that resemble AT in many respects (for review see (Shiloh, 1997). In particular NBS patients exhibit chromosome instability, immune deficiency, microencephaly and developmental delay. In addition, cells from such individuals display increased sensitivity to killing by ionizing radiation and as well as radio-resistant DNA synthesis. As previously described, the Nbs protein forms a complex with Mre11 and Rad50 that is required for the repair of DSBs by both NHEJ and HR (Carney et al., 1998). Functional analysis of this complex with immunofluorescently labeled antibodies indicates that following exposure to ionizing radiation, the Mre11/Rad50/Nbs1 complex localizes to sites of DNA damage, forming discrete nuclear foci early in the DNA damage response that remain associated with DSBs until the repair is complete (Maser et al., 1997; Mirzoeva and Petrini, 2001).

Interestingly, it has recently been shown that ATM directly phosphorylates Nbs1 on several sites that are necessary for the formation of these foci (Gatei et al., 2000; Lim et al., 2000; Wu et al., 2000; Zhao et al., 2000). This finding provides a functional link between ATM and Nbs1 that may explain the similar phenotypes associated with the corresponding syndromes.

**The breast cancer susceptibility gene, BRCA1**

BRCA1 was initially identified as a tumor suppressor gene mutated in a significant percentage of patients with familial breast cancer and/or ovarian cancer (for review see Scully and Livingston, 2000). The normal cellular function of this gene has subsequently been shown to involve the maintenance of genomic stability through its involvement in DSB repair (see above) and transcriptional regulation. The ability of BRCA1 to regulate several different cellular processes is thought to result from its association with a large protein

complex, BASC, which contains proteins involved in repair, replication and transcription (Wang et al., 2000). Following DNA damage, BRCA1 undergoes phosphorylation by both the Atm and Chk2 kinases (Cortez et al., 1999; Lee et al., 2000). This results in the subsequent dissociation of BRCA1 from its partner CtIP, a protein of unknown function that associates with the transcriptional repressor CtBP (Li et al., 1999). It is thought that dissociation of BRCA1 allows it to subsequently activate transcription of DNA-damage-response genes such as p21 and GADD45.

## Conclusions

*Saccharomyces cerevisiae* has been an outstanding model organism for studying the genetics and biochemistry of recombination processes. The extensive work that has been carried out with this organism has led to a detailed mechanistic understanding of DSB repair. The relevance of these studies to the comprehension of DSB repair in higher eukaryotes is supported by the significant conservation of genes involved in this process. However, the emergence of various tumor suppressors as potential modulators of recombination emphasizes the additional complexity of DNA repair and its consequences in mammals. Another recent advance in the field has been the discovery of numerous endogenous sources of DSBs. These lesions can be generated during specific programmed processes such as V(D)J recombination and meiosis, as byproducts of normal cellular metabolism - for example during replication or transcription - as well as by environmental factors such as ionizing radiation, radiomimetic drugs or reactive oxygen species. Thus, DSBs are lesions with which the cell is frequently confronted, and improper or inefficient repair can be extremely detrimental at both the cellular and organismal levels. This is made particularly evident by the wide-ranging defects of knockout mice lacking important DSB repair genes and by the severity of human diseases (i.e. Ataxia telangiectasia, Nijmegen breakage syndrome) associated with loss of function of DSB repair genes.

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