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Emerging models for DNA repair: *Dictyostelium discoideum* as a model for nonhomologous end-joining

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ABSTRACT

DNA double strand breaks (DSBs) are a particularly cytotoxic variety of DNA lesion that can be repaired by homologous recombination (HR) or nonhomologous end-joining (NHEJ). HR utilises sequences homologous to the damage DNA template to facilitate repair. In contrast, NHEJ does not require homologous sequences for repair but instead functions by directly re-joining DNA ends. These pathways are critical to resolve DSBs generated intentionally during processes such as meiotic and site-specific recombination. However, they are also utilised to resolve potentially pathological DSBs generated by mutagens and errors during DNA replication. The importance of DSB repair is underscored by the findings that defects in these pathways results in chromosome instability that contributes to a variety of disease states including malignancy. The general principles of NHEJ are conserved in eukaryotes. As such, relatively simple model organisms have been instrumental in identifying components of these pathways and providing a mechanistic understanding of repair that has subsequently been applied to vertebrates. However, certain components of the NHEJ pathway are absent or show limited conservation in the most commonly used invertebrate models exploited to study DNA repair. Recently, however, it has become apparent that vertebrate DNA repair pathway components, including those involved in NHEJ, are unusually conserved in the amoeba *Dictyostelium discoideum*. Traditionally, this genetically tractable organism has been exploited to study the molecular basis of cell type specification, cell motility and chemotaxis. Here we discuss the use of this organism as an additional model to study DNA repair, with specific reference to NHEJ.

1. Introduction

The genome is under continual assault from a variety of agents that induce DNA damage. As such, pathways have evolved that detect and repair specific types of DNA lesions to restore genome integrity. A particularly cytotoxic DNA lesion is the DNA double strand break (DSB). It is estimated that a single DSB can activate cell cycle arrest and, if left unrepaired, induce cell death [1]. A number of pathways exist that detect DSBs when they occur and initiate a series of events to restore genome integrity. These pathways are utilised under highly regulated circumstances to resolve DSBs induced intentionally during meiotic recombination and site-specific recombination events including V(D)J recombination during immunoglobulin maturation. However, they are also employed to resolve potentially pathological DSBs induced through errors in DNA replication, or by mutagens such as reactive oxygen species (ROS) generated during cellular metabolism or by exposure of cells to ionising radiation (IR). The importance of DSB repair is underscored by the observations that deregulation of these pathways leads to chromosomal rearrangements, such as deletions and translocations that are associated with a variety of disease states, including malignancy.

DSBs can be repaired by two distinct but complementary pathways, homologous recombination (HR) and non-homologous end-joining (NHEJ). A variety of HR sub-pathways exist, all of which require sequences homologous to the damaged DNA template to facilitate repair [2]. HR results in accurate repair of DSBs and is utilised in late S and G2 phases of the cell cycle. In contrast, NHEJ is the principle DSB repair pathway utilised in G1, although it is also active during other phases of the cell cycle [3–5]. NHEJ does not require homologous DNA sequences to facilitate repair. Instead, a variety of proteins are recruited to the break that directly re-join DNA ends [6]. The most straightforward mechanism of NHEJ is the detection and direct ligation of DNA ends. However, the majority of DSBs generated by ROS and other mutagens possess complex structures at their ends that require processing to make them compatible for ligation. These events result in loss or addition of genetic material, making NHEJ an error prone form of DNA repair. In the absence of core NHEJ factors, a variant NHEJ pathway is utilised. This pathway, termed alternative-NHEJ (A-NHEJ), functions through limited resection of the break to reveal short regions of homology that are subsequently used for DNA end synapsis and ligation [7].

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Understanding the molecular basis of DSB repair in relatively simple genetic model organisms has been instrumental in providing a greater understanding of how these pathways function in humans. This is particularly the case for HR, where studies in prokaryotes and single cell eukaryotes provided a mechanistic understanding of these pathways that was subsequently applied to vertebrates. For example, recombination genes were initially identified in prokaryotes by screening mutant strains for recombination deficiencies or sensitivity to IR, in addition to testing previously defined mutants that may conceivably function in the recombination process [8]. Similarly, in the yeasts *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe*, genetic screens for mutants with altered recombination efficiencies, defects in sporulation, or sensitivity to DNA damage led to the identification of a variety of genes required for recombination, the orthologues of which were subsequently identified in vertebrates [9,10]. A striking degree of conservation in the mechanistic basis of HR is evident between prokaryotes and eukaryotes and it is now apparent that the general principles of these pathways have been conserved throughout evolution. Therefore, although certain differences and additional complexities in the vertebrate HR pathways have become apparent, studies in model organisms have provided an important conceptual framework that has facilitated the unravelling of these pathways in humans.

In contrast to HR, genetic screens employed in prokaryotes or eukaryotic models such as yeast met with limited success in identifying components of the NHEJ pathway. Instead, the key players in this pathway were identified by cloning genes mutated in radiosensitive and/or V(D)J recombination-deficient vertebrate cell lines, and the orthologues of these genes subsequently identified in other organisms. Nevertheless, the identification and analysis of NHEJ in genetically tractable organisms has provided important advances and a wealth of information that has been invaluable to understanding how NHEJ is regulated in vertebrates. The overall mechanisms of NHEJ, namely re-joining of DNA ends, are conserved across species. However, differences in how DSBs are resolved exist between lower eukaryotes and vertebrates, whether it is conservation of a particular NHEJ or HR factor(s), or the primary pathway that is utilised to resolve these DNA lesions. Thus widening the study of DSB repair and NHEJ to other less conventional model organisms may uncover hitherto unrecognised conservation of these pathways, or where this is not the case, novel or more subtle mechanisms by which they are regulated that may be applicable to vertebrates. Here we review the recent advances in using *Dictyostelium discoideum* as a model for DSB repair and NHEJ in particular.

2. The vertebrate NHEJ pathway

The first step in vertebrate NHEJ is recognition of DNA ends by the DNA end-binding activity of Ku (Fig. 1). Ku consists of a heterodimer of Ku70 and Ku80 polypeptides that interacts with components of the NHEJ pathway *in vitro* [11–15] and is required for their assembly at sites of DNA damage *in vivo* [12,15,16]. One such factor, the DNA-dependent protein kinase (DNA-PK) catalytic subunit (DNA-PKcs), is recruited to DSBs *via* an interaction with the C-terminus of Ku80 [17,18] and this stimulates the kinase activity of the protein [11]. Loss of DNA-PKcs results in radiosensitivity and severe combined immune deficiency (SCID) due to defects in NHEJ and V(D)J recombination [11]. The kinase activity of DNA-PKcs is required for NHEJ [19–22]. Although a number of proteins phosphorylated by DNA-PKcs in response to DSBs have been identified, including core NHEJ factors, the physiological relevance of these events remains unclear [23]. One exception is DNA-PKcs which undergoes phosphorylation within several amino acid clusters following DNA DSBs, some of which have been attributed to autophosphorylation *in trans* [24]. Phosphorylation in two of these clusters spanning amino acids 2609–2647 and 2023–2056, termed the ABCDE and PQR clusters respectively, are required for efficient NHEJ [25–27]. The emerging theme is that these events influence access of factors that mediate end-processing to facilitate NHEJ or HR [26,28]. However, whereas autophosphorylation within ABCDE promotes end-processing, phosphorylation within PQR inhibits this process, indicating a reciprocal relationship between phosphorylation at these two clusters. Although the molecular basis of how these phosphorylation events regulate DSB repair remains unclear, ABCDE phosphorylation defective DNA-PKcs is retained longer at DSBs *in vivo* [16], resulting in radiosensitivity and defective HR [25,29]. This has led to the proposal that phosphorylation of DNA PKcs within this cluster induces disassembly of the NHEJ complex to promote DNA end-resection and HR.

DNA ends produced by agents such as IR and ROS are often complex structures that require processing to render them compatible for DNA end-joining. Additional NHEJ factors possess activities to facilitate this process. One such protein, Artemis, is a β-CASP metallo-β-lactamase family nuclease that mediates NHEJ by opening hairpins at coding joints during V(D)J recombination [30,31]. Following interaction with DNA-PKcs phosphorylated within the ABCDE cluster, Artemis exhibits endonuclease activity on 5′ and 3′ overhangs, in addition to DNA hairpins [32,33]. Artemis is also capable of removing 3′-phosphoglycolate groups from DNA ends *in vitro* [34]. Whilst initial reports suggested Artemis also possesses a 5′ exonuclease activity, subsequent studies indicate this is not an intrinsic property of the protein [35]. Artemis-defective cells are radiosensitive and the nuclease activity of the protein is required to repair approximately 10% of DNA lesions that are resolved more slowly following administration of IR [36]. Initially, it was thought that this represented processing more 'complex' DNA structures to facilitate NHEJ. However, more recently this has been proposed to represent repair of DSBs in specific chromatin contexts, most notably heterochromatin [37,38].

Several other factors process DSBs during vertebrate NHEJ. Polynucleotide kinase (PNK) restores 5′-phosphate groups, in addition to removing 3′-phosphate moieties by virtue of its 3′-phosphatase activity [39]. Depletion of PNK results in radiosensitivity and delayed repair of IR-induced DNA damage [40]. Consistent with a role in NHEJ, radiosensitivity of PNK-depleted cells is epistatic with DNA-PKcs [41] and PNK is required to restore terminal 5′-phosphate groups to promote NHEJ in cell free extracts [42]. Furthermore, PNK interacts with phosphorylated XRCC4 through a FHA phospho-recognition motif at the N-terminus of the protein, providing a possible explanation for how PNK is recruited to sites of DNA damage [43]. The variant DNA polymerases of the PolX family, Polμ, Polλ and terminal deoxynucleotidyl transferase (TdT) have also been implicated in processing DNA ends by performing alignment-based gap filling of DNA ends to promote NHEJ *in vitro* [44–46]. TdT, Polμ and Polλ interact with Ku, providing a link between PolX family members and their recruitment to sites of DSBs [46,47]. However, Polμ$^{-/-}$, Polλ$^{-/-}$ or Polμ$^{-/-}$Polλ$^{-/-}$ MEFs are not overtly radiosensitive [48]. Therefore, should these proteins promote NHEJ-mediated repair of IR-induced DSBs, they are likely required to process only a subset of breaks. Finally, the Aprataxin-PNK-like factor (APLF) has been implicated in DNA strand break repair [49–52] and NHEJ [53]. Although APLF contains endonuclease and exonuclease activities that process DSBs to facilitate NHEJ *in vitro* [52,54], how these activities impact on NHEJ *in vivo* remains unclear. However, APLF interacts with phosphorylated XRCC4 through its FHA domain [49,50,52], and with Ku80 through its vWA domain [55,56], and is required to facilitate accumulation of NHEJ factors at DNA lesions to promote end-joining *in vivo* [53].