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Primer

Non-homologous end joining as a mechanism of DNA repair

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In spite of its essential role as the carrier of genetic information, DNA is not an inert structure. The genome is susceptible to potentially mutagenic threats of both endogenous and environmental origin. A dramatic threat to the covalent structure of DNA is posed by breaks in the phosphate backbone affecting one or both strands of the Watson–Crick double helix. Ionizing radiation and certain chemotherapeutic drugs, for example, generate single-strand and, less commonly, double-strand breaks, as well as clustered base lesions in both DNA strands where simultaneous excision-repair has the potential to convert a single-strand to a double-strand break. The cell is not unduly troubled by single-strand breaks, as it sees these as reaction intermediates during the excision-repair of DNA base damage, and so deals with them accordingly. However, a double-strand break is an extremely dangerous lesion, posing one of the greatest threats not just to the informational integrity but also to the structural cohesion of the DNA. Unless quickly repaired, such breaks can lead to chromosomal deletion, loss, rearrangement or cytotoxicity if the cell continues its cycle of DNA replication and cell division. Not surprisingly, numerous proteins are involved in detecting and repairing DNA double-strand breaks, or checking cell-cycle progression until repair is complete.

Double-strand break repair mechanisms rely on enzymes which evolved primarily to deal with developmentally programmed double-strand breaks. The deliberate targeted introduction of DNA double-strand breaks by endonucleases and their coordinated repair underpin key biological processes such as recombination between homologous chromosomes during meiosis or V(D)J rearrangements of immunoglobulin and T-cell receptor genes. The two main repair mechanisms that the cell can use when faced with an accidentally introduced DNA double-strand break, homologous recombination and non-homologous end joining, largely recruit those enzymes involved ordinarily in meiotic and V(D)J recombination, respectively.

The strategy of homologous recombination relies on the fact that replication generates an identical copy of the cellular DNA and the undamaged copy can be used as a template for resynthesis and repair of a damaged DNA strand. In this mode, homologous recombination is restricted to the S phase of the cell cycle, and serves to restart the replication machinery by resolving unfavourable DNA structures generated when a replication fork stalls. Homologous recombination requires extensive stretches of DNA sequence homology but is then a very accurate method of repair. By contrast, non-homologous end joining is a much more robust but low fidelity form of double-strand break repair, where the broken ends are simply fused together again irrespective of DNA sequence. This process requires at most a few base pairs of homology between the two broken ends, but may lead to loss of nucleotides at the join, hence the alternative names of ‘illegitimate recombination’ or ‘error-prone recombination’. As this end joining does not require a second homologous DNA duplex, it is of major importance in the G1 phase of the cell cycle where there is only a single copy of each chromosome. This article will focus on non-homologous end joining as a mechanism of DNA repair.

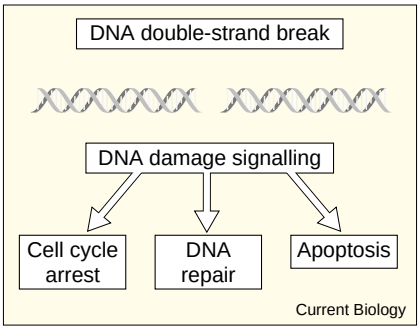
Responses to a double-strand break

Although single-strand breaks are formed as reaction intermediates during the excision-repair of DNA base lesions, these are continuously protected by the assembly of repair enzymes at the site of a lesion. Such protection is also likely to be provided by the replisome to double-strand breaks arising at a replication fork. Similarly, double-strand breaks introduced during meiosis may be physically constrained by a synaptonemal complex. In contrast, an accidental strand break is a rather exposed target, vulnerable to the unwanted attention of nucleases.

A large number of nuclear proteins specifically bind to DNA double-strand breaks. These proteins not only protect the lesion, but also signal the presence of the damage to the cell so that it can activate the appropriate repair pathway and delay the cell cycle until the damage has been repaired. Alternatively, an extensively damaged cell may execute its own death by activating apoptosis (Figure 1). Proteins binding to DNA double-strand breaks include the ATM — and probably ATR — protein kinases that signal the presence of damage to the cell cycle machinery, the RAD50–MRE11–p95 complex that may be involved in processing of DNA ends as well as damage signalling, and the homologous recombination protein RAD52. The catalytic domain of DNA-dependent protein kinase (DNA-PKcs), Ku and the DNA ligase IV–XRCC4 heterodimer are also able to bind; these are all components of the non-homologous end joining pathway.

It is still unclear how the cell chooses which pathway to use to repair a double-strand break, although the nature of the DNA ends at the break, the context in terms of cell cycle stage or nuclear compartment, as well as the balance between levels of the RAD52 and Ku proteins, all apparently affect this decision. Furthermore, the yeast *Saccharomyces cerevisiae* appears to

Figure 1



Cellular responses to a DNA double-strand break. The cell is alerted to the presence of a DNA double-strand break by DNA damage sensing and signalling mechanisms. The damage is channeled into the appropriate DNA repair pathway, the cell cycle is arrested as necessary until repair is complete or, depending on the cell type and extent of the damage, cell death (apoptosis) may be activated.

favour using homologous recombination whenever possible, whereas mammalian cells are very efficient at non-homologous end joining, although homologous recombination may be more prevalent than first thought. The basis for this difference is unclear, but may minimize the risk of gene amplifications or deletions that might otherwise result because of repetitive sequences in the mammalian genome.

Mechanism of end joining

The first requisite for repairing a double-strand break is to bind and tether the DNA ends, so that they are not only protected from further degradation but also brought into proximity for rejoining. Although several components of the non-homologous end joining pathway are able to bind DNA, the protein binding most avidly is Ku, a heterodimer of related 70 and 80 kDa subunits. In mammalian cells, Ku forms a complex with DNA-PK$_{cs}$ ('DNA-PK', without the subscript, strictly refers to this complex), recruiting it to the break, where the complex bridges the DNA ends and initiates assembly of the other components of the end joining pathway. DNA-PK$_{cs}$ is a very large and rather enigmatic protein. It does indeed, as its name suggests, have protein kinase activity, which is activated by binding Ku in the presence of DNA ends, and it phosphorylates several proteins, including other end-joining components and signalling proteins such as p53. However, there are two puzzling issues here: the kinase function is absolutely required for end joining but the essential target proteins remain unidentified; and there is no equivalent of DNA-PK$_{cs}$ in yeast, yet it is not clear whether there are fundamental differences in non-homologous end joining between mammalian and yeast cells.

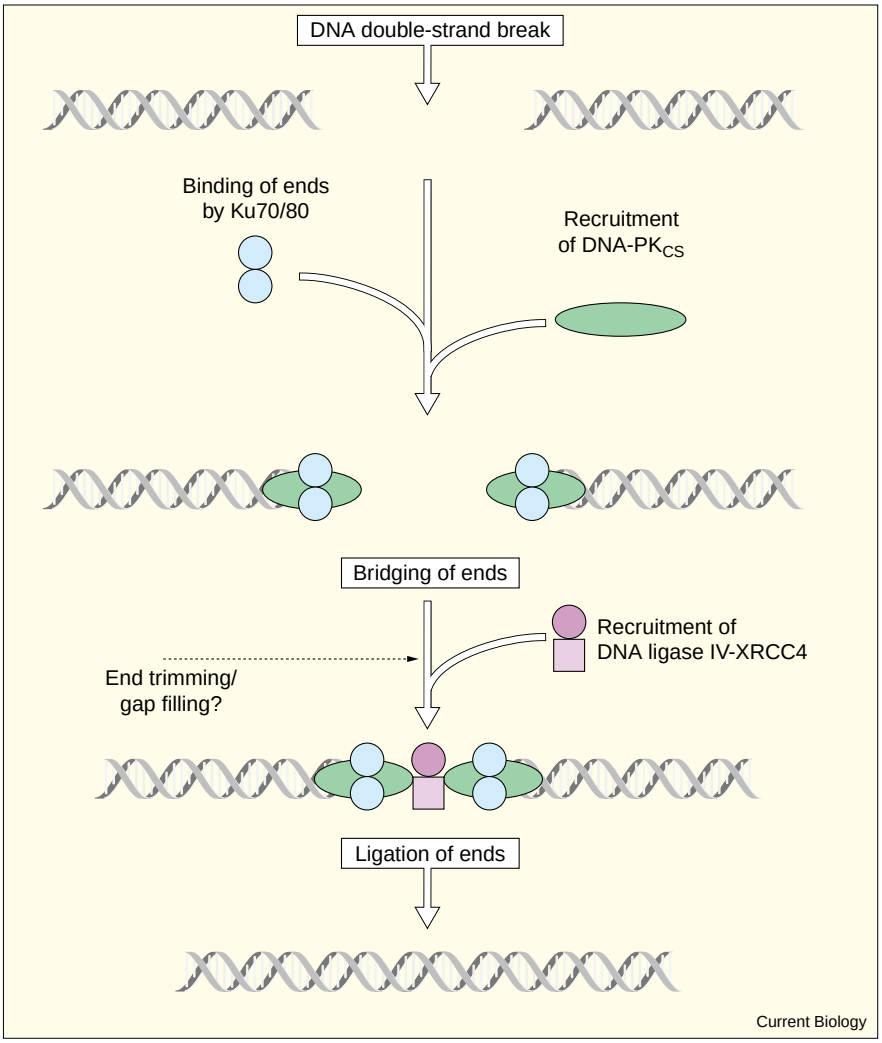
Once the DNA ends have been bound by Ku and DNA-PK$_{cs}$, another protein complex, DNA ligase IV–XRCC4, is recruited and ligates the DNA ends (Figure 2). However, the above factors are necessary but not sufficient for efficient end joining in an *in vitro* system and efforts in several laboratories are actively seeking the 'missing' components. These may not necessarily be other proteins: inositol phosphate (IP_6) is bound by DNA-PK and stimulates end joining *in vitro*. Furthermore, *in vivo*, a double-strand break rarely has ends that can be immediately rejoined by a DNA ligase; rather there could be single-strand overhangs at the break which need to be trimmed by an exonuclease and gaps to be filled in by a DNA polymerase. It is unclear which enzymes are employed in these roles, although interaction between the Ku70 and MRE11 proteins may implicate the latter's 3′→5′ exonuclease activity in non-homologous end joining.

Defective end joining

Cells with mutations in genes that encode components of the non-homologous end joining pathway are hypersensitive to ionizing radiation. Of a panel of 11 ionizing radiation-sensitive mutant rodent cell lines, four — IR4 to IR7 — are deficient in end joining factors. These lines can be complemented by human genes XRCC4 to 7, corresponding to XRCC4, Ku80, Ku70 and DNA-PKcs, respectively. (XRCC stands for X-ray cross complementing.) Furthermore, a mutant mouse strain shown to have severe combined immunodeficiency, or SCID, is also radiosensitive. These mice harbour a mutation in the DNA-PK$_{cs}$ gene so that they cannot carry out V(D)J recombination of their immunoglobulin and T-cell receptor genes. This provided the first indication of the link between V(D)J recombination, ionizing radiation sensitivity, and the repair of both developmentally programmed as well as accidental DNA double-strand breaks by non-homologous end joining. Subsequently, targeted gene disruption of DNA-PKcs in the mouse was shown to recapitulate the SCID phenotype, as do Ku70 and Ku80 knockout mice. Surprisingly, deficiencies in DNA-PK$_{cs}$ or Ku70/80 have not been reported in human SCID and it was thought that such cases might be inviable. However, a recent study of radiosensitive SCID patients identified mutations in a novel DNA double-strand break repair/V(D)J recombination protein; it will now be of great interest to establish the previously unidentified role of this protein in non-homologous end joining.

Prior to this discovery, DNA ligase IV had been unique amongst the end joining proteins in its relationship to human disease. A defect in DNA ligase IV was identified in a patient with childhood leukaemia who died from an adverse reaction to conventional radiotherapy, and cells derived from the patient were radiosensitive. Although work from knockout mouse models has shown that non-homologous end joining factors act as genome 'guardians', protecting against genome instability and cancer, it is not clear how frequently end joining defects

Figure 2



Repair of a DNA double-strand break by non-homologous end joining. The DNA ends are bound by the Ku70/80 heterodimer, which in turn binds and activates DNA-PKcs. The complex of Ku70/80 and DNA-PKcs protects the ends, directs assembly of the other factors and in some way bridges the DNA break. Finally, the DNA ligase IV-XRCC4 heterodimer is recruited and effects repair by ligating the DNA ends.

contribute to spontaneous tumours in the general population, nor whether they occur in the 1 in 4,000 cancer patients that over-respond to standard radiotherapy. In the case of DNA ligase IV or XRCC4, these would be subtle point mutations in the gene as, in contrast to DNA-PKcs and Ku70/80, targeted disruption of DNA ligase IV or XRCC4 surprisingly results in embryonic lethality in mice. These null mice show widespread apoptosis of newly generated neurons in the central nervous system. It seems that neuronal cells are particularly sensitive to undergoing apoptosis, and blocking the apoptotic pathway, by deletion of the p53 or ATM genes, rescues the null mice from both neuronal cell death and embryonic lethality. Intriguingly, the incidence, nature and source of DNA damage in the developing embryonic nervous system, that requires the function of DNA ligase IV–XRCC4, have yet to be resolved.

Context and future prospects

In a field where we are still identifying the key players, it is clear that there is much left to learn about non-homologous end joining as a mechanism of DNA repair. And any process acting on DNA must be considered in the broader context of chromatin conformation and sub-nuclear structure: there is clear evidence, largely from yeast, of interactions between non-homologous end joining and chromatin remodelling. Furthermore, certain end joining factors are essential to maintain the length of telomeres, presenting the paradox of end joining proteins binding to DNA ends that must not be joined. Ku in particular seems play a pivotal role in several cellular processes. A recent report of a functional interaction between Ku70 with the BLM protein, which is associated with homologous recombination, highlights an emerging interplay between non-homologous end joining and alternative repair pathways, as well as the coordination of repair with other cellular processes in the concerted response to a DNA double-strand break.

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