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Risky Business: Microhomology-Mediated End Joining

Supriya Sinha¹, Diana Villarreal², Eun Yong Shim³, and Sang Eun Lee^{1,3}

¹Department of Molecular Medicine, Institute of Biotechnology, University of Texas Health Science Center at San Antonio, TX 78229

²Children's Hospital of San Antonio, Baylor College of Medicine, San Antonio, TX 78207

³Department of Radiation, Oncology, University of Texas Health Science Center at San Antonio, TX 78229

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.

Keywords

microhomology; DNA double strand break repair; chromosomal; rearrangements

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) (Aparicio et al., 2014). While NHEJ repairs DSB by juxtaposing and processing

Correspondence: Sang Eun Lee, Department of Molecular Medicine, Institute of Biotechnology, University of Texas Health Science Center at San Antonio, 7703 Floyd Curl Drive, San Antonio, TX 78229-3900, Tel) 210-562-4157, Fax) 210-562-4161, ; Email: lees4@uthscsa.edu

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DNA ends permissible for ligation (Bunting and Nussenzweig, 2013; Chiruvella et al., 2013; Lieber, 2010; Radhakrishnan et al., 2014), HR restores chromosome integrity by pairing and copying the missing genetic information from sister chromatid or homologous chromosome (Symington et al., 2014). Furthermore, HR can also occur by single end invasion and repair synthesis to the end of chromosome known as break-induced replication or BIR (Malkova and Ira, 2013), or by annealing flanking repeat sequences and deleting inter-repeat sequence known as single strand annealing or SSA (Ivanov et al., 1996). All NHEJ (aka 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 (Lieber et al., 2006; Symington, 2002).

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 (Boulton and Jackson, 1996; Liang et al., 1996). Many of these residual repair events feature short stretches (3–20 bp) of complementary base pairing called microhomology (MH) at the breakpoint junctions, and thus the name MMEJ emerges (Ma et al., 2003; McVey and Lee, 2008; Radhakrishnan et al., 2014; Sfeir and Symington, 2015). 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 (Decottignies, 2013; Ma et al., 2003; McVey, 2014; McVey and Lee, 2008; Rodgers and McVey, 2016). However, even NHEJ and several other DSB repair pathways also use MH for complementary base pairing and end juxtaposition (Pannunzio et al., 2014) 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” (Bennardo et al., 2008; Deriano and Roth, 2013; Frit et al., 2014; McVey and Lee, 2008; Yan et al., 2007).

MMEJ reporters and systems

To date, the basic framework of MMEJ is assembled primarily from the studies in which artificially designed MMEJ reporters were used to induce site-specific DNA breakage flanked by various sizes of MHs (Bennardo et al., 2008; Deng et al., 2014; Liu et al., 2012; Ma et al., 2003; Oh et al., 2014; Truong et al., 2013; Yu and McVey, 2010). 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 (Villarreal et al., 2012). Concurrently, 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 (Boboila et al., 2012; Boboila et al., 2010; Cortizas et al., 2013; Lee-Theilen et al., 2011; Yan et al., 2007). 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 (Nakade et al., 2014).

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) (Lee and Lee, 2007; McVey and Lee, 2008). 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 (McVey and Lee, 2008). 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 (Pannunzio et al., 2014).

Given that the MH annealing is the key and the obligate event in MMEJ, the process can be subdivided 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 resemble 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 (Sfeir and Symington, 2015; Symington and Gautier, 2011). 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 (Bennardo et al., 2008; Bothmer et al., 2010; Lee-Theilen et al., 2011; Ma et al., 2003; Rahal et al., 2010; Truong et al., 2013; Wang et al., 2012; Xie et al., 2009; Yun and Hiom, 2009; Zhang and Jasin, 2011; Zimmermann et al., 2013). 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 (Deng et al., 2014).

Following resection, MH should be first 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. To this end, it has been proposed that MH annealing occurs spontaneously in yeast 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 (Deng et al., 2015; Deng et al., 2014; McVey, 2014). 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 (Chen et al., 1998; Deng et al., 2014). 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 (Ceccaldi et al., 2015b; Kent et al., 2015). The emergence of a dedicated MH annealing factor in mammals coincide with more efficient MMEJ usage in these organisms and may reflect an evolutionary pressure to rely on the more flexible mechanism like MMEJ to repair genome with low gene density.

After MH annealing, 3' non-homologous tails are removed by the XPF/ERCC1 nuclease to form DNA ends permissible for priming repair synthesis and triggering the deletion of inter-MH sequence (Ahmad et al., 2008; Bennardo et al., 2008; Ma et al., 2003). Interestingly, MMEJ mechanisms diverge substantially between budding yeast and metazoan cells from this point forward. In flies and mammals, Pol θ appears extending annealed DNA and concurrently counteract Rad51 to inhibit HR (Ceccaldi et al., 2015a; Chan et al., 2010; Mateos-Gomez et al., 2015; Yousefzadeh et al., 2014). Alternatively, polymerase β and λ might catalyze DNA synthesis during MMEJ (Crespan et al., 2012). However, yeasts do not have Pol θ homolog and instead depend on Pol32 and other error prone polymerases for repair synthesis and annealed intermediate stabilization (Lee and Lee, 2007).

Similarly, the ligation step of MMEJ is noticeably different in yeast and metazoan cells: in higher eukarotic cells, Ligase III seals DNA ends as the primary ligase for MMEJ (Della-Maria et al., 2011; Liang et al., 2008; Oh et al., 2014; Paul et al., 2013; Simsek et al., 2011; Simsek and Jasin, 2010). However, due to the absence of Ligase III homolog in yeast, the ligation in MMEJ rather depends on Cdc9 and Dnl4 (Ma et al., 2003). Evidence also suggests that in metazoan cells, PARP1 and the factors mutated in FA patients, which are known to play roles in inter-strand cross-link (ICL) repair, could stimulate MMEJ by some unidentified manner (Howard et al., 2015; Robert et al., 2009; Soni et al., 2014). Mismatch repair proteins such as Msh2, Mlh1, Exo1 and the single strand break repair protein Xrcc1 are also implicated in alternative end joining that produces breakpoint junctions with MH in class switch recombination in metazoan cells {Saribasak, 2011 #305} (Eccleston et al., 2009). These additional MMEJ factors in metazoan cells might contribute to the more frequent usage and/or increased versatility to wider types of DNA lesions in these organisms.

Obviously, further exploration is essential for the identification of all the MMEJ genes: the knowledge on MMEJ mechanism has just begun to emerge. To date, only the limited screens for MMEJ factors have been reported in yeast and in human cells (Howard et al., 2015; Lee and Lee, 2007). Unequivocally, the future endeavors should focus on the identification of additional MMEJ factors that will help to define biochemical steps associated with MMEJ and to elucidate the precise contribution of MMEJ for genome maintenance and repair.

Regulation of MMEJ

MMEJ is an intrinsically mutagenic event and frequently produces repair products with significant sequence deletion and/or small in-del mutations to the flanking DNA segments (McVey and Lee, 2008; Yu and McVey, 2010). Naturally, cells should tightly control the usage of MMEJ to limit critical mutagenesis resulting from the repair events. This part of the regulation is also likely to be intertwined with the way cells opt for a particular pathway over others because inhibition of MMEJ should divert the repair of DNA lesions toward other non-mutagenic pathways and thus help to sustain chromosome integrity (Aparicio et al., 2014; Shrivastav et al., 2008).

We now know more about how cells choose NHEJ or HR for the repair of a given DNA lesion, which depends largely on cell cycle stage (Ira et al., 2004; Scully and Xie, 2005;

Zhang et al., 2009b) as well as on the different kinetics and non-overlapping requirement on DNA damage signaling intrinsic to each repair event (Kakarougkas and Jeggo, 2014; Shrivastav et al., 2008). But the basic parameters dictating MMEJ usage over more conventional repair options are not defined yet and remain to be elucidated. Because both MMEJ and HR absolutely rely on resection for MH annealing and strand exchange, respectively, these two pathways might be more tightly coupled to each other and thus could compete for repairing the same pools of DSB. Indeed, inactivation of HR by deleting *RAD51* or *RAD52*, two key HR factors in yeast elevates MMEJ usage (Deng et al., 2014). Likewise, the inactivation of BRCA1 also leads to elevated MMEJ in humans (Yun and Hiom, 2009).

Similarly, the relationship between MMEJ and NHEJ is complex and poorly defined. The deletion of KU, the core NHEJ factor, increases MMEJ; but the effect could be attributed to the role of Ku inhibiting resection, and thus might not reflect a direct competitive relationship between NHEJ and MMEJ (Bennardo et al., 2008; Fattah et al., 2010). The inactivation of other NHEJ also elevates the frequency of repair events using longer MH and deletions flanking the break site (Sisek and Jasin, 2010; Yan et al., 2007). Interestingly, in class switch recombination, NHEJ and MMEJ target different substrate molecules and their activities do not overlap (Cortizas et al., 2013). Further studies are needed to define the precise relationship between NHEJ and MMEJ.

Molecularly, the regulation of MMEJ appears adopting at both temporal and spatial level by segregating MMEJ to a subset of DNA break. For instance, the accumulating evidence suggests that MMEJ is blocked at G1 phase of the cell cycle when the extensive resection and the formation of ssDNA do not occur (Truong et al., 2013). However, MMEJ uses MHs that often exist very close to the break, and in principle should not rely on extensive resection as HR does (Deng et al., 2014). Indeed, the deletion of two extensive resection factors in yeast did not suppress and rather stimulated MMEJ (Deng et al., 2014). The apparent cell cycle distribution of MMEJ may thus be caused by other unidentified reasons besides resection. Alternatively, it is possible that a subset of MMEJ could still operate in G1 under certain genetic conditions (Xiong et al., 2015).

In humans, MMEJ could be associated with unique sub-nuclear compartment at the nuclear membrane and operate at certain cell cycle stage (Lemaitre et al., 2014; Lemaitre and Soutoglou, 2015). The DNA breaks localized at the nuclear membrane are repaired by a Lig3/Xrcc1 and PARP-1 dependent process, even though the junctional sequences were not analyzed (Lemaitre et al., 2014; Nagai et al., 2008). The spatial segregation of MMEJ to nuclear periphery is particularly intriguing because the same location has been implicated as the repair site for persistent DNA lesions and mutagenic repair in yeast (Nagai et al., 2008). In yeast, the existence of sumoylation-directed E3 ubiquitin ligases Slx5 and Slx6 at the nuclear periphery might trigger proteolytic degradation of DNA repair factors (Li et al., 2007; Nagai et al., 2008). Interestingly, the inhibition of MMEJ by protease inhibitor treatment indicates that MMEJ is regulated by the factors that are subjected to proteolytic degradation at the onset of repair (Howard et al., 2015). Currently the putative MMEJ inhibitors that are subjected to proteolytic degradation are unknown.

Targeting MMEJ to treat recombination deficient cancers

Implicit for pathological chromosomal translocation formation, MMEJ has long been regarded as the mechanism underlying chromosomal instability and the wide range of diseases associated with repair defects in multiple organisms. Under the conditions where repair could proceed by intra or inter-chromosomal joining, MMEJ and the related SSA process trigger promiscuous joining between chromosomes (Haber and Leung, 1996; Villarreal et al., 2012). Thus the efficient inter-chromosomal MMEJ explains the formation of frequent chromosomal rearrangements featuring MH at the junctions, especially from cells deficient in classical end joining (Zhang and Jasin, 2011). Puzzlingly, cell type specificity might impinge on the types and the pathways for chromosomal rearrangements because two site-specific DNA breaks induce chromosomal translocations by classical NHEJ in humans but by MMEJ in mouse ES cells (Ghezraoui et al., 2014). Alternatively, the diminished role of MMEJ in chromosomal translocations in human cells could be explained by the spatial and temporal difference in MMEJ and NHEJ usage in the formation of chromosomal rearrangements.

Most recently, the assembly of chromosomal fragments by MMEJ-like mechanism has been attributed to the formations of chromothripsis in human cells, which involves massive chromosome fragmentation and rejoining, and frequently associates with tumorigenesis (Kloosterman et al., 2012; Zhang et al., 2015). The eroded telomeres also lead to telomeric fusion with MH at the joints that depend on known MMEJ factors (Jones et al., 2014; Letsolo et al., 2010; Mateos-Gomez et al., 2015; Zimmermann et al., 2013). Besides, the unexpected decline in more conventional DNA repair mechanisms in aging cells also leads to elevated production of chromosomal translocations and the repair events featuring MH at the junctions (Vaidya et al., 2014). Based on these premises, MMEJ is thought to be a highly versatile mechanism and is capable of joining a wide range of DNA break types under extremely stressful conditions.

Consistent with competitive relationship between HR and MMEJ, HR-deficient cells rely heavily on MMEJ for repairing DNA lesions than HR proficient cells, which could be exploited clinically to target cancer cells with HR defects (Fan et al., 2010). Indeed, Polθ is over-produced in multiple types of cancer cells including ovarian cancer and the inhibition of Polθ greatly sensitizes cancer cells to PARP inhibitors (Ceccaldi et al., 2015a; Chan et al., 2010; Mateos-Gomez et al., 2015; Yousefzadeh et al., 2014). Further studies also demonstrate that therapy-resistant breast cancers express high levels of two alternative NHEJ factors, PARP1 and LIGIII, and become sensitized to PARP1 and LIGIII inhibitor treatment (Tobin et al., 2012). The inhibitors to PARP1 and LIGIII that disrupt MMEJ are also effective to tyrosine kinase inhibitor resistant chronic myelogenous leukemia that might utilize alternative NHEJ to repair DNA DSBs (Tobin et al., 2013). Collectively, targeting MMEJ should be a powerful strategy for the development of therapy against cancers that specifically use MMEJ as the key repair option.

Even though MMEJ inhibition promises the reasonable strategy as therapeutics for certain cancers, the key challenge still remains and corresponds to the lack of knowledge on the genetic factors that are almost exclusively involved in MMEJ. Such gap in knowledge

impedes the precise assessment of physiological role of MMEJ in cells as well as the pathophysiology of organisms deficient in MMEJ. The identification of unique MMEJ factors via systematic screen for more MMEJ genes is thus imperative and will enable scientists to develop approaches for specific therapeutic targeting of cancers without affecting normal cells and organs.

Is MMEJ a variant of NHEJ or SSA?

The mechanistic similarity between MMEJ and NHEJ raised a considerable concern if MMEJ is simply a variant of NHEJ that operates under distinct genetic context (Pannunzio et al., 2014). This hypothesis is further supported by the elevated MMEJ frequency in NHEJ deficient or HR deficient cells (Deng et al., 2014; Yan et al., 2007). The plasticity of NHEJ and other repair processes were experimentally documented thus supporting the view that MMEJ might not be a stand-alone pathway, but instead corresponds to the variants operating under specific conditions (Pannunzio et al., 2014). However, given the unique temporal and spatial distributions between MMEJ, HR and NHEJ, it is tempting to suggest that MMEJ might be a distinct process even if it shares some features with other repair options. The robust MMEJ frequency in cells proficient in canonical NHEJ and HR further supports the model that MMEJ represents a pathway distinct from other DSB repair pathways (Ma et al., 2003; Nakade et al., 2014).

Of note, MMEJ operates differently according to the size of MH and the types of DNA breaks, posing further complexity to defining the relationship between MMEJ and other mechanistically related processes (Villarreal et al., 2012). It was shown that MMEJ mediated by MH larger than 15 bp operates more similarly to SSA but those mediated by less than 5–6 bp MH resembles NHEJ more. Overall, the results emphasize that MMEJ, like NHEJ, is also extremely versatile in nature, but the operational or genetic parameters of the processes associated with MH, MMEJ and NHEJ become significantly overlapping at the borderline conditions. Alternatively, one cannot rule out the possibility of the prevalence of multiple mechanisms of MMEJ to yield repairs with MH at the junctions according to the MH size.

Are junctional MHs the hallmark of MMEJ?

The development of high-resolution sequencing techniques was instrumental to reveal that many breakpoint junctions in human copy number variants are associated with MH at a frequency which is far exceeding than random probability (Conrad et al., 2010; Kidd et al., 2010; Ottaviani et al., 2014). The robust appearance of MMEJ events in certain genetic conditions and their preponderance among pathogenic chromosomal structural aberrations not only established the causal relationship between MMEJ and human diseases but also deepened the interests about the dissection of underlying mechanisms and regulation among research community (Bentley et al., 2004; Rodgers and McVey, 2016)(Boboila et al., 2010; Yan et al., 2007).

Nevertheless, these studies also led to the major confusions regarding the concept of MMEJ and their contributions to chromosomal aberrations because the types of responsible repair events were often deduced simply based on the sizes of MHs at the breakpoint junctions.

Conceptually, the presence of MHs at the breakpoint junction should not be used as the sole marker of MMEJ event because MMEJ might still be responsible for the formation of repair junctions without MHs, such as the acquirement of MH (Fig. 2) during non-processive repair synthesis (Yu and McVey, 2010) in synthesis dependent MMEJ (SD-MMEJ). Such joints often associate with small sequence insertions instead of MHs at the breakpoints and Polθ dependent in flies. Assigning the repair events simply based on MH size at the breakpoints could significantly underestimate the role and the contributions of MMEJ to genome rearrangements. Another important point to consider that all repair events with MH at the junctions are not necessarily derived from MMEJ. As presented in details in Fig 3, several other repair options including fork-stall template-switch (FoSTeS) and microhomology-mediated break-induced replication (mmBIR) could produce MH at the breakpoint junctions and be highly mutagenic (Colnaghi et al., 2011; Hastings et al., 2009; Lee et al., 2007; Zhang et al., 2009a). Both FoSTeS and mm-BIR have been implicated in the repair of broken replication forks and promote chromosomal rearrangements with MH at the breakpoint junctions in copy number variations and complex rearrangements. Even classical NHEJ often joins DNA ends using 1–2 bp of complementary base pairing and could leave MHs at the breakpoint junctions (Pannunzio et al., 2014). Therefore, the breakpoint sequence based studies appear to have substantial limitations in elucidating the underlying mechanisms and should not be relied to elucidate the detailed biochemical and genetic underpinnings.

To date, no means are available to distinguish the types of MH mediated repair events simply by analyzing the breakpoint junctions. Undoubtedly, the lack of such pivotal tool hinders the precise assessment of the role of MMEJ and other MH-mediated pathways in genome destabilization and mutagenesis. At this stage, the efforts for the development of a tool that allows accurate detection of MMEJ or other MH associated repair events should be forthcoming. By and large, this critical tool should empower scientists to resolve some of the existing discrepancies in the research findings based on the sequence analyses of the genome rearrangements.

Conclusion and future perspectives

MMEJ is a surprisingly diverse process that is widely different between organisms, depending on the size and the type of MH near the break. The development of highly reliable reporters can lead to a sensitive readout for MMEJ across organisms and in tissues. Further scientific exploration will uncover universal features associated with most MMEJ and elucidate the accurate account of MMEJ contributions to diseases and normal cell physiology.

Regardless of whether MMEJ is a unique DNA repair pathway or a salvage mechanism, MMEJ produces chromosomal rearrangements that contribute to the development and resistance of many types of human cancers. The oncogenic role of MMEJ underscores the need to know how MMEJ operates and is controlled inside the cell. Such mechanistic information can be attained by comprehensive genetic and biochemical reconstitution of this process in a model system and will also preface the development of cancer therapeutics.

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Fig. 1. Basic MMEJ mechanism

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.

Fig. 2. Synthesis dependent MMEJ

MMEJ could occur between MHs (z) that forms by repair synthesis of the same chromosome (**A**) or other chromosome (**B**) after annealing of DNA ends using limited homology (shown in x). The broken end (Ai) is resected (Aii), forms a loop after unwinding and uses nearby sequences as template to synthesize DNA (Aiii). After synthesis, the loop dissociates or unwinds (Aiv) to generate a 3' overhang that has microhomology (z) complementary to the break for annealing (Av). The resulting repair product formed due to MMEJ leads net insertion at the breakpoint junction (Avi).

Alternatively, the broken end (black chromosome) switches template (grey chromosome) using microhomology (Bi). Sequence from another genomic locus is copied (Bii) to acquire microhomology (z), which is used to anneal after switching back to the original template (Biii). Such repair products do not have flanking MHs at the breakpoint junctions as predicted but may carry insertions or duplications of the sequence (y) adjacent to newly copied MHs.

Fig. 3. Microhomology-mediated break induced replication (mmBIR) and Fork Stalling/Template Switching (FoSTeS)

A. Microhomology-mediated break-induced replication (MMBIR). (i) The ongoing replication fork may encounter a nick or lesion on the template strand, which leads to replication fork collapse. (ii) The replication fork is then collapsed forms a double stranded break. The 5' end of the broken end undergoes resection, exposing a 3' tail. (iii) After resection, the 3' end invades different template (grey) using microhomology (*mh1*). Replication is re-initiated but of low processivity. (iv and v) The extended broken end, now carrying the different sequence (green) dissociates and reinvades different templates (pink) using another microhomology (*mh2*). (v and vi) This process of template switching continues until the extended end anneals back with the original single-stranded sequence (black) using microhomology (*mh3*). The fully replication fork is re-formed and the synthesis resumes till the end of the replicon.

B. Fork Stalling and Template Switching (FoSTeS)

(i) Replication fork stalling can be caused by the formation of secondary structures, lesions or shortage of deoxynucleotide triphosphates in lagging strand template. (ii) The 3' primer end of the DNA strand becomes dissociated from their templates and might align to single-stranded DNA templates in other nearby replication forks (grey) that share microhomology

(*mhl*). (iii) This process could occur multiple times by repetitive dissociation and reinvasion into nearby replication forks. (iv) According to the position of the other replication fork, the resolution of this intermediate might lead to the formation of chromosomal rearrangements like duplication, deletion or translocation.

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