COMMENTARY

Sensing and repairing DNA double-strand breaks

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The DNA double-strand break (DSB) is the principle cytotoxic lesion for ionizing radiation and radio-mimetic chemicals but can also be caused by mechanical stress on chromosomes or when a replicative DNA polymerase encounters a DNA single-strand break or other type of DNA lesion. DSBs also occur as intermediates in various biological events, such as V(D)J recombination in developing lymphoid cells. Inaccurate repair or lack of repair of a DSB can lead to mutations or to larger-scale genomic instability through the generation of dicentric or acentric chromosomal fragments. Such genome changes may have tumorigenic potential. In other instances, DSBs can be sufficient to induce apoptosis. Because of the threats posed by DSBs, eukaryotic cells have evolved complex and highly conserved systems to rapidly and efficiently detect these lesions, signal their presence and bring about their repair. Here, I provide an overview of these systems, with particular emphasis on the two major pathways of DSB repair: non-homologous end-joining and homologous recombination. Inherited or acquired defects in these pathways may lead to cancer or to other human diseases, and may affect the sensitivity of patients or tumour cells to radiotherapy and certain chemotherapies. An increased knowledge of DSB repair and of other DNA DSB responses may therefore provide opportunities for developing more effective treatments for cancer.

Introduction

The DNA within our cells is continually being exposed to DNA-damaging agents. These include ultraviolet light, natural and man-made mutagenic chemicals and reactive oxygen species generated by ionizing radiation (IR) or by processes such as redox cycling by heavy metal ions and radio-mimetic drugs (1,2). Of the various forms of damage that are inflicted by these mutagens, probably the most dangerous is the DNA double-strand break (DSB). DNA DSBs are generated when the two complementary stands of the DNA double helix are broken simultaneously at sites that are sufficiently close to one another that base-pairing and chromatin structure are insufficient to keep the two DNA ends juxtaposed. As a consequence, the two DNA ends generated by a DSB are liable to become physically dissociated from one another, making ensuing repair difficult to perform and providing the opportunity for inappropriate recombination with other sites in the genome. Another barrier to rapid and error-free DSB repair is the fact that the DNA termini have often also sustained base damage, meaning that DSB ligation cannot occur until processing by DNA polymerases and/or nucleases has taken place.

Despite posing major threats to genomic integrity (see below), DSBs are nevertheless sometimes generated deliberately and for a defined biological purpose. Probably the best characterized example of this in higher eukaryotes is the pathway of V(D)J recombination, which occurs in developing B- and T-lymphocytes to provide the basis for the antigen-binding diversity of the immunoglobulin and T-cell receptor proteins. In this pathway, DNA DSBs are generated at specific loci by a site-specific nuclease composed of the RAG1 and RAG2 proteins, and the DSBs are subsequently repaired by proteins that also function in repair of DSBs that have been generated by mutagenic agents (for reviews see refs 3,4). Although tight controls are imposed on events such as V(D)J recombination, they can sometimes go awry, with potentially devastating consequences for the cell or for the organism.

DSBs are potent inducers of mutations and of cell death. In metazoa, just one DSB can kill a cell if it leads to the inactivation of an essential gene or, more commonly, triggers apoptosis (5). Furthermore, there is experimental evidence for a causal link between the generation of DSBs and the induction of mutations and chromosomal translocations with tumorigenic potential (6–10). Indeed, it is generally accepted that such chromosomal translocations must have arisen through the generation of one or more chromosomal DNA DSBs that were subsequently ligated together by a cellular DNA repair system. Many cancers of lymphoid origin bear oncogenic chromosomal rearrangements that have arisen as a consequence of the defective DSB repair of V(D)J recombination intermediates (11–13). A classical example of this is provided by the B-cell malignancy, Burkitt’s lymphoma, where the c-MYC gene is often juxtaposed by genome rearrangement to the immunoglobulin heavy-chain genes. Furthermore, the loss and/or amplification of chromosomal material that is characteristic of many cancer cells—and is associated with the inactivation of tumour suppressor loci and activation of proto-oncogenes, respectively—is most easily explained as having arisen through inappropriate DSB repair events. In addition, and as discussed further below, mutations in many of the factors involved in DSB signalling and repair lead to increased predisposition to cancer in people and in animal models. Indeed, defects in cellular responses to DSBs may be a frequent initiating event of carcinogenesis (4,7).

The DNA-damage response

As shown in Figure 1, cells respond to DNA DSBs through the actions of systems that detect the DNA lesion and then trigger various downstream events. At least in some cases,
these systems can be viewed as classical signal-transduction cascades in which a ‘signal’ (DNA damage) is detected by a ‘sensor’ (DNA-damage binding protein) that then triggers the activation of a ‘transducer’ system (protein kinase cascade), which amplifies and diversifies the signal by targeting a series of downstream ‘effectors’ of the DNA-damage response. Clearly, such systems need to be exquisitely sensitive and selective, as they must be triggered rapidly and efficiently by low numbers of, and maybe just one, chromosomal DNA DSB, yet must remain inactive under other conditions.

One cellular response to DSBs is to activate and/or induce the levels of DNA repair proteins, which are then physically recruited to the site of the DNA lesion to bring about its repair (Figure 1). In addition, dividing cells respond to DNA DSBs by slowing down progression through the cell cycle. When damage arises in the G1 or S cell-cycle phases, for example, entry into S-phase is prevented or progress through S-phase is slowed, respectively. This presumably provides time to allow DNA repair to occur before the lesions are encountered by a replicative DNA polymerase. Similarly, DNA DSBs present in G2-phase prevent entry into mitosis, thereby preventing the mis-segregation of chromosomal fragments during cytokinesis (for recent reviews see refs 7,14,15). Such pauses in cell-cycle progression are often termed ‘cell-cycle checkpoints’. Recently, however, it has become clear that DNA damage-induced cell-cycle checkpoint pathways actually regulate several other events and, moreover, that various aspects of these responses may even take place in cells that are not actively dividing. Therefore, in some circumstances, a more appropriate term for these events is simply ‘the DNA-damage response’.

A crucial component of the DNA DSB signalling cascade in mammalian cells is the protein kinase, ATM (for reviews see refs 16,17). ATM deficiency leads to the human cancer predisposition and neurodegenerative syndrome ataxia-telangiectasia (A-T). At the cellular level, ATM deficiency is manifested by increased sensitivity to ionizing radiation and other agents that yield DNA DSBs but little or no hypersensitivity to other forms of DNA damage. In addition, A-T cells are markedly impaired in ionizing radiation-induced G1–S, intra-S and G2–M cell-cycle checkpoints (16,17). Recent data suggest that ATM is recruited to and activated at sites of DNA DSBs (18). Once activated, ATM then phosphorylates various downstream substrates, including p53, the checkpoint kinase CHK2, BRCA1 and NBS1, leading to a variety of effects on DNA repair, cell-cycle progression and apoptosis (for detailed reviews see refs 7,14–17; also see below). ATM homologues also exist in *Saccharomyces cerevisiae* (Tel1p) and *Schizosaccharomyces pombe* (Tel1), where they are also involved in genome surveillance and controlling telomere length.

Another DNA-damage surveillance protein that is related to ATM is ATR (for review see ref. 17). Disruption of the gene for ATR leads to early embryonic lethality in the mouse (19,20) and to cellular inviability in mouse or chicken DT40 B-lymphocyte cells. The reason for this lethality is not yet clear but is likely to reflect a role for ATR in the recognition and repair of DNA replication complexes that have stalled at sites of DNA damage. Overexpression of catalytically inactive dominant-negative mutants of ATR leads to hypersensitivity to several DNA-damaging agents and to the DNA replication inhibitor hydroxyurea (21,22). Homologues of ATR also exist in *S.cerevisiae* and *S.pombe* (Mec1p and Rad3, respectively) and play key roles in the DNA-damage response (for reviews see refs 14,15,17). The available evidence indicates that ATR phosphorylates an overlapping set of targets to ATM and responds to a distinct spectrum of lesions from those that trigger ATM activation. It is also clear that ATR plays a particularly important role in signalling DNA damage during S-phase (for example see ref. 23). As discussed further below, both ATM and ATR share homology in their kinase domains with the DNA DSB repair protein DNA-PKcs.

An aspect of the DNA-damage response that may be particularly important for non-dividing cells is the elevation of the levels of deoxyribonucleotides, which are necessary for the DNA synthesis-dependent steps of DSB repair. In mammalian cells, this is achieved at least in part by the p53-dependent transcriptional induction of the ribonucleotide reductase subunit p53R2 (24), whereas in *S.cerevisiae* it is mediated by the post-translational modification of the ribonucleotide reductase inhibitor, Sml1p (25). Other non-cell-cycle aspects of the DNA-damage response include changes in factors bound to yeast telomeric DNA (26–28) and the reorganization of chromatin structure. In yeast, this latter response is brought about by the phosphorylation of histone H2A (29), whereas in mammals it is triggered by phosphorylation of the histone H2A isoform, H2AX (30,31). This could lead to alteration of chromatin structure at the site of DNA damage so that recruitment of DSB repair factors can take place efficiently (29,32). Finally, under conditions where the extent of DNA damage is too great, cells can instead enter an apoptotic programme. Although the details of how this decision is reached are not yet clear, it appears that this pathway involves the actions of proteins that also function in other aspects of the DNA-damage response (5,33,34).

**DNA DSB repair pathways**

There are two main pathways for DNA DSB repair—homologous recombination (HR) and non-homologous end-joining (NHEJ). These pathways are largely distinct from one another and function in complementary ways to effect DSB repair (35–38). During HR, the damaged chromosome enters into synopsis with, and retrieves genetic information from, an undamaged DNA molecule with which it shares extensive sequence homology. In contrast, NHEJ, which brings about the ligation of two DNA DSBs without the requirement for

![Fig. 1. Schematic representation of cellular response to DNA DSBs.](image-url)
Fig. 2. Schematic representation of the pathway of DNA NHEJ, indicating the known players in this pathway in vertebrates. NHEJ rejoining the two broken ends directly and generally leads to small deletions of DNA sequence. It requires Ku, which binds to free ends and recruits DNA-PKcs. Ku then recruits XRCC4 along with DNA ligase IV, and DNA-PKcs-mediated phosphorylation of XRCC4 may influence its activity. Ligase IV then brings about the physical religation of the DNA ends. The MRE11–RAD50–NBS1 complex, which contains exo- and endo-nuclease and helicase activities, may also function in NHEJ, particularly if the DNA ends require processing before ligation. However, it is possible that other nucleases are involved in addition to or instead of the MRE11 complex, and candidates for such a nuclease include mammalian Artemis (shown) and \textit{S.cerevisiae} Rad27p. In many cases, NHEJ may also require the actions of a DNA polymerase(s).

extensive sequence homology between the DNA ends, does not need suppression of the broken DNA with an undamaged partner DNA molecule. Both pathways are highly conserved throughout eukaryotic evolution but their relative importance differs from one organism to another. Simple eukaryotes such as the yeasts \textit{S.cerevisiae} and \textit{S.pombe} rely mainly on HR to repair radiation-induced DNA DSBs. In contrast, in mammals the NHEJ pathway predominates in many stages of the cell cycle—particularly in \textit{G}₀ and \textit{G}₁—although HR is also of importance, particularly during \textit{S}- and \textit{G}₂-phases (39). The basic mechanisms of these pathways and the factors involved are outlined below.

DNA NHEJ

The basic mechanism and factor requirements of NHEJ are described in Figure 2. Central to NHEJ in organisms from yeast to man is the Ku protein, a heterodimer of two subunits called Ku70 (~69 kDa in man) and Ku80 (~83 kDa in man; also known as Ku86) (40–42). Biochemical studies of mammalian Ku have revealed that it binds to DNA in a non-sequence-dependent fashion and in a manner that relies on DNA DSBs (43). These features have been explained recently by the crystal structure of Ku, both alone and bound to double-stranded DNA (44). In brief, Ku forms an open ring-type structure that can be threaded onto a DNA end. One side of the ring forms a cradle that protects one surface of the DNA double helix, whereas the other side is much more open, presumably to allow other NHEJ factors to access the DSB.

In vertebrates, Ku serves as the DNA targeting subunit of the DNA-dependent protein kinase catalytic subunit (DNA-PKcs), which together with Ku forms the DNA-PK holoenzyme (41). DNA-PKcs is an ~465 kDa polypeptide, the C-terminal region of which has homology to the catalytic domains of proteins of the phosphatidylinositol 3-kinase-like (PIKK) family (17,41,45). Strikingly, this family includes two other mammalian proteins implicated in responses to DNA damage, ATM and ATR, along with their counterparts in other organisms. Despite their homology to lipid kinases, DNA-PKcs and these proteins seem to be exclusively protein kinases. DNA-PKcs itself has affinity for DNA ends and its activation appears to be triggered by its interaction with a single-stranded DNA region derived from a DSB (46,47). As a consequence of this and the DNA binding properties of Ku, DNA-PK is activated by DNA DSBs \textit{in vitro}, and presumably this is also the case \textit{in vivo}. Once bound to DNA DSBs, DNA-PK displays protein Ser/Thr kinase activity with preference for the consensus sequence Ser/Thr-Gln (48,49). Likely \textit{in vivo} substrates for DNA-PK include XRCC4 (50) and replication factor A2 (51).

Phosphorylation of these factors presumably facilitates NHEJ. Although no clear homology exists between DNA-PKcs and other regions of ATM and ATR, the recent findings that these proteins are also physically recruited to sites of DNA damage \textit{in vivo} raises the possibility that these kinases share a common general mechanism for activation in response to disruptions in genome integrity (for recent review see ref. 52).

Despite the existence of Ku in all eukaryotes examined, DNA-PKcs has so far only been identified in vertebrates. One possibility is that the functions of DNA-PKcs in higher eukaryotes are carried out in lower eukaryotes by other members of the PIKK family. Indeed, although the mechanism for the effect has not yet been defined, in \textit{S.cerevisiae}, mutations in one member of this family, Mec1p, do lead to reduced NHEJ (53). Alternatively, the functions of DNA-PKcs might indeed be restricted to higher organisms. For example, DNA-PKcs may enhance the efficiency of NHEJ by helping to bridge the two DNA ends and/or by preventing these from being degraded or becoming engaged in other recombination events. DNA-PKcs might also facilitate NHEJ by helping to overcome the repressive effects of chromatin, particularly the more condensed chromatin states that are prevalent in higher eukaryotic genomes. Finally, it is possible that DNA-PKcs allows the NHEJ machinery to tackle more difficult DNA end-structures than a Ku-based system could alone. Indeed, unlike the situation in higher eukaryotes, NHEJ is inefficient in yeast unless the two DNA ends bear simple, mutually cohesive overhanging termini (54,55).

Another mammalian NHEJ factor is DNA ligase IV, which brings about DNA strand-joining events by this pathway and functions in a tight complex with the protein XRCC4 (56–58). Work in yeast has shown that Ku is required for the recruitment of the analogous Dnl4p–Lif1p complex to chromosomal DSBs \textit{in vivo} and that Lif1p appears to act as an adaptor between Ku and Dnl4p (59). Consistent with this, biochemical studies in the mammalian system have shown that Ku can load the XRCC4–ligase IV complex onto DNA ends and stimulate DNA end-ligation (60–62). Recently, it has been established that NHEJ in \textit{S.cerevisiae} is regulated in a cell-type-specific
manner by a Lif1p-interacting protein, Nej1p/Lif2p (63–66). This positively acting NHEJ protein is not expressed to a significant level in diploid cells, meaning that NHEJ only functions effectively in the haploid state. Whether a Lif2p homologue exists in mammals is not yet known.

Most DNA DSBs that are generated by mutagenic agents cannot be directly religated and, instead, some limited processing and/or DNA polymerization must take place before NHEJ can ensue. Consequently, NHEJ is rarely error-free and sequence deletions of various lengths are usually introduced. 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 in vitro (67,68) and has been shown by immunocytochemical studies to localize to sites of DNA DSBs in mammalian cells (69,70). Analysis of the MRE11–RAD50–NBS1 system in vertebrates has been particularly difficult due to the inviability of cells totally lacking components of this complex (71–74). However, *S.cerevisiae* strains deficient in components of the analogous Mre11p–Rad50p–Xrs2p complex are deficient in NHEJ (75–77), and recent work indicates that the complex can juxtapose DNA ends and stimulate Ku- and Dnl4p-catalyzed NHEJ in vitro (78,79). Another nuclease linked to NHEJ in yeast is the 5′ flap-specific endonuclease Rad27p, which is homologous to mammalian FEN-1 (80). In mammalian cells, another candidate for a factor that processes DNA DSBs before NHEJ can occur is the Artemis protein (81). This factor has weak sequence homology to the metallo-β-lactamase superfamily, some members of which possess hydrolytic activities. As described below, defects in Artemis occur in a group of radiosensitive human patients with severe combined immune-deficiency. The identity of the DNA polymerase(s) that might function in NHEJ is not yet clear, although work in yeast has indicated an involvement of Pol4p, the homologue of mammalian DNA polymerase β (82).

Inactivation of the genes for NHEJ proteins in cells or animals leads to radiosensitivity that is associated with an impairment of DSB religation but little or no hypersensitivity to agents that do not yield DSBs (for reviews see refs 10,41). The rejoining of V(D)J recombination intermediates is also severely impaired in these mutants, leading to severe combined immune-deficiency (SCID) at the animal level (83,84). No people deficient in Ku or DNA-PKcs have so far been identified but a group of radiosensitive SCID patients has defects in Artemis (81). Significantly, the loss of Ku strongly affects rejoining of both V(D)J coding and signal sequences, whereas loss of DNA-PKcs or Artemis has a less pronounced effect on signal join formation than on coding join formation (81,85). As coding ends, but not signal ends, pass through a DNA hairpin intermediate (3) this suggests that DNA-PKcs or Artemis might be, or might modulate the actions of, a hairpin-opening endonuclease; such a nuclease could also be required for the limited processing of radiation-induced DSBs.

DNA-PKcs-deficient mice are overtly normal in appearance. In contrast, Ku−/− mice are small and display various features suggestive of premature ageing (for review see ref. 10). This may indicate that Ku is required for the repair of a larger repertoire of naturally arising DSB lesions than DNA-PKcs, or could indicate that Ku has other cellular functions that are not shared with DNA-PKcs. Strikingly, inactivation of the genes for DNA ligase IV or XRCC4 in the mouse leads to embryonic lethality associated with extensive apoptosis of newly generated post-mitotic neurons in the central nervous system (for review see ref. 10). This might be due to defects in hypothetical genome rearrangement events taking place in these cells but the current consensus is that it reflects an inability to deal effectively with spontaneously arising DNA DSBs, which are then repaired inappropriately. In line with this idea, NHEJ-deficient cells in culture have high rates of spontaneous chromatid and chromosome breaks (86–91). Why does the inactivation of ligase IV or XRCC4 in the mouse lead to a more severe phenotype than the inactivation of Ku? One explanation is that in XRCC4 or ligase IV-deficient cells, Ku and DNA-PKcs can still bind to DSBs, leading to a non-productive complex that prevents access by the other repair components, such as those involved in HR. In contrast, in Ku-deficient animals, such alternative DSB pathways are not inhibited, allowing them to compensate to some degree for a loss of NHEJ. Support for this idea comes from work in chicken DT40 cells that showed that LIG4−/− cells are more radiosensitive than Ku70+−/− cells but that Ku70+/−LIG4−/− double-mutant cells have a similar sensitivity to Ku70−/− cells (92).

The presence of persistent DNA DSBs in NHEJ mutants could also trigger the activation of DNA DSB signalling events, thus contributing to the slow growth rates, spontaneous apoptosis and premature senescence phenotypes of cells lacking Ku or ligase IV–XRCC4. Consistent with this idea, p53 or ATM deficiency rescues the embryonic lethality and neuronal apoptosis of XRCC4−/− or LIG4−/− mice (90,93,94) and the premature senescence of Ku-deficient cells in culture (95). As V(D)J recombination and radio-resistance were not restored in these studies, it seems that the severe phenotype of XRCC4- or ligase IV-deficient mice and the poor growth and premature senescence of mouse fibroblasts lacking XRCC4, ligase IV or Ku, is not due to the presence of persistent DSBs per se but reflects the induction of p53-dependent apoptotic or cell-cycle arrest programmes.

Loss of DNA-PKcs or Ku function has been associated with increased rates of lymphomas in the mouse (for example refs 96–98). This phenotype is exacerbated when NHEJ-deficient mice also lack p53 function (88,99–101). The predominant cause of death in these doubly mutant mice is due to aggressive progenitor B-cell lymphomas that bear translocations between the c-Myc gene and the immunoglobulin heavy-chain locus—a translocation seen in Burkitt’s lymphomas. It seems probable that such translocations in the mice arise as a consequence of inappropriate repair of DNA DSBs generated at the immunoglobulin locus by the RAG1/2 enzyme, a hypothesis that is supported by the observation that lymphoma incidence in DNA-PKcs−/−, p53−/− mice is suppressed in the RAG2−/− background (101). Recently, it has been found that mouse cells haploinsufficient for the gene for DNA ligase IV contain chromosomal translocations, amplifications and deletions and, when this ligase IV haploinsufficiency is combined with homozygous loss of the INK4A/ARF locus, this leads to an elevated incidence of soft-tissue sarcomas (102).

Finally, some of the features of Ku- and DNA-PKcs-deficient cells and animals may reflect the function of the proteins at telomeres—the nucleoprotein caps at the ends of linear eukaryotic chromosomes. Recent studies have shown that cells lacking functional Ku or DNA-PKcs are particularly prone to chromosome end-to-end fusions, presumably due to a loss of telomere capping functions (86,91,103–105). These findings are consistent with work in yeast, which has shown that Ku
but not Dnl4p functions in telomere length maintenance in <i>S. cerevisiae</i> (55,106,107).

In light of the above, it is tempting to speculate that defects in NHEJ may be associated with carcinogenesis and/or clinical radiosensitivity in people. Indeed, 180BR cells, which were derived from a radiosensitive leukaemia patient, contain a point mutation in a highly conserved amino acid residue in the catalytic domain of DNA ligase IV (108,109). Although this mutation is clearly the cause of the radiosensitivity, unlike NHEJ-deficient mice the patient was not overtly immunodeficient and was not reported to have any neurological defects. This may be because the mutation in 180BR cells does not totally abolish ligase IV activity. Presumably, the residual ligase IV activity is sufficient for generation of enough productive V(D)J junctions to yield a functional immune system and for the repair of endogenous damage but is insufficient to repair larger amounts of damage inflicted by exposure to clinical doses of radiotherapy. Recently, mutations in ligase IV have been found in a set of patients showing immunodeficiency combined with developmental and growth delay (110). The cells from these patients are radiosensitive, display chromosomal instability but are proficient in cell-cycle checkpoint responses. It seems that the mutations in these individuals do not totally inactivate ligase IV function but are more deleterious than those in the patient giving rise to the 180BR cells. The human glioma cell line, MO59J, also has a NHEJ deficiency. This is associated with a defect in DNA-PKcs that was probably acquired subsequent to the generation of the tumour or upon passage of the cells in culture (111,112).

Perhaps surprisingly, other than the group of SCID patients defective in Artemis (81), no other people have been found who are fully deficient in a NHEJ component. Although there are other potential explanations, this suggests that full deficiency in Ku or DNA-PKcs might not be compatible with human life. Recently, it was shown that targeted disruption of just one allele of the <i>KU80/KU86</i> gene in the human HCT116 colon cancer cell line leads to an increase in polyploid cells, slow growth and elevated p53 levels (113). Moreover, inactivation of the second <i>KU80</i> allele in this study led to cells that could only divide a limited number of times before succumbing to apoptosis, presumably due to an inability to repair endogenously arising DSBs. In contrast, the gene for DNA ligase IV has been homozygously inactivated in a human pre-B-cell line to yield radiosensitive but viable cells (114). This suggests that the requirement of NHEJ factors for cell viability may vary from one human cell type to another. Given the above results, it currently seems most likely that variations in the levels or activity of NHEJ components—rather than their total inactivation—will be of greatest medical relevance. Consistent with this idea, variations in the levels of Ku70 have been correlated with clinical outcome in carcinoma of the cervix (115).

HR

The molecular basis and genetic requirements of HR were initially defined by studies in bacteria and yeast but it has become clear that this pathway is well conserved in higher organisms (for recent reviews see refs 4,35,36,116). In brief, genetic analysis of <i>S. cerevisiae</i> identified a set of genes— <i>RAD50</i>, <i>RAD51</i>, <i>RAD52</i>, <i>RAD54</i>, <i>RAD55</i>, <i>RAD57</i>, <i>RAD59</i>, <i>MRE11</i> and <i>XRS2</i>—whose products play important roles in HR and whose defects lead to increased sensitivity to ionizing radiation. Mammalian homologues of essentially all of these factors in the ‘RAD50 group’ have now been described (117). The events of HR are complex and, based on analyses of HR under various biological circumstances and in different organisms, there are various models for precisely how they take place (see ref. 35). An outline of one model is given in Figure 3.

An early event in yeast HR is believed to be the nucleolytic resection of the DNA DSB in the 5’ to 3’ direction. The efficiency of this reaction <i>in vivo</i> relies upon, and probably involves, a complex containing Rad50p, Mre11p and Xrs2p (NBS1 in humans). The ensuing 3’ single-stranded DNA tails are then bound by Rad51p in a process that is influenced by a range of other proteins, including replication protein A (RPA), Rad52p and Rad54p. Notably, human RAD52 has been shown to preferentially bind to DNA DSBs, leading to the proposal that competition between it and Ku for DNA ends may determine which of the two DSB repair pathways is employed (118). The Rad51p nucleoprotein filament then interacts with an undamaged DNA molecule and, when a homologous region has been located, Rad51p catalyzes strand-exchange events in which the damaged molecule invades the other DNA duplex, displacing one strand as a D-loop. These events are influenced by the other members of the RAD50 group of proteins and by other factors such as RPA (for example ref. 119). The 3’ terminus of the damaged DNA molecule is then extended by a DNA polymerase that copies information from the undamaged partner, and the ends are ligated by DNA ligase I (Figure 3). Finally, after migration, the DNA crossovers (Holliday junctions) are resolved by cleavage and ligation to yield two intact DNA molecules. Although HR is generally accurate and non-mutagenic, an exception to this can occur when direct repeats flank the two
DNA ends. In this case, HR by the pathway of single-strand annealing may lead to loss of one of the two direct repeats and the intervening DNA (36).

Although mammalian homologues exist for all of the known *S.cerevisiae* HR factors, the details of HR are likely to be considerably more complex in higher eukaryotes. One indication of this is the existence of several RAD51 paralogues, such as RAD51B, C and D, and other proteins with weaker homology to the catalytic domain of RAD51, such as XRCC2 and XRCC3 (117). At least some of these factors interact directly with RAD51 and their functions appear to be to help the assembly of the RAD51 nucleoprotein filament and/or the selection and interaction with the appropriate recombination substrate. Indeed, where analyzed, these factors have important roles in the HR process (for example refs 120–124).

Recent work has established strong links between HR and the breast cancer susceptibility proteins, BRCA1 and BRCA2, which do not appear to have direct homologues in yeast (for reviews see refs 125–127). Specifically, loss of function of either BRCA1 or BRCA2 in mammalian cells markedly reduces the efficiency of accurate homology directed DNA repair (128–131). Furthermore, mutation of BRCA2 stimulates error-prone homology directed repair of DNA DSBs that have been generated between repeated sequences (132). It is not yet clear exactly how these effects are brought about but they may reflect the binding of BRCA1 and BRCA2 to RAD51 (for example refs 133,134). Indeed, it has been shown that, through its BRC motifs, BRCA2 directly interacts with RAD51 thereby affecting both the nuclear localization and DNA binding properties of RAD51 (135) and permitting RAD51 to form foci at sites of DNA damage within the cell (127,136,137). The presence of BRCA1 in complexes involved in chromatin remodelling and/or the control of transcription (138,139) raises the possibility that it may also affect HR by changing chromatin structure at sites of DNA DSBs or could influence HR indirectly through its involvement in transcriptional responses to DNA damage.

Loss of HR in vertebrate cells leads to an inability to successfully traverse S-phase, and this is probably due to an inability to restart replication at sites where DNA replication forks have collapsed as a consequence of encountering endogenously generated lesions such as DNA single-strand breaks. Presumably as a consequence of this, inactivation of genes such as *RAD51, BRCA1* and *BRCA2* leads to inviability of vertebrate tissue culture cells and to early embryonic lethality in the mouse (140–143; also see above). The inviability of vertebrate cells disrupted for *MRE11, RAD50* or *NBS1* may also be related to defects in HR (71–74). Nevertheless, hypomorphic mutations or conditional alleles of the above genes have been described that have allowed cellular viability and, in some cases, the development of mice to maturity. For instance, mice bearing one truncation mutant of Brca2 are small and radiation sensitive and have an increased incidence of thymic lymphoma (144,145). Cells bearing this or another truncation mutant were shown to be radiosensitive, to display high levels of spontaneous chromosomal rearrangements and/or to senesce prematurely in culture (146,147). It should be noted, however, that HR can be involved in mechanisms of telomere maintenance (in mammals, called the alternative lengthening of telomeres (ALT) pathway) that do not rely on telomerase (148,149). It is therefore possible that some of the phenotypic effects of loss of HR factors could reflect deficiencies in telomere metabolism.

For some other HR genes viable cells and animals have been generated. A good example of this is provided by *RAD54*, mice or chicken DT40 B-cells in which the *RAD54* gene has been homozgyously disrupted are radiosensitive and have reduced rates of HR but are viable (37,150). Furthermore, when these mutations are combined with deficiencies in Ku, this results in greater radiosensitivity than the single mutants, thus providing strong support for the idea that HR and NHEJ act in complementary ways to repair radiation-induced DNA damage in vertebrates (37,38). In addition, DT40 cells lacking RAD51B or mouse cells lacking *XRCC2* or *XRCC3* have reduced rates of HR and high levels of chromosomal aberrations and/or mis-segregation of chromosomes at mitosis (120–124,151,152).

Finally, recent data reveal links between the ATM- and ATR-dependent systems of DNA-damage signalling and the activation of the HR pathway. For example, work in the chicken DT40 system has shown genetically that ATM functions, at least in part, by affecting the HR pathway (153). Further evidence for such a link is provided by the findings that a subgroup of patients with patients with A-T do not have mutations in ATM but instead bear hypomorphic mutations in *MRE11* (154) and that hypomorphic defects in NBS1 cause the related human disorder, Nijmegen breakage syndrome (NBS) (155,156). These diseases are characterized by radiosensitivity and defects in the intra-S-phase DNA-damage checkpoint. The latter appears to be dependent on the phosphorylation of NBS by ATM in response to DNA DSBs (157–160; for review see ref. 161). In addition, it seems likely that the lethality associated with disruption of ATR function in mouse or DT40 cells is linked with an involvement of ATR in triggering HR as a mechanism to help the resolution of stalled DNA replication complexes. The *S.cerevisiae* Mre11p complex also functions in DNA-damage responses, including the S-phase checkpoint, thus suggesting a high degree of evolutionary conservation for its roles in DNA-damage signalling (162–164). Perhaps phosphorylation affects the ability of the vertebrate and yeast MRE11 complexes to process DSBs. Alternatively, or in addition, the effects of ATM and ATR and their yeast homologues on HR might reflect the ability of these kinases to phosphorylate histone H2AX and thus bring about changes in chromatin structure at the sites of DNA DSBs. It is also possible that ATM and ATR affect HR indirectly by influencing events such as cell-cycle progression or the control of deoxyribonucleotide synthesis.

**Future directions**

Over the past few years, there has been much progress in our understanding of how cells detect, signal the presence of, and repair DNA DSBs, and we are beginning to understand how defects in these events are associated with carcinogenesis in humans. However, there is still much to learn. Major goals for future research will be to characterize DNA DSB responses in greater molecular detail and to identify further components of these pathways. Another key issue will be to understand how the cell coordinates the activities of the multiple systems that respond to DNA DSBs and how the relative importance of these different pathways is modulated during the cell cycle and in different cell types. For example, how are DNA-PKcs, ATM, ATR regulated and what are their individual and overlapping roles? Another key issue will be ascertaining how DNA DSB repair and signalling occur in the context of
chromatin. Finally, it will be of great interest to further define the roles of DSB response proteins in other cellular functions, including telomere maintenance and programmed genome changes such as V(D)J recombination, class-switch recombination, meiotic recombination and possibly immunoglobulin somatic hypermutation.

Progress in the above areas will be achieved by combining clinical knowledge with information gleaned from experiments in model organisms. A key long-term goal for this work will be to understand how DSB response pathways protect against carcinogenesis and how somatic or inherited deficiencies in these events may lead to carcinogenesis in humans. It also seems likely that this increased knowledge will lead to more effective treatments for cancer. For instance, genotyping or phenotyping individual cancers or patients for DNA DSB response pathways may lead to better predictions of how they will respond to radiotherapy and certain chemotherapies that will respond to radiotherapy and certain chemotherapies that may trigger the development of novel anticancer tumour. Moreover, increased knowledge of DSB response pathways may trigger the development of novel anticancer drugs that target proteins involved in DSB responses in order to bring about more effective and more selective killing of cancer cells.

Since submitting this article, Ma, Pannicke, Schwarz and Lieber have reported that an Artemis/DNA dependent protein kinase complex opens DNA hairpin structures and processes overhangs during NHEJ and V(D)J recombination (Cell: 2002, in press).

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