DNA double strand break repair and chromosomal translocation: Lessons from animal models

David O Ferguson[3,5] and Frederick W Alt*[,1,2,3,4]

[1]Howard Hughes Medical Institute, Harvard Medical School, Boston, Massachusetts, MA 02115, USA; [2]The Children's Hospital, Harvard Medical School, Boston, Massachusetts, MA 02115, USA; [3]The Center for Blood Research, Harvard Medical School, Boston, Massachusetts, MA 02115, USA; [4]Department of Genetics, Harvard Medical School, Boston, Massachusetts, MA 02115, USA; [5]Department of Pathology, Brigham and Women's Hospital, Boston, Massachusetts, MA 02115, USA

The maintenance of genomic stability is one of the most important defenses against neoplastic transformation. This objective must be accomplished despite a constant barrage of spontaneous DNA double strand breaks. These dangerous lesions are corrected by two primary pathways of double strand break repair; non homologous end joining and homologous recombination. Recent studies employing mouse models have shown that absence of either pathway leads to genomic instability, including potentially oncogenic translocations. Because translocations involve the union of different chromosomes, cellular machinery must exist that creates these structures in the context of unrepaired double strand breaks. Evidence is mounting that the pathways of double strand break repair that are so important for survival may themselves be the culprits that generate potentially fatal translocations. Evidence and models for the dual roles of double strand break repair in both preventing, and generating, oncogenic karyotypic changes are discussed. *Oncogene* (2001) **20**, 5572–5579.

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Introduction

Neoplastic transformation of a cell results from alteration of the inherited genetic material such that growth controls are abrogated. The offending lesions are most commonly comprised of changes to the DNA sequence, although epigenetic phenomena may also contribute (Baylin and Herman, 2000). Alterations to the DNA sequence come in many forms and all can contribute to neoplasia (Loeb and Loeb, 2000). These include simple nucleotide mutations, and events effecting genomic regions, such as deletion, duplication and amplification. However, the karyotypic hallmark of oncogenesis is the translocation. These readily identifiable abnormal chromosomes have served as

reliable markers for accurate diagnoses of tumors and their ever growing number of subtypes, as well as a reagent to elucidate mechanisms of oncogenesis through identification of genes fused at translocation break points. However, while much has been learned about the abrogation of cellular growth controls caused by fusion genes, until recently little has been learned about mechanisms that catalyze the formation of translocations in the first place.

Translocations are abnormal chromosomes comprised of material deriving from two (or more) different chromosomes. In general, these entities come in two varieties; reciprocal, in which two chromosomes have swapped portions of their arms resulting in two translocated products, or non-reciprocal in which only a single translocated chromosome is identified and the reciprocal product has either been lost, or was never generated. Regardless of the nature or location of a translocation the one common thread is that at some point at least one chromosome was broken, resulting either from a DNA double strand break (DSB) or two nearby single strand breaks. DNA ends cannot ligate to one another without catalysis by proteins, implying that translocations are actively generated by cellular machinery (Pfeiffer *et al.*, 2000). Numerous recent studies employing mouse models and vertebrate cell lines have begun to elucidate the complex events leading to translocation, and have demonstrated the central role of DSB repair (DSBR) pathways in both their prevention, and their creation.

The repair of DSBs in eukaryotic cells is carried out by two main pathways, non homologous end joining (NHEJ) and homologous recombination (HR) (Haber, 2000). NHEJ repairs DSBs by directly re-ligating DNA ends, which may effect perfect repair, or create a deletion if sequences surrounding the lesion were lost (Lieber, 1999). HR repairs breaks through interaction of a free DNA end with intact homologous sequences which are used as a template to copy missing information prior to re-ligation (Haber, 1999; see Figure 1). Because of the ability to fill in gaps by copying information from a sister chromatid or homologous chromosome, HR avoids creating deletions, but runs the risk of causing loss of heterozygosity by gene conversion, or generating rearrangements through interaction of similar sequences on non homologous chromosomes.

*Correspondence: FW Alt, The Children's Hospital, Enders Building, Room 861, Mail Stop EN-334, 320 Longwood Avenue, Boston, Massachusetts 02115, USA;
E-mail: alt@rascal.med.harvard.edu

Non Homologous End Joining
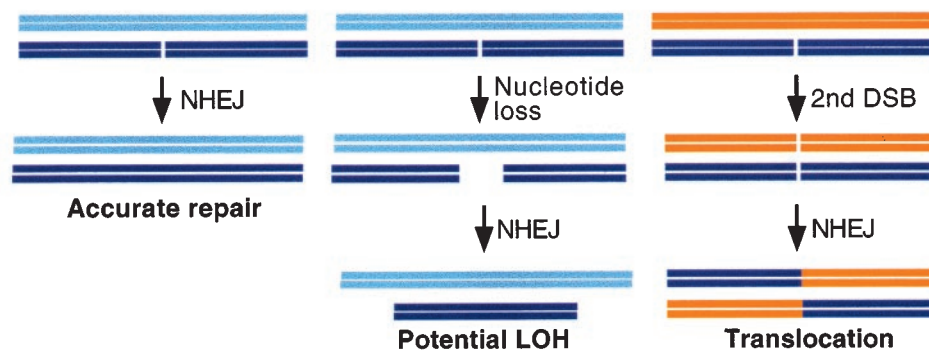
Homologous Recombination
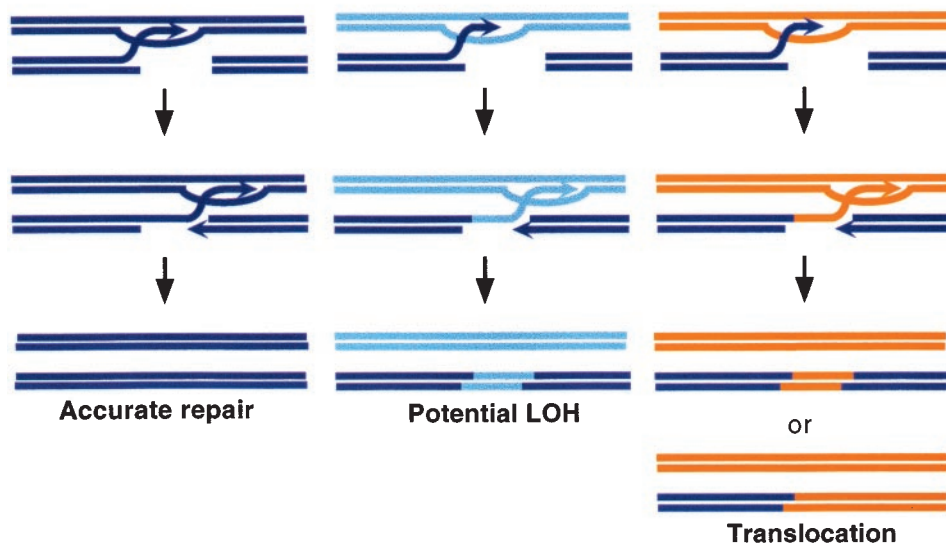
Figure 1 The benefits and risks of double strand break repair. The pairs of parallel lines represent chromosomes. The dark blue and light blue chromosomes are homologues, while red indicates a non homologous chromosome. The left column depicts how NHEJ and HR can effect accurate repair, with NHEJ catalyzing ligation without nucleotide loss, and HR using the sister chromatid as a replication template. The center column depicts how both pathways can generate LOH, with NHEJ catalyzing ligation after sequence loss due to nucleolytic processing or two nearby DSBs, and HR effecting gene conversion using the homologous chromosome as template. The right column depicts how either pathway can generate translocations, with NHEJ acting upon two DSBs on different chromosomes, and HR initiating replication in similar sequence on a non homologous chromosome, which could potentially extend to the end of a chromosome arm as has been shown in yeast (Bosco and Haber, 1998). The events shown are an oversimplification, as it has been demonstrated that lagging strand polymerases are also involved in HR (Holmes and Haber, 1999), and the two DSBR pathways may cooperate in some circumstances (Richardson and Jasin, 2000a)

In addition to general genome maintenance, both of the DSBR pathways are also utilized in specialized recombination reactions that are initiated by intentionally induced DSBs. NHEJ is used to complete V(D)J recombination in developing lymphocytes, while HR catalyzes recombination in meiosis.

Non-homologous end joining and genomic stability

Two multi-protein complexes have thus far been proven as factors in the NHEJ pathway. The first is the DNA-dependant protein kinase (DNA–PK) which is comprised of the Ku70 and Ku80 proteins which form a complex (Ku) that binds to DNA ends, plus the DNA–PK catalytic subunit (DNA–PKcs) which is catalytically activated by DSBs via the end-bound Ku complex. The second complex is comprised of DNA Ligase4 (Lig4) and XRCC4 which catalyze ligation during NHEJ. All of the NHEJ factors are evolutionally conserved from yeast to human, except the DNA–PKcs which is not found in yeast (Lieber, 1999) and is apparently not present in the completed genome databases of *Drosophila* or *C. elegans*. DNA–PKcs is

a large protein with homology to the phosphoinositol 3 kinases, and currently provides one of the more intriguing mysteries of DNA repair because no *in vivo* substrates have yet been identified. A new member of the NHEJ pathway, referred to as Artemis, has recently been identified through positional cloning of a gene harboring the mutation responsible for the rare human immunodeficiency disorder RS – SCID (Moshous *et al.*, 2001). Its relationship to other proteins in the pathway and roles in genomic stability are not yet known.

The critical role of the NHEJ pathway in DSBR was first illustrated by the extreme sensitivity of NHEJ deficient cells to ionizing radiation or compounds that preferentially induce DSBs, and resistance to UV or agents that induce single strand lesions (Jeggo, 1998). The central role of this pathway in metabolism of DSBs was further illustrated by the discovery that NHEJ is necessary for ligating the DSBs formed during V(D)J recombination (Taccioli *et al.*, 1993). V(D)J recombination is a process whereby individual V, D, and J gene segments are rearranged to produce a vast diversity of immunoglobulin genes. The reaction is initiated by the lymphocyte-specific RAG1/2 endonuclease which induces DNA DSBs specifically at recognition signal sequences (RSS) adjacent to the unrearranged gene coding segments (Fugmann *et al.*, 2000). The free coding ends are liberated as closed hairpins that are opened by unknown factors, and then acted upon by the lymphocyte-specific terminal deoxynucleotydal transferase (TdT), which increases diversity by adding non-templated random sequences (Komori *et al.*, 1993). The process is completed by members of the NHEJ pathway which join RAG-liberated V, D or J gene segments (Lieber, 1999). In addition, the NHEJ proteins join the RAG-liberated RSS ends, which have 5′ phosphorylated ends with no hairpin, to generate episomal circles or inversions depending on the relative orientation of the participating gene segments. Contrary to the other four factors, DNA – PKcs and Artemis are relatively dispensable for joining of the blunt RSS ends, suggesting that they may be more important for hairpin end-processing prior to ligation (Gao *et al.*, 1998; Moshous *et al.*, 2001).

Mice deficient in each of the components of the NHEJ pathway have been generated (except Artemis) and reveal common phenotypes, as well as surprising differences (Sekiguchi *et al.*, 1999). Deficiencies in Ku70, Ku80, XRCC4, and Lig4 generate a set of qualitatively similar phenotypes including premature cellular senescence, and defects in growth, V(D)J recombination, and neurogenesis – consistent with all phenotypes resulting from disruption of the basic, evolutionally-conserved NHEJ pathway. Notably, end-joining in XRCC4- and Lig4-deficient cells appears even more severely impaired than in Ku-deficient cells. This difference may contribute to the higher degree of neuronal cell death and embryonic lethality in the context of the XRCC4/Lig4-deficiency, as compared to Ku-deficiency. Finally, while Ku70, Ku80 and DNA –

PKcs are all considered subunits of DNA – PK, DNA – PKcs-deficiency generally has a much milder phenotype than that of Ku-deficiency, with no premature cellular senescence or major defects in RS joining, growth, or neurogenesis.

The cellular senescence and apoptosis phenotypes conferred by mutation in any of the four conserved NHEJ factors, as well as the embryonic lethality of Lig4/XRCC4 deficiency, implies that spontaneous double strand breaks occur frequently in normally dividing cells and the NHEJ pathway is critical for their repair. Indeed, a vital role for NHEJ in maintaining genomic stability was demonstrated by several groups who reported surprisingly frequent spontaneous chromosome and chromatid breaks in NHEJ deficient cells (Bailey *et al.*, 1999; Karanjawala *et al.*, 1999; Difilippantonio *et al.*, 2000; Ferguson *et al.*, 2000; Gao *et al.*, 2000). Given the role of the NHEJ pathway in ligation, it did not come as a surprise that breaks were observed when traditional banding techniques were employed. However, reliable examination of more complex events required the use of new technologies that allow for karyotyping of murine cells, a task nearly impossible by banding techniques routinely used on human cells. Chief among these techniques is Spectral Karyotyping (SKY) which has allowed significant advances in the use of mice as a model for genomic stability and tumorigenesis (Liyanage *et al.*, 1996). This technology uses a single DNA probe mixture labeled with five separate fluorochromes, with DNA probes specific for each chromosome labeled with a unique subset of the fluorochromes. A device known as an interferometer, under control of a computer, scans the entire visible spectrum and can classify each chromosome based on the specific subset of fluorescent colors. This powerful technology can also be used on human chromosomes and has successfully solved karyotypes that were challenging by traditional methods (Hilgenfeld *et al.*, 1999). Surprisingly, untransformed NHEJ deficient fibroblasts growing in normal culture conditions were shown using SKY to harbor spontaneous translocations that were non clonal and random (Difilippantonio *et al.*, 2000; Ferguson *et al.*, 2000; Gao *et al.*, 2000).

While simple chromosome breaks can easily be reconciled with the ligation function of NHEJ, translocations cannot. In fact, it might have been expected that the propensity to translocate would be decreased in the context of NHEJ deficiency because of the notion that this pathway catalyzes promiscuous ligation of broken DNA ends. Indeed, it has been reported that the simultaneous induction of two double strand breaks in different chromosomes induced by the rare cutting endonuclease I-SceI can lead to reciprocal translocations in which deletions of varying sizes were found at the breakpoints (Richardson and Jasin, 2000b). The presence of deletions led to the conclusion that NHEJ was involved, although this has not been formally tested by performing the assay in end-joining deficient cells. While simple ligation of free DNA ends in solution is random, this is not likely to be the case

for NHEJ *in vivo*. This notion is supported by studies on mouse embryonic fibroblasts (MEFs) doubly deficient for Lig4 and p53, which progress through the cell cycle after exposure to ionizing radiation to allow for karyotypic analysis (whereas Lig4$^{-/-}$ p53$^{+/+}$ cells arrest and cannot be examined). In comparison to irradiated wild type or p53 deficient cells, which showed only one or two chromosomal aberrations per cell 24 h after treatment, doubly deficient cells displayed massive chromosomal fragmentation (Ferguson *et al.*, 2000). Assuming the cells suffered similar numbers of double strand breaks after the same dose of IR, it is remarkable that the NHEJ pathway was able to so accurately rebuild the genome of wild type and p53 deficient cells such that the vast majority returned to growth by 48 h. If NHEJ were highly promiscuous, the karyotypes of these irradiated cells would have had many more translocations. Therefore, the degree of promiscuity assigned to end joining may be overestimated. It must be kept in mind that most assays employing I-Sce I rely on selection or observable deletion, which may enrich for aberrant events, and cannot identify NHEJ mediated perfect repair of the endonuclease target site, which could plausibly be the most common event.

Several of the phenotypes of end-joining deficient mice suggested that DNA damage responsive cell cycle checkpoints were being activated, likely as a result of persistent double strand breaks. These phenotypes included premature senescence of cultured embryonic fibroblasts and massive apoptosis of newly generated post-mitotic neurons (Sekiguchi *et al.*, 1999). Deficiency in DNA–PKcs does not confer either of these phenotypes, consistent with its overall relative dispensability in NHEJ. To test the role of checkpoints several groups crossed NHEJ deficient mice to those with p53 deficiency. The crucial interplay between p53 and NHEJ was dramatically highlighted by the ability of p53 deficiency to rescue the embryonic lethality and neuronal apoptosis of Lig4 or XRCC4 deficiency, indicating that these phenotypes were due to the cellular response to DSBs, and not to the damage *per se* (Gao *et al.*, 2000; Frank *et al.*, 2000). Furthermore, fibroblast premature senescence, but not V(D)J recombination, was rescued in Ku80, Lig4, and XRCC4 deficiencies (Difilippantonio *et al.*, 2000; Gao *et al.*, 2000; Frank *et al.*, 2000). Together, these studies demonstrated that V(D)J recombination fully relies on NHEJ and cannot be resurrected through removal of checkpoints, or substituted for by another repair pathway such as HR.

While removal of a checkpoint in the context of a severe DSBR defect may extend development to maturity, the consequences of unchecked genomic instability cannot be avoided for long. XRCC4 or Lig4 deficient mice, rescued by p53 deficiency, begin dying at 6 weeks of age and live a maximum of approximately 12 weeks. Ku80 (Difilippantonio *et al.*, 2000) or DNA-PK deficient (Guidos *et al.*, 1996; Nacht *et al.*, 1996; Vanasse *et al.*, 1999) mice, which normally are fairly long-lived, suffer from shortened life spans in the absence of p53. In fact, despite the dramatic differences in the phenotypes of the various end joining deficiencies, when p53 is absent all have virtually indistinguishable life spans. Remarkably, the cause of death in the vast majority of these double mutant mice were aggressive widespread progenitor B cell (Pro-B) lymphomas that usually harbored translocations between the immunoglobulin heavy chain (IgH) locus on mouse chromosome 12 and c-*myc* on chromosome 15 (Figure 2). Such translocations resemble the classic translocations found in human Burkitt's lymphomas and mouse plasmacytomas (Korsmeyer, 1992). One translocation in a Ku$^{-/-}$ p53$^{-/-}$ tumor contained a 12;3 translocation as determined by SKY, but was shown by FISH to have a complex event involving 12, 15 and 3. Such 'jumping' translocations have also been found in human tumors involving the c-*myc* and the IgH locus (Coleman *et al.*, 1999). In addition, several translocations from DNA-PKcs$^{-/-}$ p53$^{-/-}$ Pro-B tumors were reported to involve chromosome 15 near, but not at, the c-*myc* gene (Vanasse *et al.*, 1999). Whether these events involved a different oncogene or caused over expression of c-*myc* from a distance has not been resolved.

A crucial question raised by these findings is what cellular apparatus generates translocations in the absence of NHEJ? As all of the events in the Pro-B tumors involve the IgH locus, which is the first target of the RAG endonuclease in the development of the B lymphocyte lineage, it is reasonable to hypothesize that the initiating lesion is a RAG induced double strand break. This hypothesis is supported by the elimination of Pro B cell lymphomas in DNA-PK$^{-/-}$ p53$^{-/-}$ RAG2$^{-/-}$ (Vanasse *et al.*, 1999) and in preliminary data, XRCC4$^{-/-}$ p53$^{-/-}$ RAG2$^{-/-}$ (F Alt, unpublished) triple mutant mice. The subsequent steps are a mystery, with two likely possibilities. In the absence of a single component of NHEJ, the pathway may become inaccurate and catalyze the aberrant events. This is unlikely since significant residual catalysis of V(D)J recombination is not detected in the absence of XRCC4 or Lig4, where the pathway appears totally disabled. Alternatively, other main pathways of DSBR, including HR, may produce these events if a small region of sequence similarity between the two partners exists.

Two aspects of the translocations in the Pro-B lymphomas distinguish them from those most commonly found in Burkitt's lymphomas and mouse plasmacytomas. First, the Pro-B events in XRCC4/p53 double deficient mice only contained a derivative 12 translocation, with no derivative 15 observed (Gao *et al.*, 2000), suggesting the translocations were non-reciprocal, while the aforementioned tumors usually contain reciprocal partners (Korsmeyer, 1992). The consistent non reciprocal nature of translocations in these mice was confirmed in NHEJ deficient MEFs, indicating that this is not specific to RAG induced breaks, and not limited to the lymphoid lineage (Ferguson *et al.*, 2000). Second, nearly all of the Pro-B tumors displayed dramatic amplification of the IgH-
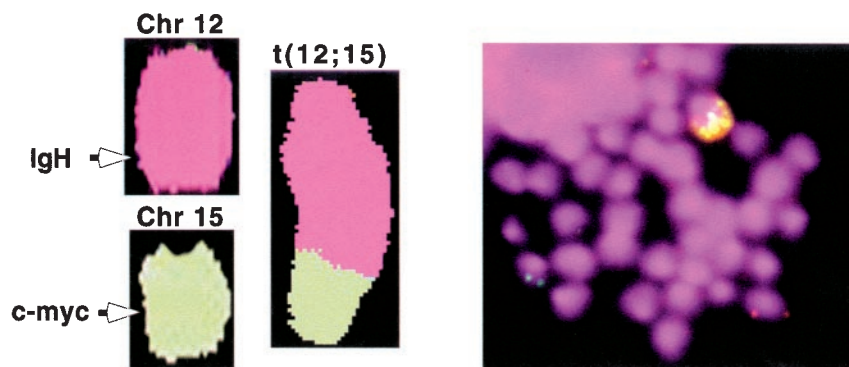
Figure 2 Left – 12;15 translocation from a pro-B cell lymphoma arising in an XRCC4 p53 double deficient mouse. The classified SKY colors are 12-pink and 15-green. Right – FISH using probes for IgH (red) and c-*myc* (green). The single copies of these genes can be seen faintly in the lower right and lower left corners respectively. The large yellow signal indicates colocalization and massive amplification of IgH and c-*myc*

c-*myc* fusion (Figure 2) (Difilippantonio *et al.*, 2000; Gao *et al.*, 2000; Frank *et al.*, 2000). The region was present in as many as 20 copies, and the amplification unit extended beyond several hundred kilobases in the IgH locus. It is not known whether this is also a property of translocations in NHEJ deficient MEFs as these were not clonal rearrangements, and thus could not be examined by Southern or FISH analyses.

Homologous recombination and genomic stability

Homologous recombination (HR) has been carefully studied in *E. coli* and single celled eukaryotes since the early 1960s, and in fact strains defective in this pathway were the earliest radiation sensitive mutants identified (Howard-Flanders and Boyce, 1966; Holliday, 1967). HR effects repair through interaction of a broken DNA duplex with homologous sequences on a sister chromatid or homologous chromosome. Several early findings led to the belief that, while simple eukaryotes preferentially use HR to repair DSBs, multicellular eukaryotes predominantly use NHEJ (Jeggo, 1998). These findings included the following; (i) while the earliest DSBR mutants identified in simple eukaryotes affected HR, the earliest mammalian mutants affected NHEJ (Jeggo, 1998); (ii) the mammalian genome was found to be replete with repetitive sequences which could wreak havoc on attempts to find appropriate homologous partners; (iii) Homologous gene targeting using transfected DNA is far less efficient in mammalian cells than in simple eukaryotes such as *Saccharomyces cerevisiae*. However, several recent studies indicate that HR in vertebrates is a frequently used double strand break repair pathway, whose functions are essential for survival.

HR in eukaryotes involves numerous proteins, many of which are evolutionarily related to *E. coli* RecA which catalyzes DNA strand-pairing and exchange reactions that are central to the interaction of homologous duplexes during repair. The main eukar-

yotic factor in the large family of RecA-like proteins is RAD51, which is highly conserved in all eukaryotes, contains similar *in vitro* activities to RecA, and when mutated in single-celled organisms cripples HR in mitosis and meiosis (Shinohara and Ogawa, 1999). Functions in multi-cellular organisms are presumed to be similar, but this has been difficult to demonstrate because mutation of Rad51 confers cellular lethality (Sonoda *et al.*, 1998) Similar to *S. cerevisiae*, multi-cellular eukaryotes each contain multiple RAD51 paralogs that likely act as accessory factors. In fact, the recently completed sequence of the human genome uncovered seven RAD51-like genes (Wood *et al.*, 2001). Numerous additional HR factors that are not related in sequence to RAD51 (or *E. coli* RecA), such as RAD52, RAD54, RAD50 and Mre11 also have conserved mammalian orthologs and in some cases have related paralogs (Haber, 2000). Why evolution has selected for such diversification of functional HR homologues while maintaining single copies of conserved NHEJ genes is an intriguing question with no answers at present.

A role for HR in maintaining genomic stability in vertebrate cells was firmly established using the DT40 chicken B cell lymphoma cell line, which allows for exceptionally efficient gene inactivation through homologous targeting. These studies have demonstrated that cells conditionally disabled for RAD51 cease dividing in late G2 phase and display a large number of chromatid breaks (Sonoda *et al.*, 1998). In a variety of experimental systems it has now been demonstrated that mutation of several of the Rad51 paralogs as well as RAD54 and other HR homologues leads to chromosomal instability to varying degrees (Haber, 2000).

A direct link between HR defects and oncogenesis has come from studies of the BRCA1 and BRCA2 genes, which are found mutated in familial breast and ovarian cancer syndromes (Welcsh and King, 2001). Both proteins interact directly or indirectly with RAD51 and colocalize with numerous HR proteins in radiation induced foci (Scully and Livingston, 2000;

Davies et al., 2001; Moynahan et al., 2001). Similar to RAD51, complete inactivation of either BRCA gene confers very early embryonic lethality, but hypomorphic or conditional alleles have proven useful in the generation of animal models. Germ line homozygous mutations affecting the C-terminus of BRCA2 allowed development to maturity, but caused a wide range of defects including radiation sensitivity, small size, improper differentiation of tissues, absence of germ cells, and development of lethal thymic lymphomas (Connor et al., 1997; Patel et al., 1998). Cultured MEFs underwent premature senescence and displayed spontaneous chromosomal abnormalities including chromatid breaks and radial structures. SKY was not performed, but it would be fascinating to determine if translocations are frequent and if they are typically reciprocal, as opposed to the non-reciprocal translocations associated with NHEJ deficiency. V(D)J recombination in developing lymphocytes was shown to be unaffected by BRCA2 deficiency, indicating that its function does not overlap with NHEJ. Therefore, the karyotypic changes causing the thymic lymphomas were likely produced by a mechanism distinct from that which produced the pro-B cell lymphomas in NHEJ/p53 double deficient mice, which were generated by incorrect repair of RAG induced DSBs. Why end joining deficiency led to tumors of the B lymphocyte lineage while BRCA2 deficiency led to those of T lymphocyte lineage is currently a mystery. A role for BRCA1 in maintaining genomic stability was demonstrated through the use of an allele in which a single exon was deleted specifically in mammary tissues, which led to tumor formation associated with recurrent translocations of chromosome 11 (Xu et al., 1999).

Genomic instability in telomerase deficient mice

Genomic instability has been observed in mutant backgrounds in which the inactivated gene is not directly involved in NHEJ or HR. The most dramatic example was seen in mice deficient for mTERC, the RNA component of telomerase. Telomeres serve to 'cap' the ends of chromosomes and ensure their protection and accurate replication (McEachern et al., 2000), and absence of telomerase led to genomic instability and tumor formation when the defect was propagated for several generations (Blasco et al., 1997; Rudolph et al., 1999). When combined with p53 deficiency, these mice suffered from a spectrum of epithelial cancers that resembled those that afflict humans and are rarely seen in mice (Artandi et al., 2000). These tumors harbored large numbers of Robertsonian fusions as well as non-reciprocal translocations which likely were generated by the fusion-bridge breakage cycle originally described in plants (McClintock, 1941). This cycle initiates in the context of short or absent telomeres, which results in 'unprotected' chromosome ends that subsequently fuse to form dicentric chromosomes, which are broken as the centromeres are pulled apart by the spindle pole apparatus. The free DNA ends then enter into subsequent rounds of fusion-bridge breakage until oncogenic karyotypic changes occur. This cycle involves two separate events that likely rely on the functions of DSBR pathways; the initial fusion of short or absent telomeres, and the subsequent conversion of DSBs into translocations. The roles of NHEJ and HR in this context are not understood and are compelling mysteries whose answers will have a strong impact on our understanding of human carcinogenesis. Use of mouse models combining telomerase and DSBR deficiencies should provide important answers.

Interplay between NHEJ and HR in maintaining genomic stability

The demonstration that both main pathways of DSBR function to prevent tumorigenesis by inhibiting the formation of translocations raises three fascinating questions: (i) Why are there two separate pathways of DSBR? (ii) Why is loss of one not compensated for by the other? (iii) In the absence of one pathway, how are potentially oncogenic translocations created?

Likely reasons for the existence of two DSBR pathways include action upon different substrates, and separation into different phases of the cell cycle. At present there is no convincing evidence to indicate that the two pathways discriminate between different types of DSBs (i.e. blunt versus overhang). However, there is evidence suggesting that the two DSBR pathways each predominate in different phases of the cell cycle. Studies using the DT40 cell line have demonstrated that Ku70 deficiency leads to radiation sensitivity predominantly in G1 and early S phase, while RAD54 deficiency effects survival in cells irradiated in late S and G2 (Takata et al., 1998). When Rad51 was conditionally inactivated in DT40, cells accumulated at the G2/M boundary and contained numerous isochromatid type (single chromatid) breaks, suggesting lack of repair after, but not before, replication (Sonoda et al., 1998). Furthermore, many proteins involved in HR co-localize in discrete nuclear foci during late S phase, even in the absence of exogenous DNA damage (Petrini, 2000). In contrast, V(D)J recombination is restricted to the G1 phase, and in cells deficient for NHEJ, HR cannot catalyze significant levels of productive rearrangements (Gao et al., 2000; Frank et al., 2000). Separation of DSBR pathways by cell cycle phase also makes for an appealing teleologic argument. Successful HR in G1 may be deleterious because gene conversion can cause loss of heterozygosity (LOH), whereas correct repair by NHEJ will not (Figure 1). After replication, HR may be preferred because it can restore the exact sequence missing at a DSB by using the sister chromatid as a template. Indeed, it has been demonstrated that in mouse embryonic stem cells the sister chromatid is used by HR far more frequently

than the homologous chromosome (Johnson and Jasin, 2000).

A pure separation of NHEJ and DSBR by cell cycle phase would be an oversimplification as significant overlap likely exists. For example, it has recently been suggested some double strand breaks may be repaired by coupled action of both HR and NHEJ (Richardson and Jasin, 2000a). In this study it was found that gene conversion at the site of a DSB could use a heterologous chromosome as template, and that the conversion extended beyond a small region of homology into non homologous regions. Deletions of various sizes found on the repaired chromosome led to the proposal that after homologous strand invasion and DNA synthesis, NHEJ terminates the reaction. In addition, reciprocal translocations were not observed, consistent with models of homologous recombination that do not employ a Holliday junction intermediate which would lead to frequent crossovers (Ferguson and Holloman, 1996; Malkova et al., 1996; Johnson and Jasin, 2000). The cooperation among pathways could function to maintain genomic stability by using NHEJ to limit the extent to which homologous recombination extends into non-homologous sequences. A prediction of this model is that in NHEJ deficiency, gene conversion tracts should be dramatically lengthened, and could in theory extend to the end of a chromosome arm, as has been documented to occur in yeast (Bosco and Haber, 1998). Consistent with this model, the spontaneous translocations observed in NHEJ deficient fibroblasts were non-reciprocal (Ferguson et al., 2000).

Conclusions

Extensive use of animal and animal cell models has recently shed much light on the roles of DSBR pathways in the generation of translocations. NHEJ and HR are both required to maintain genomic stability, and absence of either leads to potentially oncogenic translocations and other karyotypic changes. While both pathways exist to effect accurate and 'safe' repair, both have the potential to mis-repair and generate deleterious products. In light of this, it is easy to understand the strong evolutionary pressures that created mechanisms of apoptosis and cellular senescence, in which it is better to eliminate a cell with chromosomal damage from a dividing population rather than risk errant repair and oncogenic transformation. It is therefore also easy to appreciate the selective pressure during transformation to mutate the proteins responsible for this elimination, such as p53. The dual role of the DSBR pathways in both preventing and generating oncogenic translocations raises an intriguing potential therapeutic approach. Chemical inhibition of either pathway would sensitize tumor cells to local radiation or radiomimetic chemotherapy, but increase the risk of further transformation by inducing translocations and/or LOH. However, simultaneous inhibition of both pathways could lead to dramatic sensitization and potentially deny cells of the mechanisms needed to produce additional karyotypic changes. Further use and development of animal models will be required to explore this uncharted territory.

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