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Alternative end-joining pathway(s): Bricolage at DNA breaks

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ABSTRACT

To cope with DNA double strand break (DSB) genotoxicity, cells have evolved two main repair pathways: homologous recombination which uses homologous DNA sequences as repair templates, and non-homologous Ku-dependent end-joining involving direct sealing of DSB ends by DNA ligase IV (Lig4). During the last two decades a third player most commonly named alternative end-joining (A-EJ) has emerged, which is defined as any Ku- or Lig4-independent end-joining process. A-EJ increasingly appears as a highly error-prone bricolage on DSBs and despite expanding exploration, it still escapes full characterization. In the present review, we discuss the mechanism and regulation of A-EJ as well as its biological relevance under physiological and pathological situations, with a particular emphasis on chromosomal instability and cancer. Whether or not it is a genuine DSB repair pathway, A-EJ is emerging as an important cellular process and understanding A-EJ will certainly be a major challenge for the coming years.

1. Introduction

DNA double strand breaks (DSBs) are the most deleterious lesions inflicted on the genome. Discontinuity on both DNA strands may prove lethal for the cell if left unrepaired, or lead to chromosome aberrations and promote tumor development when misrepaired [1,2].

DSBs can arise from endogenous sources, mainly corresponding to accidental events like replication fork collapse following stalling or arrest at DNA damage or telomere deprotection [3]. More specialized mechanisms of DSB formation also exist that rely on development-associated programmed processes like meiosis during gametogenesis [4], or V(D)J recombination [5] and class-switch recombination (CSR) [6] which facilitate the rearrangements of antigen receptor genes in lymphogenesis. Aside from these endogenous sources, DSBs are also produced by environmental or medical sources of clastogenic injuries such as ionizing radiation (IR), radiomimetic chemicals or topoisomerase inhibitors [3,7].

Cells have evolved two main repair pathways to cope with DSB genotoxicity: homologous recombination (HR) which uses homologous DNA sequences as repair templates [8–10], and non-homologous end-joining (NHEJ) involving direct sealing of DSB ends [3,7,11,12].

In addition to the extensively studied predominant NHEJ pathway, an alternative end-joining mode (A-EJ) has emerged during the last two decades. Here, we review its mechanism and compare arguments suggesting that A-EJ relies on a single pathway, on various (sub)pathways or on no defined pathway. In addition, we report mounting evidence that although A-EJ may have little physiological relevance in normal cells due to several concurrent locks, A-EJ may particularly contribute to cancer through promotion of genetic instability and chromosomal translocation.

2. Historical overview and definition of A-EJ

Over the past two decades, the dominant NHEJ pathway has been thoroughly investigated and its genetic and mechanistic bases have been largely clarified (for reviews, see [3,7,11–13]). Briefly, the reaction is initiated by the binding of the Ku complex at each DNA end. Ku is a ring-shaped heterodimer composed of two subunits (Ku70 and Ku80) able to encircle the free ends. Once bound, Ku recruits the remaining components of the reaction including the catalytic subunit of the DNA-dependent proteine

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kinase (DNA-PKcs). Together these form an active serine/threonine DNA-PK holoenzyme belonging to the phosphatidylinositol 3-kinase-related kinases (PIKKs) family. Besides its essential catalytic function, DNA-PK has a major role in maintaining both DNA ends in close proximity, although recent findings have indicated a substantial role of the ligase complex in stabilizing this synapsis [14]. Both the DNA-PK and the ligase complexes are important for DNA end processing which is frequently required to make ends ligatable [15]. One of the processing activities is carried out by the structure-specific endonuclease Artemis, a DNA-PKcs partner which is also involved in hairpin opening during V(D)J recombination (see below, section 4). The ligation complex is composed of DNA ligase IV (Lig4), a homodimer of XRCC4 (X-Ray repair cross-complementing protein 4) which is indispensable for Lig4 stability, and the more recently identified Cernunnos homodimer (also known as XRCC4-like factor, henceforth referred to as Cer-XLF) whose exact function remains unclear. The Lig4 complex has no known function aside from its essential role in NHEJ, whereas components of the DNA-PK complex, especially Ku70/Ku80, have been implicated in multiple important cellular functions such as telomere maintenance [16], replication [17], transcription [18] or apoptosis [19].

The major role of NHEJ in response to IR-induced DSBs or during the V(D)J recombination is underlined by the cellular radiosensitivity (RS) and the severe combined immunodeficiency (SCID) phenotype routinely observed when one of the corresponding genes is mutated, whether in animal models or in human patients with hereditary RS-SCID syndromes [20,21].

Although NHEJ plays a critical role in DSB repair, a residual end-joining activity was reported in yeast mutated for Ku80, Ku70 or Lig4 [22,23]. The resulting repair products exhibited deletions and were strikingly characterized by a strong dependence on short homologous sequences at the junctions [22]. Similar NHEJ-independent end joining activities were also found in mycobacteria [24], *Arabidopsis* [25], *Caenorhabditis elegans* [26], *Xenopus* [27], chicken [28], as well as in rodent [29–32] and human cells [32–34]. Furthermore, when analyzed, the junctions consistently exhibited a greater use of microhomology (MH), compared to NHEJ. This novel universal alternative end-joining mode was susbequently termed MH-mediated end-joining (MMEJ), as opposed to the *canonical* or *classical* NHEJ, hereinafter called C-NHEJ. Although MMEJ resembles single-strand annealing (SSA), the MMEJ mechanism differs by being independent of Rad52 [35,36] and by using significantly shorter direct repeats [37]. Although the C-NHEJ-independent end-joining pathway is biased toward an increased use of MH, direct end joining and MH usage are not exclusive attributes of C-NHEJ and alternative end-joining pathway, respectively [3,38]. Moreover, apparently direct joints may yet arise from a MMEJ process using occult MH through non-templated nucleotide insertion by a TdT-like polymerase activity of polμ [39] or templated insertion by polθ [40,41]. Consequently, it seems prefereable to use the generic name alternative end-joining (A-EJ) instead of the restrictive term MMEJ and to define A-EJ as any Ku- or Lig4-independent end-joining process [42].

3. Molecular mechanism of A-EJ

3.1. A-EJ tools

A-EJ investigations have benefited from the development of a large number of assays that have been set up to study DSB repair by either NHEJ or HR pathways. Historically, *in vitro* end-joining assays as reviewed in Pastwa and coll. [43] first established important features of the NHEJ mechanism. The technique is based on incubation of linearized plasmid DNA or oligonuclotides bearing or not modified ends with cell extracts or purified proteins. These *in vitro* assays brought early insights into the mechanism of A-EJ [30,34,44–50]. Notably, low Mg^{2+} concentration were found to favor DNA-PKcs-dependent end-joining activity [51], whereas high Mg^{2+} concentration (10 mM) facilitated DNA-PK-independent reaction [34]. In addition, A-EJ preferred high DNA ends/protein molar ratios [44,52] or was favored by volume excluders like PEG [53], possibly indicating a weak intrinsic synapsis activity at DNA ends.

More recently, *in vivo* end-joining assays developed to study NHEJ have also contributed to establish features of A-EJ (Table 1). They use transient transfection of linearized reporter substrates followed by plasmid rescue, or cutting of intrachromosomal GFP-based reporter substrates by the rare endonuclease I-SceI [54]. End-joining is monitored by restoration of reporter gene expression combined with PCR amplification and DNA sequencing of the junctions. A drawback of transient transfection assays may be that significant differences in MH usage and/or end-joining efficiency are obtained depending on the transfection method employed [32,55]. Limitations of intrachromosomal assays concern first, the low frequency of double I-SceI cut at a single locus and second, underestimation of accurate NHEJ efficiency because iterative I-SceI cutting tends to select inaccurate repair.

Assessing repair of endogenous DSB generated during the two physiological processes of V(D)J and class-switch recombination (CSR) that relies on end-joining has also been useful to characterize A-EJ under conditions of C-NHEJ deficiency (Table 1). Although repair is measured on endogenous substrates, conclusions from these assays may not be entirely transposable to repair of any DSB. V(D)J recombination breaks are preferentially constrained to C-NHEJ and repetitive context sequence of CSR breaks favors MH usage. Finally, we have used a cellular fractionation protocol to study A-EJ in native chromatin. After treatment with a strong DSB inducer followed by western-blotting or immunostaining, recruitment of A-EJ proteins to damaged chromatin can be studied in cells engineered for Ku depletion [56].

3.2. A-EJ players

Based on the C-NHEJ mechanism, the A-EJ reaction likely relies on at least three main steps (Fig. 1). First, the two DNA ends must be recognized and held together. Second, although sometimes dispensable, most of DNA ends require processing to make them ligatable. Several enzymatic activities may participate in this step such as various types of nucleases, dRP lysases, kinases, phosphatases, helicases and polymerases. Third, the final step requires a DNA ligase and according to the proposed definition of A-EJ, this step in mammals should rely on DNA ligase III (Lig3) and/or DNA ligase I (Lig1).

In the next sections, we review the protein components potentially involved in the recognition/synapsis, processing and ligation steps of A-EJ.

3.2.1. End recognition and tethering of DSB ends

Several reports have established a role for poly(ADP-ribose) polymerase 1 (PARP1) in early steps of A-EJ. PARP1 is a sensor of DNA damage that binds to single strand breaks (SSBs) and DSBs, gets activated and catalyzes the poly(ADP-ribosyl)ation of proteins at DNA damage sites (reviewed in [57], [58]). The well documented role of PARP1 in SSB repair is to recruit factors including the ligation complex XRCC1/Lig3 to promote repair *via* DNA end processing and ligation [59]. PARP1 also participates in the initial accumulation of the MRE11/RAD50/NBS1 (MRN) complex to DSBs [60].

Concomitantly to the finding that PARP inhibitors increase the sensitivity of DNA-PK-deficient cells to radiomimetic-induced DSBs [45], biochemical experiments [45,48,49] and plasmid assays in Ku-deficient cells [61] substantiated the involvement of PARP1 in a non