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Risky business: Microhomology-mediated end joining

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ABSTRACT

Prevalence of microhomology (MH) at the breakpoint junctions in somatic and germ-line chromosomal rearrangements and in the programmed immune receptor rearrangements from cells deficient in classical end joining reveals an enigmatic process called MH-mediated end joining (MMEJ). MMEJ repairs DNA double strand breaks (DSBs) by annealing flanking MH and deleting genetic information at the repair junctions from yeast to humans. Being genetically distinct from canonical DNA DSB pathways, MMEJ is involved with the fusions of eroded/uncapped telomeres as well as with the assembly of chromosome fragments in chromothripsis. In this review article, we will discuss an up-to-date model representing the MMEJ process and the mechanism by which cells regulate MMEJ to limit repair-associated mutagenesis. We will also describe the possible therapeutic gains resulting from the inhibition of MMEJ in recombination deficient cancers. Lastly, we will embark on two contentious issues associated with MMEJ such as the significance of MH at the repair junction to be the hallmark of MMEJ and the relationship of MMEJ to other mechanistically related DSB repair pathways.

1. Introduction

DNA double strand break (DSB) is the most lethal form of DNA damage and the repair of the broken DNA ends must occur so that cells can survive. Collectively, two main pathways of DSB repair exist: (i) homologous recombination (HR) and (ii) non-homologous end joining (NHEJ) [2]. While NHEJ repairs DSB by juxtaposing and processing DNA ends permissible for ligation [9,14,48,62], HR restores chromosome integrity by pairing and copying the missing genetic information from sister chromatid or homologous chromosome [74]. Furthermore, HR can also occur by single end invasion and repair synthesis to the end of chromosome known as break-induced replication or BIR [52], or by annealing flanking repeat sequences and deleting inter-repeat sequence known as single strand annealing or SSA [34]. All NHEJ (a.k.a. classical EJ) in eukaryotic cells essentially depend on Ku and Ligase IV whereas HR is dependent on Rad51 and several proteins that help load Rad51 on DNA molecules [49,72].

While this generalization of repair into two separate pathways is largely true given separate genetic requirements and predominant cell cycle stages, the flexibility of DSB repair is more complex. Limited repair still occurs when both of these repair options are disabled [8,46]. Many of these residual repair events feature short stretches (2–20 bp) of complementary base pairing called microhomology (MH) at the breakpoint junctions, and thus the name MMEJ emerges [51,55,62,67]. Of note, MMEJ is genetically distinct from classical NHEJ (i.e., Ku and/or Ligase IV-independent) and HR (Rad51, Brca2-independent), and is loosely defined based on the obligate requirement of flanking MH longer than 2-bp for repair [19,51,54,55,65]. However, even NHEJ and several other DSB repair pathways also use MH for complementary base pairing and end juxtaposition [60],and therefore the presence of MHs at the breakpoint junctions should not be used as a sole test for the recognition of MMEJ event (see the later part of this review). Furthermore, not all repair events in NHEJ deficient cells feature MH at the breakpoints and therefore MMEJ corresponds to the subset of so called "alternative end joining" [3,23,27,55,83].

2. MMEJ reporters and systems

To date, the basic framework of MMEJ is assembled primarily from the studies in which artificially designed MMEJ reporters were

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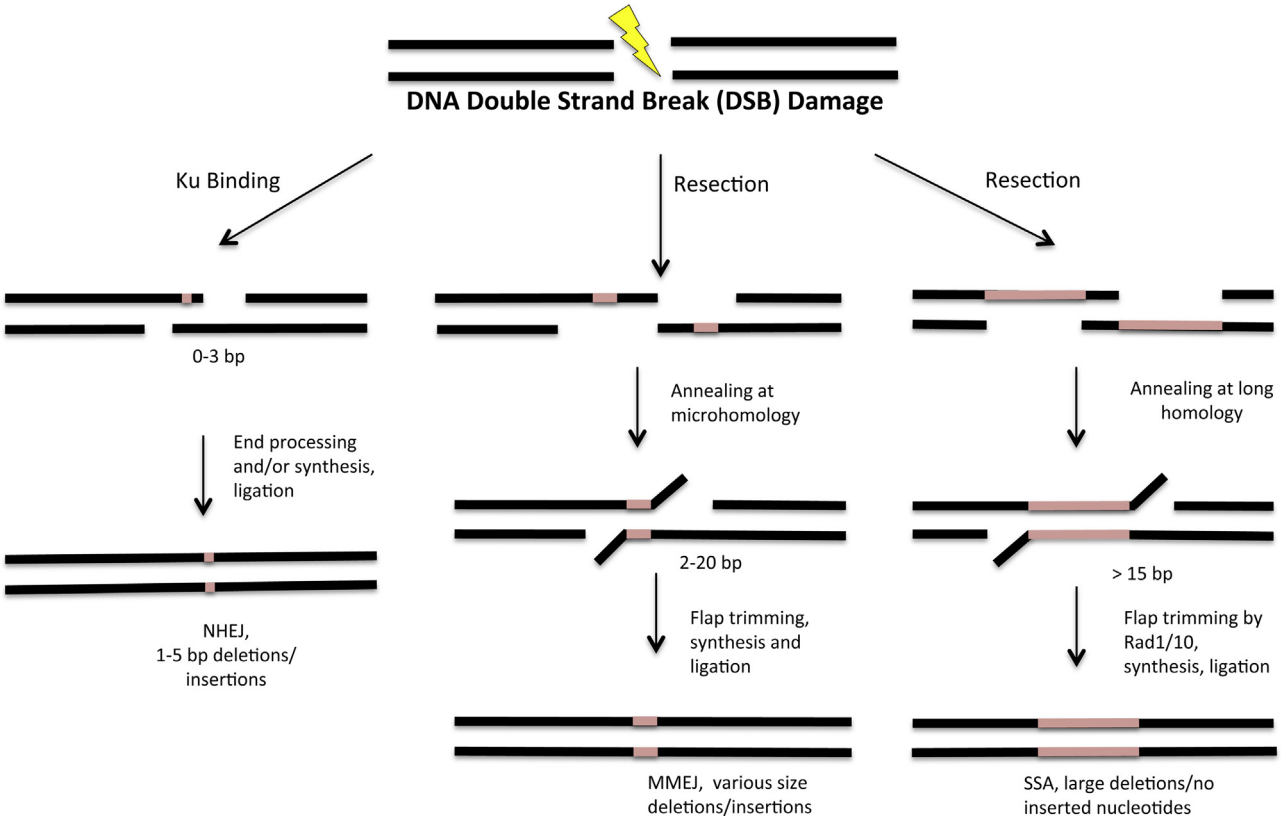

Fig. 1. Basic MMEJ mechanism. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.). NHEJ preferentially repairs DNA breaks with limited or no resection. Binding of Ku impedes resection and 0–3 bp MHs (pink boxes) helps juxtaposing DNA ends, yielding repair products with 1–5 bp deletions/insertions at the repair junctions. Alternatively, DNA resection exposes 2–20 bp (MMEJ) or >15 bp (SSA) homology (pink boxes) at the flanking sequence for annealing DNA ends in MMEJ and SSA. In both MMEJ and SSA, homology annealing is followed by 3′ flap trimming, DNA synthesis and ligation, producing MMEJ products with various size of deletions/insertions or SSA products with large deletions but no inserted nucleotides.

used to induce site-specific DNA breakage flanked by various sizes of MHs [3,22,50,51,58,77,85]. These studies defined the optimal sizes and the relative positions of MHs with regard to DNA break to mediate efficient MMEJ event. The outcomes of these studies also help identify unique genetic requirements associated with MMEJ that are distinct from HR and NHEJ. However, the MMEJ reporters in these studies employed the different sizes and the sequence of MHs as well as their placement to the DNA breaks, which likely contribute to the variations in the frequency and the genetics of MMEJ. Indeed, the study in yeast demonstrated that the difference in the sizes and the position of MHs dictate the mechanistic parameters of ensuing MMEJ process [79]. Additionally, the compilation of the genetics of MMEJ was performed by analyzing end-joining events in class switch recombination from classical end joining deficient cells that produce repair joints with MH at the breakpoint junctions [5,6,17,42,83]. Recent genome editing techniques such as those using CRISPR-CAS9 in HR and NHEJ proficient cells further support the role of MMEJ in the repair of DNA breaks [57].

3. Basic Mechanisms of MMEJ and associated MMEJ factors

The simple model of MMEJ was proposed based on their operational resemblance to single strand annealing (SSA) or NHEJ (Fig. 1) [41,55]. The length of MH (2–20 bp) used in MMEJ is indeed an intermediate in size between those found in NHEJ (0–3 bp) and SSA (>15 bp), underscoring the mechanistic similarity among these events [55]. Partially overlapping size in MH requirement of three pathways further blurs the boundary between these mechanisms, fueling the argument of MMEJ as SSA or NHEJ variants [60].

Given that the MH annealing is the key and the obligate event in MMEJ, the process can be sub-divided into those occurring before and after MH annealing (a.k.a. pre-annealing and post-annealing). In principle, the pre-annealing events in MMEJ should resembles those in HR because MMEJ should also depend on the 5′ to 3′ resection of DNA ends that expose imbedded MHs flanking the break site [67,73]. The resulting single stranded 3′ DNA (ssDNA) then mediates strand annealing and pairing of broken DNA ends via annealing of MHs. Accordingly, the factors regulating the extent of resection including those carrying out nuclease activity such as CtIP, Mre11 complex, BLM and 53BP1 all impinge on the frequency and the types of MMEJ products [3,7,42,51,63,77,80,81,86,89,91]. The resection also likely dictates the types of MH usage in MMEJ because more extensive resection will allow additional MHs flanking the break site to contribute to annealing process [22].

Following resection, MH should be annealed to form a repair intermediate with 3′ flap and gaps on both sides of the break. However, the mechanism of MH annealing remains obscure to date. In yeast, it has been proposed that MH annealing occurs spontaneously by a thermodynamically-driven fashion, which could then be inhibited by single strand binding RPA complex via its ability to disassemble secondary structure formation in ssDNA [21,22,54]. Expression of hypomorphic *rpa1* mutants thus increases MMEJ between 12 bp MHs up to ~350-fold and accumulates gross chromosomal rearrangements featuring MHs at the breakpoint junctions [13,22]. In metazoan cells, additional annealing factors such as Polθ might promote more efficient MH annealing by stabilizing the annealing intermediates or counteracting anti-annealing factors such as RPA [11,37]. The emergence of a dedicated MH annealing factor in mammals coincide with more efficient MMEJ