

## DNA Double Strand Break and Repair: Mechanisms and Involvement in Human Cancer

Shiladitya Sengupta and Susanta Roychoudhury\*

*Human Genetics and Genomics Division, Indian Institute of Chemical Biology, 4 Raja S.C  
Mullik Road, Kolkata 700 032, West Bengal, India*

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**ABSTRACT** The most frequent damage on a cell is the DNA double-strand break (DSB). This is sensed and repaired by normal cellular DSB response pathways. Depending on the phase of the cell at which the DSB is sensed, there are two different pathways for the repair of this lesion, the non homologous end joining (NHEJ) repair and the homologous recombination (HR) repair. Defects in these sensing and repair pathways leads to no repair or inappropriate/ abnormal repair. This causes genome instability that results in different disorders among which cancer is the most significant one. We describe how cells repair DSB and the relationship between the defects in this repair system and cancer.

### INTRODUCTION

The property common to many carcinogens is their reactivity with cellular biomolecules. These carcinogens by themselves, or one or more of their metabolites interact with the biomolecules and cause damage to it. Cellular responses to such damage in mammalian cells include repair, cytotoxicity, apoptosis, mutagenesis and transformation to malignancy. These biochemical processes are the basis for both maintaining cellular integrity and genome stability or set the cell on a path to mortality or malignancy. Initially, after the discovery of DNA double helix the prevailing hypothesis was that key proteins are the targets of carcinogens. Later, it was demonstrated that the potency of a series of carcinogens correlated with their ability to bind to DNA *in vivo* and not with that to protein or RNA (Brookes 1964). This led to the acceptance of DNA as the critical target in carcinogenesis. The damage can occur at any stage of the cell cycle; if damage is permanent then cell replicates with the damage resulting in formation of a colony of unhealthy cells that gradually accumulate mutations and proceed towards malignancy. The defective progeny cells will have faulty genome with respect to both quality and quantity and such genome instability

is a characteristic feature of all cancer cells. While it is widely assumed that DNA damage is an early and obligatory event in the process of carcinogenesis, it is by no means a sufficient event. It is believed that a person will develop cancer only when such somatic events are present in the background of germline defects that are inherited. Nevertheless, cancer doesn't occur in the absence of DNA damage and interventions that repair the damage result in inhibition of carcinogenesis. So the mechanisms how different repair pathways fix DNA damage is key to the understanding of the role of DNA repair pathways for maintaining genome stability and inhibiting tumorigenesis.

The outcome of DNA damage is diverse and generally adverse. Acute manifestation results from disturbed DNA metabolism, which triggers cell cycle arrest or cell death. The cell cycle machinery somehow senses DNA damage and arrests at specific checkpoints in G1, S, G2 and M to allow repair of the lesions before they are converted into permanent mutations (Lukas 2004). If damages escape normal DNA repair mechanisms or if the damages are repaired incorrectly due to defective or altered repair pathways and persist, then there are two alternative strategies the cells can follow. One is apoptosis, which is beneficial to the system. On the other hand, if cells undergo division with the damaged DNA then it will gradually accumulate mutations resulting in genomic instability ranging from simple point mutations to large insertions, deletions, rearrangements and aneuploidy. These

\*Address for correspondence: Dr. Susanta Roychoudhury  
Human Genetics and Genomics Division, Indian Institute  
of Chemical Biology, 4, Raja S. C. Mullick Road  
Kolkata 700 032, West Bengal, India  
Telephone: 91-033-473 3491 / 3493 / 6793 (Office)  
Fax: 91-033-473-0284 / 5197  
Email: susanta\_rc@yahoo.co.in

changes, when occur in a critical region of the genome that harbor tumor suppressor genes or oncogenes, will drive cells towards malignancy.

DNA damage can take several forms including breaks in the sugar phosphate backbone of the molecule either in one of the two strands or in both the strands and covalent binding of the carcinogen or its meta-bolite resulting in the formation of a chemically altered base in DNA, termed as DNA adduct. Mainly four major pathways repair the DNA damages. Among the different damages, the double strand DNA break (DSB) repair is crucial for the maintenance of genome stability. Defects in the DSB repair pathways in the background of damage inducing environment have potential for the development of genome instability and thus driving cells towards malignancy. This article reviews the DSB repair pathway in connection to its involvement in the process of maintaining genome stability and suppressing carcinogenesis.

### THE DIFFERENT REPAIR SYSTEMS

In the background of different types of lesions no single repair process can cope with all kinds of damage. Evolution has molded an orchestra of highly sophisticated, interwoven DNA repair systems that guard all the insults inflicted on a cell's vital genetic information. These DNA repair systems have arisen early in evolution and this explains why all known repair pathways are highly conserved usually across the pro/eukaryotic evolutionary border. There are at least four main, partly overlapping damage recognition pathways operating in mammals; nucleotide excision repair pathway (NER), base excision

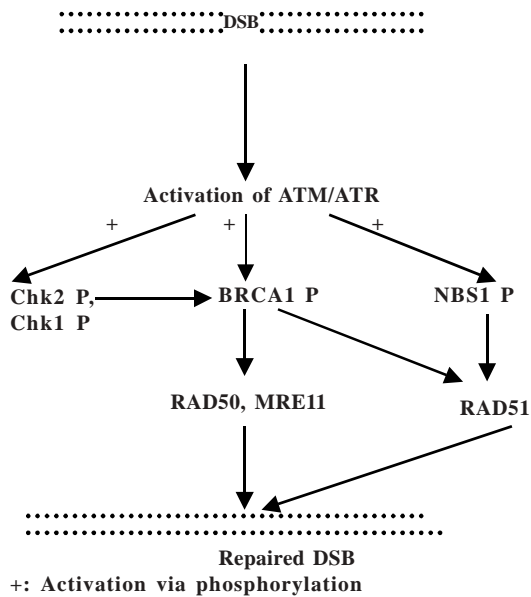
repair pathway (BER), mismatch repair pathway (MMR), and double strand break repair pathway (DSBR; Lindahl 1999). Table 1 illustrates briefly the source of different types of DNA damage (considering both generated by exogenous carcinogens and endogenous processes) and involvement of the corresponding repair pathway. Initially, different forms of DNA damage have different fates; single strand break (SSB) is repaired by the BER and any defect in the repair of such lesion leads to a DSB after replication of the SSB template; oxidized bases are repaired by BER involving a SSB intermediate and likewise any defects in this pathway leads to a DSB. Similar is the case with the DNA adduct clearance system. So double strand break repair pathway is the ultimate guard to a cell preventing genome instability.

### SENSING DOUBLE STRAND BREAK IN DNA

Cells respond to DNA DSBs through the actions of various systems that detect the DNA damage and then trigger various downstream events. A crucial component of DSB signaling cascade in mammalian cells is the protein kinase ATM, which is recruited to and activated at sites of DSBs (Kurz 2004). This activation signals the presence of DNA damage by phosphorylating and activating targets (ATM independent phosphorylation events are mediated primarily by the ATM-related protein i.e., ATR) involved in cell cycle checkpoints, DNA repair and stress response (Kurz, 2004). ATM phosphorylates and activates the downstream targets such as p53, MDM2, Chk1, Chk2, BRCA1 and NBS1 (Kurz

**Table 1: DNA damages and the repair pathways**

<i>Agent</i>	<i>Source</i>	<i>DNA Damage</i>	<i>Repair Pathway</i>
X-Ray	Exogenous	DNA single strand break	BER
	Exogenous	DNA double strand break	DSBR
Oxygen radical	Exo & Endogenous	Abasic site	BER
		DNA single strand break	BER
		Oxidised base adduct (e.g. 8-oxoguanine)	BER
Alkylating agent	Exo & Endogenous	Alkyl adduct (e.g. O6-methylguanine)	Direct repair
Spontaneous reaction	Endogenous	Cytosine replaced by Uracil	BER
UV-Ray	Exogenous	Cyclobutane pyrimidine dimer, Pyrimidine (6-4) pyrimidone photoproduct	NER
Polyaromatic hydrocarbon	Exogenous	Bulky adduct	NER
Replication of DNA single strand break	Exo & Endogenous	DNA double strand break	DSBR
Anti-tumor agent	Exogenous	DNA Interstrand cross link	DSBR
Replication error	Endogenous	Mismatch, Insertion, Deletion	MMR



**Fig. 1. DSB sensing pathway initiated by ATM: in response to a DSB, ATM gets activated and phosphorylates the effector proteins, which then carry out their downstream functions via a signaling cascade to repair the DSB**

2004). In the BASC (BRCA1 associated genome surveillance complex), BRCA1 acts as a protein scaffold for putative DNA damage sensors such as ATM, RAD50-MRE11-NBS1 and mismatch repair proteins MSH2/6 and MLH1 that orchestrates the repair or signaling pathway, depending on the type of lesion encountered (Wang 2000). Figure 1 illustrates the role of ATM/ATR for signaling the DNA repair pathway involving BRCA1, NBS1, MRE11, RAD50, RAD51, Chk2 and Chk1. Another early event in the sensing phase is the phosphorylation of histone H2AX in the DNA domain next to the DSB over a megabase distance and this depends upon ATM, ATR and DNAPKcs (Celeste 2003), which may be required for providing a local chromatin state for the complex repair reactions.

### DOUBLE STRAND BREAK REPAIR

Two different types of mechanism repair DSBs. One permits non-homologous joining (NHEJ) of two DNA DSBs without the requirement for extensive sequence homology between the DNA ends particularly in the G0 and G1 phases of the cell cycle and the other promotes homologous recombination (HR) after obtaining

instructions from the undamaged sister or homologous chromosome and entering into synapsis for proper repair of breaks, operating particularly in the S and G2 phases (Johnson 2000). Often these pathways are overlapping: there are evidences that DNA ligase IV, a NHEJ protein and RAD54, a HR protein function in a cooperative fashion to maintain chromosomal stability (Mills 2004). Recently in the context of V(D)J rearrangement RAG proteins have been reported to act like molecular shepherd that guides DSB to the proper NHEJ pathway (Lee 2004).

### DOUBLE-STRAND BREAK REPAIR BY NON-HOMOLOGOUS END JOINING (NHEJ)

By the name it appears that non-homologous end joining results in random joining of any two ends, however, this does not appear to be the case. In most cases, NHEJ rejoins the correct ends, thus preventing chromosome rearrangements. To accomplish this, the two ends must be held together until they can be ligated. This is accomplished by the nucleosome structure, Ku-Ku interactions and DNA-PKcs interactions. However, this pathway usually results in gain or loss of nucleotide sequences at the break point. The basic steps of NHEJ in mammalian cells are illustrated in the Figure 2. The Ku heterodimer consisting of 69 kDa Ku70 (XRCC6) and 83 kDa Ku80 (XRCC5) with ATPase and helicase activity binds strongly to DNA ends, to stem-loop and bubble structures, and to transitions between double-stranded DNA and two single strands (Walker 2001). Once Ku binds to a DNA molecule at its ends, it can translocate along the DNA. Ku recruits other proteins, including XRCC4, DNA ligase IV, and DNA-PKcs to DNA ends (NickMcElhinny, 2000). The 465-kDa XRCC7 is the catalytic subunit (cs) of a DNA-dependent protein kinase (DNA-PK) activity. The Ku heterodimer interacts with and regulates DNA-PKcs. Thus DNA-PK is frequently considered to be a trimeric protein consisting of DNA-PKcs, Ku70 and Ku80 (Thacker 2004). DNA-PKcs itself has affinity for DNA ends and its activation appears to be triggered by its interaction with single stranded DNA region derived from a DSB (Martensson 2002). Although DNA-PKcs binds DNA ends in the absence of Ku, its affinity for ends is increased about 100-fold when Ku is already bound to those ends. Once bound to

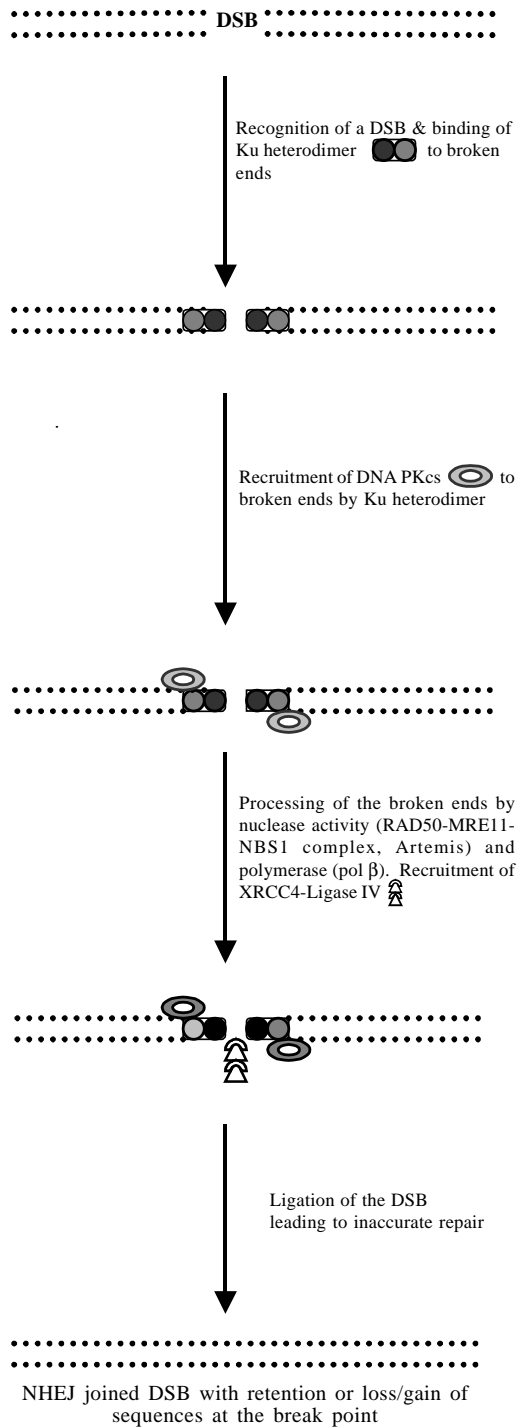


Fig. 2. Possible steps in NHEJ pathway

DNA DSB, DNA-PK displays protein Ser/Thr kinase activity with preference for the consensus sequence Ser/Thr-Gln (Chan 2002). Recently it has been proposed that autophosphorylation of DNA-PKcs at certain sites is important for remodeling of DNA-PK complexes at DNA ends prior to DNA end joining (Chan 2002; Block 2004). The most likely *in vivo* substrate for DNA-PK include XRCC4 and the replication factor A2 (Binz 2004) whose phosphorylation presumably facilitates NHEJ. Most DNA DSBs cannot be directly ligated and limited processing and/or polymerization must take place before NHEJ can ensue. One candidate for an enzyme involved in the nucleolytic processing stages of NHEJ is the mammalian MRE11-RAD50-NBS1 complex. This complex possesses exonuclease, endonuclease, and DNA unwinding activities (Paull 1999). In the presence of DNA ends the Artemis protein (another factor for processing DNA DSBs before NHEJ) is an important substrate of DNA-PK (Moshous 2001). Artemis binds to DNA-PKcs and is thus recruited to Ku-bound DNA ends along with DNA-PKcs. Once bound to a DNA end, DNA-PKcs becomes active as a kinase, and it phosphorylates Artemis. This phosphorylation stimulates and extends the nuclease activity of Artemis, so that Artemis becomes capable of opening hairpin loops (if present) and cutting away protruding single-stranded regions at DNA ends by complexing with the DNA dependent protein kinase (Noordzij 2003) and creating blunt double-stranded structures that are good ligase substrates. Then DNA ligase IV functions in a tight complex with the protein XRCC4 and Ku recruits the XRCC4-ligase IV complex onto DNA ends and stimulates DNA end-ligation (Chen 2000). The resulting "healed" DNA molecule is likely to have altered DNA sequence, with the extent of alteration depending on the amount of damage at the break and the extent of processing that was required to make the break ligatable. In some cases, NHEJ takes advantage of short stretches (1-4 nucleotides) of nucleic acid sequence complementarity near the ends of the broken molecules, while in other cases it does not. In this case it requires microhomologies at DNA ends. Some reports suggest that direct NHEJ and microhomology directed end joining constitute biochemically and genetically distinct DSB repair pathways (Verkaik 2002). Recently it has been found that BRCA1 is involved in microhomology mediated NHEJ mainly by its interaction with the

MRE11-RAD50-NBS1 complex (Zhong 2002). The identity of the DNA polymerase(s) that is involved in NHEJ is not yet clear, but many reports have indicated the involvement of DNA polymerase  $\beta$  in NHEJ (Wilson 1999). Recently it is known that DNA polymerase lambda is the primary gap-filling polymerase for accurate nonhomologous end joining, and that the Brca1 C-terminal domain is required for this activity (Lee 2004).

#### **DOUBLE-STRAND BREAK REPAIR BY HOMOLOGOUS RECOMBINATION (HR)**

The proteins encoded by genes in the RAD52 epistasis group of *S. cerevisiae* (or their homologs in human) are important for this process. These proteins include RAD51, RAD52, RAD54, RAD55, RAD57 and RAD59 (Dudas 2004). The RAD51 protein contains a central core that is rather similar to the RecA protein of *E. coli* and forms a structured nucleoprotein complex with single-stranded DNA. An early event in HR is the nucleolytic resection of the DNA DSB in the 5'-3' direction. This reaction involves the complex of Rad50, MRE11 and NBS1 (Cromie 2001). Rad51 then binds the ensuing 3' single stranded DNA tails in a process that is influenced by a range of other proteins, including replication protein A (RPA), Rad52 and Rad54 (Petukhova 1998). Human Rad52 interacts and co-localizes with Rad51, induces Rad51 activity, binds preferentially to DNA DSBs and protects them from exonuclease activity (Haber 1999). These observations led to the proposal that competition between Rad52 and Ku for DNA ends may decide which of the two DSB repair pathways is to be employed (Haber 1999). The Rad51 nucleoprotein filament then interacts with an undamaged DNA and when a homologous region is located, Rad51 catalyses strand exchange events in which the damaged molecule invades the homologous DNA duplex, displacing one strand as a D-loop (Cromie 2001). RAD52 also functions independently of RAD51 by binding to single stranded DNA and thereby promotes annealing of complementary strands (Kumar 2004). Mammalian Rad51 also functions in concert with BRCA1 and BRCA2. The precise mechanism by which BRCA1 and BRCA2 function are currently unknown, possibly they function in HR by modulating chromatin structure (Bochar 2000). It has been shown that, through its BRC motifs,

BRCA2 directly interacts with RAD51 thereby affecting both the nuclear localization as well as DNA binding properties of RAD51 (Davies 2001). The presence of BRCA1 in complexes involved in chromatin remodeling and/or the control of transcription (Bochar 2000) raises its possibility to affect HR by changing the chromatin structure at sites of DSBs and also by transcriptional regulation in response to DNA damage. Although there is still considerable debate about the details of the pathways that utilize homologous recombination to repair double-strand breaks, two types of pathway appear well established, at least in general outline: synthesis-dependent strand annealing (SDSA) and single-strand annealing (SSA).

It appears that most or all mechanisms that utilize information from a sister or homologous chromosome to repair a double-strand break with homologous recombination use some variation of the SDSA pathway. One of the simplest versions is shown in the Figure 3. The 5' ends of a DSB introduced into one of the two homologous chromosomes are resected by Rad50-MRE11-NBS1 complex to expose the 3' ends in single-stranded form (Cromie 2001). With the help of Rad51, the 3' ends locate complementary regions in the sister (or homologous) chromosome. This is sometimes called "strand invasion." Then the 3' ends are used as primers for new DNA synthesis, using the donor chromosome strands as template. After sufficient synthesis, to permit the new strands to anneal with each other, the new strands are unwound from the template and allowed to anneal with each other. Any overhangs are removed by a flap endonuclease, and any gaps are filled in by a polymerase. Remaining nicks are sealed by a ligase. The DSB containing chromosome is thus repaired, but it contains information from the homologous chromosome in the newly synthesized region.

DSB repair by single-strand annealing (SSA) as illustrated in Figure 4 begins in similar fashion. After a break is introduced, the 5' ends are resected. However, this resection exposes complementary regions within the 3' strands (due to repeat sequences) flanking the DSB. In mammalian DNA, with its abundance of short repeat sequences, it is not unlikely that repeat sequences of sufficient length (several hundred base pairs) should be found flanking the DSB. After flap removal (by a FEN1-like nuclease) and ligation, the double-strand break is repaired, but

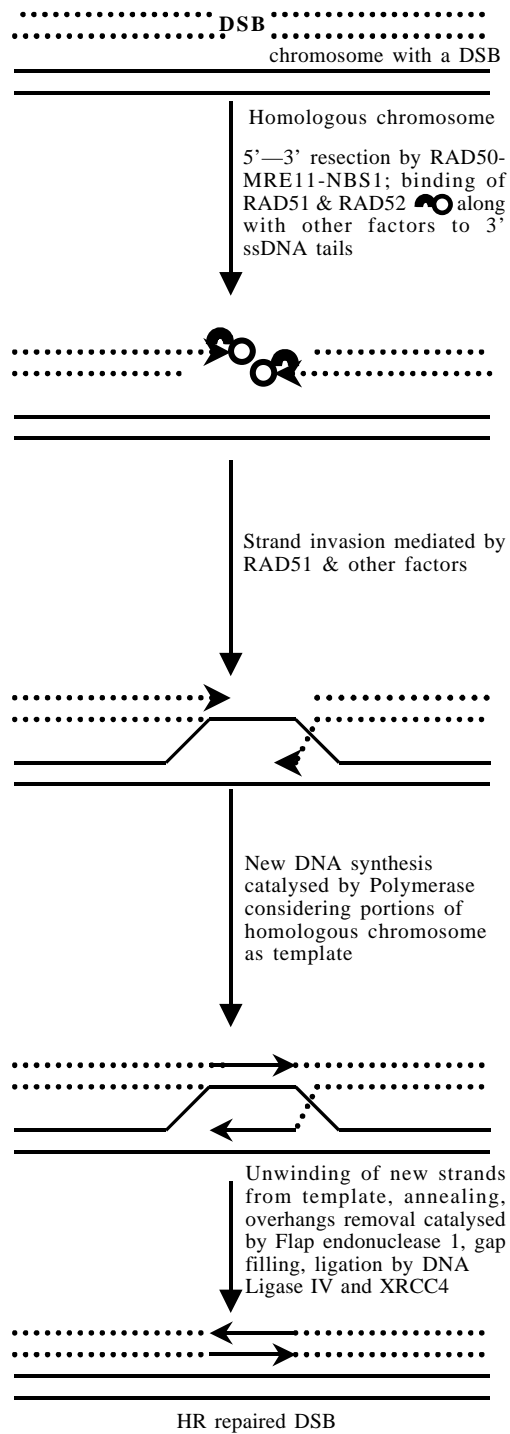


Fig. 3. Possible steps in SDSA pathway

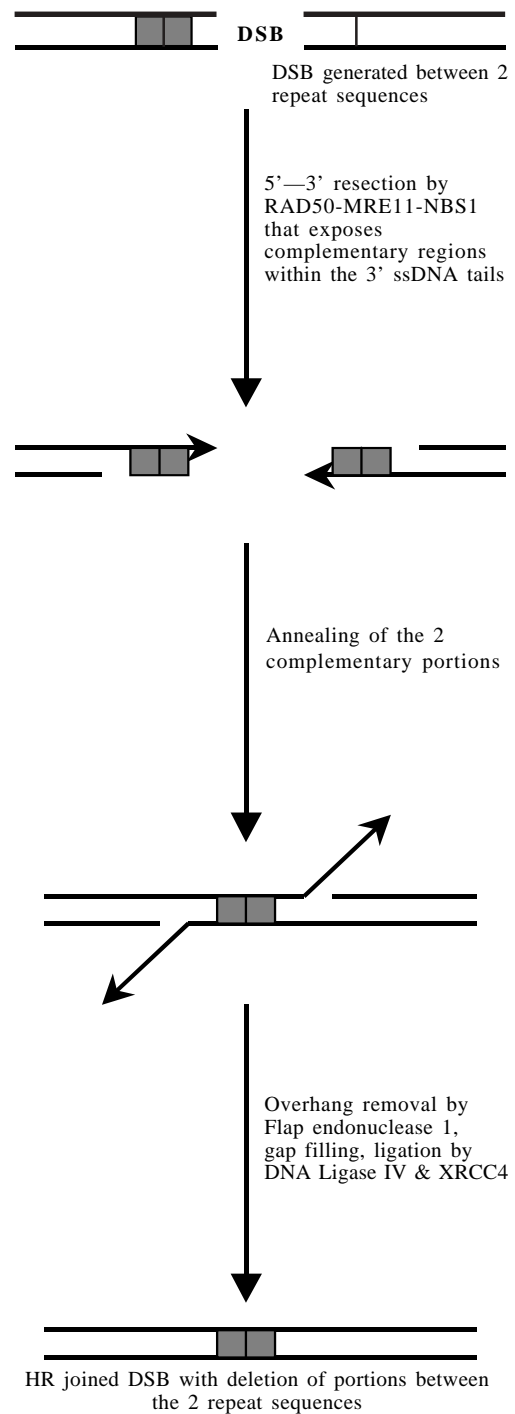


Fig. 4. Possible steps in SSA pathway

at the price of deletion of the stretch of DNA between the two repeated sequence (Haber 2000).

#### DEFECTIVE DOUBLE STRAND BREAK REPAIR AND HUMAN DISEASES

DNA double strand breaks are the most potent inducers of genome instability resulting in oncogenic transformations and cell death. Experimental reports suggest that DSB in response to a wide variety of endogenous and exogenous factors is a vital phenomenon to a living system, whose total absence is lethal but altered/suppressed/heightened function has carcinogenic potential. Mutations in many of the factors involved in the sensing, signaling and repair of DSB lead to increased predisposition of cancer.

*DNA-PKcs* and *Ku70* mutant mice have high incidence of T-cell lymphomas (Smith 1999). *Ku70*<sup>-/-</sup> mice have increased rates of fibroblast transformation with chromosomal instability including breakage, translocations and aneuploidy (Smith 1999). Mouse cells lacking *ligase IV* undergo numerous chromosome translocations after DNA damage by ionizing radiation, but wild-type cells do not (Ferguson 2001) implying that functional NHEJ in wild type cells must preferentially join correct ends of ds breaks. *DNA-PKcs*-deficient mice are overtly normal in appearance but *Ku*<sup>-/-</sup> mice are small and display various symptoms of premature ageing (Ferguson 2001). This indicates that Ku is required for the repair of a larger repertoire of naturally arising DSB lesions than *DNA-PKcs*. Inactivation of *DNA ligase IV* or *XRCC4* leads to embryonic lethality in mouse associated with extensive apoptosis of neurons in the central nervous system (Ferguson, 2001). Thus, inactivation of *ligase IV* or *XRCC4* leads to a more severe phenotype than the inactivation of *Ku*; this can be explained by the fact that in *XRCC4* or *ligase IV* deficient cells, Ku and *DNA-PKcs* still bind to DSBs, leading to nonproductive complex that prevents access by the other repair components such as those involved in HR. In contrast, in *Ku* or *DNA-PKcs* deficient animals, such alternative DSB repair pathway is not inhibited, allowing them to compensate to some degree for a loss of NHEJ. It has been found in chicken DT40 cell line that *ligase IV*<sup>-/-</sup> cells are more radiosensitive than *Ku70*<sup>-/-</sup> cells but *Ku70*<sup>-/-</sup>: *ligase IV*<sup>-/-</sup> double mutants have a similar sensitivity to *Ku70*<sup>-/-</sup> cells (Adachi 2001). However in culture, NHEJ-

deficient cells have high rates of spontaneous chromosome breaks (d'Adda 2001). Recently it has been found that mouse cells haploinsufficient for *DNA ligase IV* exhibit gross chromosomal instability involving deletion, amplification and translocation and ultimately results in elevated incidence of soft tissue carcinoma (Sharpless 2001). Human leukaemia cell line 180BR with a point mutation in a highly conserved amino acid residue in the functional domain of *ligase IV* is unlike the NHEJ deficient mouse, and the patients having such mutation are not immune-deficient with any neurological defects; such mutation may not totally abolish the *ligase IV* activity and the residual *ligase IV* activity is sufficient for proper NHEJ during V(D)J rearrangement and repair of endogenously generated lesions but insufficient for the repair of larger amounts of damage due to environmental exposures and may be the cause for such type of cancer (Riballo 2001). Recently, it has been found that targeted disruption of one allele of *Ku70/Ku80* gene in human colon cancer cell line HCT116 leads to an increase in polyploidy and elevated p53 levels (Li 2002); however inactivation of the second *Ku80* allele leads to an increase in apoptotic cells presumably due to inability to repair endogenously generated lesions. It has also been found that ectopic expression of *DNA polymerase β* induces aneuploidy and promotes tumorigenesis in nude immunodeficient mice (Bergoglio 2002). Thus it may be stated that complete deficiency of the NHEJ components may not be suitable for the viability of human cells, their repression, over expression or altered NHEJ reactions are actually significant in terms of different human diseases particularly cancer. Also the requirement of NHEJ components for normal cell viability differs from tissues to tissues and that not the total inactivation of NHEJ pathway or its components but their altered levels of activity are of great medical significance.

There are strong links between HR and the breast cancer susceptibility proteins, BRCA1 and BRCA2. It has been found that loss of function of either *BRCA1* or *BRCA2* in mammalian cells markedly reduces the efficiency of accurate homology directed DSB repair (Moynahan 2001) and mutation of *BRCA2* stimulates error prone repair of such damages that are generated between repeated sequences (Tutt 2001). Inactivation of the genes such as *RAD51*, *BRCA1*, *BRCA2*, *MRE11*, *RAD50*, *NBS1* leads to mortality of cells

in culture (Zhu 2001). Mutations in *BRCA1* or *BRCA2* result in elevated cancer incidence, at least in part due to defective HR, which in turn leads to genome instability. Loss of wild type *BRCA1* and *BRCA2* leads to aneuploidy accompanied by centrosomal amplification and chromosomal mis-segregation (Xu 1999). *p53* is frequently mutated in *BRCA1* and *BRCA2* associated familial cancers (Philips 1999) and conditional inactivation of *BRCA1* leads to the development of malignancy in a *p53* null background (Xu 1999). *RAD51* overexpression promotes alternative DSB and results in aneuploidy and multiple chromosomal rearrangements (Richardson 2004). In contrast with mice lacking *RAD51*, *RAD54* deficient mice are viable (Essers 1997); nevertheless *RAD54* is important for mammalian HR because *RAD51* foci do not form effectively in the *RAD54* background. *RAD54* deficient embryonic stem cells are hypersensitive to DSB inducing agents and have defective HR (Essers 1997). Mutations of *RAD54* have been observed in lymphoma, colon cancer and breast cancer suggesting a possible causative link (Matsuda 1999). *RAD54* null chicken DT40 cells have reduced rates of HR but are viable (Essers 1997) and when this deficiency is combined with deficiencies in *Ku*, this results in greater radiosensitivity (Essers 2000). This provides strong evidence for the action of HR and NHEJ in a complementary fashion. Certain mutations in the human *NBS1* gene cause "Nijmegen breakage syndrome" (NBS), a rare autosomal recessive disease (Varon 1998), which is characterized by microcephaly, immunodeficiency and increased frequency of hematopoietic cancers. Cells from NBS patients suffer from frequent chromosome breakage due to defects in the intra S-phase DNA-damage checkpoint and this is dependent on the phosphorylation of *NBS1* by *ATM* in response to DNA DSBs (Zhao 2000). In normal cells, the *RAD50*, *MRE11*, and *NBS1* proteins all co-localize in numerous spots (foci) within nuclei after induction of DSB by ionizing radiation (Petrini 2000). DSB induced co-localization in nuclear foci does not occur in cells from NBS patients. Many of these symptoms are identical to those of the checkpoint disease, ataxia telangiectasia (AT), in which the key checkpoint signalling protein, *ATM*, is mutated (Zhao 2000). Similar symptoms are also present in "Ataxia telangiectasia-like disease" (ATLD), which has recently been found

to be due to mutations in the *MRE11* gene (Stewart 1999).

### FUTURE DIRECTIONS

In future, DSB responses are to be characterized in a greater molecular detail with discovery of additional components of these pathways. Also, a key issue would be the elucidation of the coordinated activities of the multiple pathways that respond to DSB and the modulation of these different pathways during the cell cycle and in different tissues. An important question is to ascertain the DSB signaling and repair processes in the context of chromatin structure.

It remains to be established, to what extent mutations or polymorphisms in genes encoding the DSB response proteins are associated with carcinogenesis in the general human population and whether such association is influenced by the ethnic background and habit of an individual. This will lead to better predictions of how patients respond to radiotherapy and certain chemotherapies. The ultimate goal is to develop novel anti-cancer agents that target proteins involved in DSB responses to bring about more effective and selective killing of cancer cells.

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