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Multi-Invasion-Induced Rearrangements as a Pathway for Physiological and Pathological Recombination

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Abstract

Cells mitigate the detrimental consequences of DNA damage on genome stability by attempting high fidelity repair. Homologous recombination templates DNA double-strand break (DSB) repair on an identical or near identical donor sequence in a process that can in principle access the entire genome. Other physiological processes, such as homolog recognition and pairing during meiosis, also harness the HR machinery using programmed DSBs to physically link homologs and generate crossovers. A consequence of the homology search process by a long nucleoprotein filament is the formation of multi-invasions (MI), a joint molecule in which the damaged ssDNA has invaded more than one donor molecule. Processing of MI joint molecules can compromise the integrity of both donor sites and lead to their rearrangement. Here, two mechanisms for the generation of rearrangements as a pathological consequence of MI processing are detailed and the potential relevance for non-allelic homologous recombination discussed. Finally, it is proposed that MI-induced crossover formation may be a feature of physiological recombination.

Keywords

copy number variation; genomic instability; homologous recombination; non-allelic homologous recombination; repeated sequence; structural variant; translocation

1. Introduction

Homologous recombination (HR) repairs structural lesions of the DNA double helix, such as double-strand breaks (DSBs) and single-stranded DNA (ssDNA) gaps, by referencing an

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intact dsDNA donor as a template for repair. The universal RecA family protein (Rad51 in eukaryotes) forms a right-handed filament on single stranded DNA in a gap or at a resected DSB. This nucleoprotein filament catalyzes homology search by inter-segmental contact sampling, whereby a single filament searches for homology in multiple genomic locations at the same time.[1,2] Successful homology encounter is followed by DNA strand invasion that results in a displacement loop (D-loop; Box 1). This multiplexed search process suggests that homology can be encountered and DNA strand invasion can occur on independent donor molecules along the filament. We recently provided physical evidence in vitro with yeast and human HR proteins, as well as in vivo in *Saccharomyces cerevisiae* cells, for such DNA strand exchange intermediates that we named multi-invasions (MI; Figure 1).[3,4] MI joint molecules (JM) contain two heteroduplex DNA regions, at least one of which is internal (i.e., not containing a 3′-OH extremity). Importantly, we found that MI processing can lead to the translocation of the engaged donors.[4] Here, we delineate two general mechanisms for MI-induced rearrangements (MIR) and discuss some of their features and unknown parameters. Moreover, we propose that MIR provides a potential mechanism for non-allelic homologous recombination (NAHR) with less constraints than the current model. We also point out a potential physiological role of MI joint molecules during meiotic recombination to rationalize segregation patterns during yeast meiosis that are not easily explained by the standard model involving double Holliday junctions.

2. Mechanisms of Multi-Invasion-Induced Rearrangements

2.1. MIR: Overview and Requirements

We identified a chromosomal rearrangement mechanism in budding yeast that stems from the processing of MI joint molecules, which we named multi-invasion-induced rearrangement (MIR; Figure 1).[4] This tripartite recombination mechanism results in rearrangement (translocation) of the donors engaged in the MI and the generation of additional single-ended DSBs, which depending of the sequence context are either faithfully repaired or lead to additional rearrangements at high frequencies (Figure 1). MIR depends on the core HR proteins Rad52, Rad51, and Rad54,[4] and is stimulated by the Rad51 paralogs, Rad55-Rad57 (Piazza and Heyer, unpublished results). Ligase IV, a central NHEJ factor, is not required. Structure-selective endonucleases (SSE) are redundantly required for MIR, presumably to cleave the multiple DNA branch-points of MIs (see below). MIR also requires different extents and types of DNA synthesis, which at least partly rely on Pol32, a non-essential subunit of Polδ and Polζ.

The minimal sequence requirement for MIR is a stretch of ssDNA that bears regions of homology to two different dsDNA donors. Shared homology between the donors is not required. Importantly, any intervening sequence between the two repeats in the ssDNA is inserted at the breakpoint junction (Figure 1), establishing MIR as a mechanism to form insertions. Consequently, MIR can occur between repeats at allelic loci as well as between ectopic repeats. MIR is strongly stimulated by factors enhancing concomitant homology encounters of both donors, such as the length of homology and the physical proximity of the three DNA partners, especially the donors.[4] The relative positions of the donors (allelic vs. ectopic, inter- vs. intra-chromosomal) allows for two specific MIR mechanisms with

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consequences for their propensity to generate additional rearrangements. We delineate two general MIR sub-pathways that parsimoniously account for the segregation of the translocation and the associated rearrangements observed with donors in different sequence contexts.[4] For brevity, we refer to the internal and terminal D-loops as I and T, respectively, and their 5′ and 3′ junctions as I5, I3, T5, and T3, respectively (Figure 2A). We will refer to MIR mechanism 1 and 2 as MIR1 and MIR2, respectively.

2.2. MIR1: Endonucleolytic Resolution

The MIR1 mechanism proceeds by cleavage and differential processing of the DNA strands of the I and T invasions (Figure 2A). The cleavage and ligation of the top strands at the I5 and T3 junctions create a translocated DNA strand. The sequence between the two repeated regions in the presynaptic filament will be inserted between the I3 and T5 cleavage sites, which constitute the translocation junction. The cleavage of the bottom strand at, or near, the base of the I3 and T5 junctions produces DNA ends for DNA synthesis and ligation. Specifically, the 3′-OH end generated upon cleavage of the T5 junction is extended by a DNA polymerase using the invading strand as a template. Hence, MIR1 translocation does not require DNA strand displacement synthesis. It is akin to the second synthesis step of the synthesis-dependent strand annealing pathway (SDSA, Box 1). Ligation of the extension product to the cleaved I3 junction generates the MIR translocation. Hence, the I3 cleavage must have occurred before being reached by the DNA synthesis initiated at the 3′-OH end of T5. The processing of the other branch points can occur in any order.

The immediate products of MIR1 are a translocated molecule associated with two MI-induced single-ended DSBs and the original DSB that has lost the sequence of the 3′-ending tail that invaded the two donors.[4] The sequence context of the donors dictates the repair opportunities for MI-induced DSBs and the formation of additional rearrangements. In the case of allelic donors, the sequence homology between the two MI-induced DSB ends and their sister chromatids or the translocation chromatid grants opportunities for accurate repair using canonical HR pathways (see Box 1 and Figure 4B). However, DSBs at ectopic donors lacking nearby homologies will lead to various types of secondary rearrangements (detailed in Figure S8, Supporting Information in Ref. [4]). A subset of these secondary repair events are likely produced by break-induced replication (BIR, Box 1), and thus depends on extensive displacement DNA synthesis. In the particular case in which the I and Tdonors are on the same chromatid, the DSBs will delimit a fragment that can either be degraded by resection or generate a substrate amenable to SSA. The original DSB can be repaired using the sister chromatid or the homolog as a template.

2.3. MIR2: Endonucleolytic Resolution and Displacement DNA Synthesis

In MIR2, the processing of the I invasion remains identical to MIR1, but the processing of the T invasion resembles SDSA (Figure 3). The displacement DNA synthesis primed from the invading molecule at T3 copies the downstream sequence. If an identical sequence is present downstream of the I donor, as is the case at an allelic locus or near repeats, this synthesis step provides opportunity for annealing to the resected single-ended DSB generated following cleavage of the I invasion. Following annealing and flap removal, the translocation is completed as in SDSA, by dual DNA synthesis off the resected ssDNAs and

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final ligation. In MIR2, the immediate products are a rearranged chromosome, the restored chromatid with the Tdonor sequence, and the original DSB truncated as in MIR1, which can be repaired using the sister chromatid or homolog as a template (Figure 3). Hence, MIR2 generates chromosomal insertions.

2.4. Features and Requirements of Both MIR Mechanisms

MIR1 is applicable in any donor context, does not require the invading molecule to prime DNA synthesis (i.e., it could be initiated at ssDNA gaps see Figure 2B), and propagates additional single-ended DSBs that can generate further rearrangements. In contrast, MIR2 requires a free 3′ extremity and displacement DNA synthesis. It is restricted to contexts in which the I and T donors share downstream sequence homology. MIR2 does not propagate additional DSB. Hence, MIR1 cangenerate complex chromosomal rearrangements while MIR2 generates insertions whose scale is directly limited by the extent of displacement DNA synthesis and the proximity to identical sequences downstream of both donors. A prediction of this model is that mutants defective for long-range DNA displacement synthesis should be specifically deficient for MIR2. Accordingly, a *pol32* mutant defective for displacement DNA synthesis by Polδ exhibits a modest, yet significant, twofold decrease in MIR frequency[4] and an altered segregation profile of the translocated molecule suggestive of a loss of MIR2 events (Piazza and Heyer, unpublished results).

We emphasize that MIR results from the processing of invasions by a single end of the DSB, without contribution from the second end. This opens the potential for other events initating MIR such as single-ended DSB generated during replication fork collapse or reversal. In addition, MIs form in vitro with substrates mimicking ssDNA gaps flanked either by dsDNA tails or ssDNA heterologies.[3,4] Hence, ssDNA gaps could also be conducive to a variant of MIR1 in which the T invasion is processed in a mirror fashion as the I invasion (Figure 2B). The long trailing ssDNA generated upon semi-conservative BIR synthesis[5] could provide an opportunity for allelic or ectopic MIR.[6] Accordingly, physical examination of BIR intermediates by two-dimensional gel electrophoresis recently provided experimental support for additional joint molecules formed behind the migrating bubble.[7] These possibilities, which would greatly expand the physiological and pathological contexts prone to MIR, still need to be addressed experimentally.

2.5. Some Unknown Parameters of MIR

Several open questions remain regarding key steps of the MIR pathway. First, the nature of the internal invasion and the structure of the DNA strand exchange junctions remain elusive, since in the absence of a freely rotating extremity strand intertwining cannot occur. Hence, the internal invasion may remain paranemic (i.e., not topologically linked to the donor). Alternatively, the internal invasion might be converted to a plectoneme by a nickase or topoisomerase. Whether such conversion occurs and is required or inhibitory for MIR is unknown. Second, it is unclear whether both invasions have a required order and need for being simultaneous. Third, cleavage of the DNA strand exchange junctions (T5, T3, I5 and additionally I3 in MIR1) are integral to MIR, but uncertainties remain regarding their precise nature and position of the junction. The initial cleavage on the invading DNA strand could occur anywhere downstream of I5. In particular whether the cleavages are coordinated and

readily ligatable or if they require branch migration and/or DNA synthesis is unknown. The endonucleases Mus81-Mms4, Slx1-Slx4, and Yen1 are redundantly required for MIR.[4] Given their relatively broad selectivity for branched substrate, these SSEs could redundantly process all junction types.[8] For example, detailed biochemical analysis of Mus81-Mms4 substrates is compatible with the processing of I3 and T5 (Figure 2A).[9,10] The cleavage of the splayed arms of I5 or the T3 junction by Mus81-Mms4 is, however, less likely based on its known in vitro activity. It is also possible that the MI junctions may significantly differ from model substrates. Alternatively, these SSEs may only process a subset of junctions and other unidentified activities process the remainder. Potential candidates include mismatch repair (MMR)-related factors such as the dsDNA nickase MutLγ (Mlh1-Mlh3) that binds with high affinity to various branched DNA substrates[10–14] and, together with Sgs1 and Exo1, promotes joint molecule resolution into crossover during yeast meiosis.[15–17] In addition, the nucleotide excision repair 5′-flapase XPG (Rad2 in *S. cerevisiae*) could be involved, for example in T3 cleavage.[18] Finally, the regulation of MIR throughout the cell cycle and by the replication and DNA damage checkpoints has not yet been investigated. Given its requirements for HR and SSE, we suspect MIR to mainly occur in G2/M.[19,20]

3. Implications of MIR for Allelic and Non-Allelic Homologous Recombination

3.1. The DSBR Model: A Paradigm for the Formation of Crossovers

Several HR models have been advanced to account for the genetic or physical outcomes of DSB repair (reviewed in Ref. [21]). These include SDSA, the double-strand break repair (DSBR) model, and BIR. The tenet of the DSBR model is the topological linkage of the broken and donor molecules in the dHJ intermediate (Box 1 and Figure 4A).[22] dHJs involve both ends of the DSB, and result from DNA synthesis, branch migration, and ligation of the extended 3′ extremities to the resected 5′ extremities (Figure 4A). dHJs were physically detected following site-specific DSB formation in both mitotic and meiotic budding yeast cells.[23,24] Importantly, the dHJ intermediate is amenable to formation of a crossover (CO) believed to occur upon HJ cleavage across different planes by specialized endonucleases (Figure 4A). The DSBR model satisfyingly explained the association of CO with gene conversion during yeast meiosis,[22,25] and has remained the foundation of our current understanding of the physical and genetic HR events of the first meiotic division, and the formation of most types of repeat-mediated chromosomal rearrangements (see below).

Inspection of hDNA and conversion tracts in diverged yeast hybrids deficient for mismatch correction revealed that 12% of the NCO-associated and 85% of the CO-associated segregation patterns violated the expectations of the classic SDSA and DSBR (hHJ) models, respectively[26,27] (see Ref. [27] for older references). Similar observations were also made in mitotic cells in which only two thirds (2/3) of the events followed model expectations.[28,29] Notably, conversion tracts and CO regions were often found at a distance from the Spo11-mediated DSB site, a phenomenon that was also documented in mice.[30] Approximately 10% of NCO events exhibited hDNA on more than one chromatid, and 5% of the COs involved more than two chromatids.[26,27] Paralleling these genetic observations, physical examination of HR intermediates during wild-type yeast meiosis revealed the

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unexpected existence of multi-chromatid joint molecules (mcJMs).[31] Finally, the individual nucleases involved in CO formation are efficient on flap and branched JM substrates, but their activity on HJ/dHJ substrates is weak, with the exception of Yen1/GEN1 and the mammalian SLX1–4/MUS81-EME1/XPF-ERCC1 complex.[9–13,32–36] These additional genetic, physical, and biochemical insights suggest a greater complexity in the processing of DNA strand exchange intermediates formed during meiosis (and mitosis) than previously anticipated, and led to the proposal that at least a subset of COs could arise through dHJ-independent pathways.[8,26,27,37–40]

3.2. MIR Can Explain Unexpected Features of Meiotic and Mitotic Recombination

Applying the concept underlying MIR opens the possibility that inter-homolog MIR1 and MIR2 events contribute to NCO or CO formation during meiosis (and mitosis) (Figure 4B and Figure S1, Supporting Information). From a genetic stand point, MIR readily explains i) the one-sidedness of both NCO and CO events; ii) the presence of conversion patches and of CO regions not overlapping the initial DSB site; iii) NCO events involving two chromatids; iv) CO events involving three chromatids; and v) the complexity and patchiness of certain conversion tracts. Considering only the least ambiguous NCO and CO events involving respectively two and three chromatids, it places a lower estimate on the contribution of MIR in meiotic HR events to ≈5–10%.[27] The presence of either hDNA and/or conversion patches distant from the DSB site is inherent to MIR, as it results from the insertion of the invading sequence between the I and T invasions, and in certain instances the gap generated by the resection of the I invasion (indicated by an orange bar in Figure 4B). These patches are signatures for MIR events and can only be accounted for with more difficulty by dHJ migration or two inter-homolog template switches. The repair of the secondary DSBs provides additional opportunities for complex, intermingled hDNA tracts (Figure 4B and Figure S1, Supporting Information). From a biochemical stand point, MIR forms COs independently of HJs. From a physical stand point, MI can be converted to mcJMs exclusively bearing parental strands[31] upon involvement of the second end of the Spo11-mediated DSB (see Figure 4C). Consistently, the Sgs1-Top3-Rmi1 (STR) complex inhibits both MIR in mitotic cells[4] and the formation of mcJMs[31,41–43] and complex multi-chromatids NCO/CO products in meiosis.[27] Likewise, the SSEs Mus81-Mms4, Slx1-Slx4, and Yen1 cleave MI junctions, thus promoting MIR,[4] and inhibit formation and/or resolve mcJMs.[41–45] Furthermore, inactivation of STR and SSEs causes a synergistic increase in the amount of mcJMs,[42,45] consistent with their independent roles for MI joint molecule disruption and processing, respectively. Hence, mutant contexts that cause MI persistence also lead to the accumulation of mcJMs, suggesting that MI joint molecules could be a precursor of mcJMs.

Finally, unlike mitotic DSB repair,[46] the two ends of a meiotic DSB do not remain spatially associated in budding yeast.[47] This intriguing observation, supported by additional cytological evidence in other species[48,49] and asymmetric recombination intermediates at the *HIS4::LEU2* hotspot in yeast,[50,51] suggested that the genome-wide homology search is carried out by a single pioneer end of the DSB, while the other extremity remains associated with the initial axis. The pioneer end can cause inter-sister or inter-homolog MI joint molecules (example in Figure 4B and Figure S1, Supporting Information), which physically

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links undamaged chromatids. Secondary DSBs could explain the unexpected presence of ssDNA of opposite polarity relative to the Spo11 cleavage hotspot observed upon genome-wide mapping of dsDNA-ssDNA junctions in meiotic yeast cells.[52] Importantly, their formation requires Dmc1, the meiosis-specific RecA homolog required for strand invasion.[52,53] It suggests that these ssDNAs reflect either extended D-loops per se (as proposed by Mimitou et al.[52]) or a downstream intermediate resulting from their processing. Both MIR models predict that D-loop processing by SSEs generates secondary DSBs at a distance from the Spo11-inflicted DSB site and ssDNA of the opposite strand relative to the initial resection tract (see I invasion, Figure 4B). The evolutionary advantage for this apparently conserved one-ended homolog engagement[49] becomes evident in light of the MIR model: its critical genetic advantage is to displace the conversion and crossover events away from the sites initiating the recombination process, thus limiting "hotspot erosion."[30]

Altogether, MIR may account for a subset of NCO and CO events during meiotic (and mitotic) recombination. This model explains a number of cytological, physical and genetic observations not easily accounted for by other models.

3.3. MIR and Formation of Structural Variants of the Genome

Eukaryotic genomes contain, to variable degrees, repeated regions in the form of kilo- to megabases-long tandem arrays, such as the telomeric, centromeric, and ribosomal DNA repeats, as well as dispersed repeats such as remnants of transposable elements (0.1–0.7 kb) and large segmental duplications (>5 kb).[54] Recombination between repeats is a major source of several types of recurrent and non-recurrent chromosomal rearrangements involved in various human pathologies (reviewed in Refs. [55–57]). A conceptual framework for the formation of these ectopic recombination events (also referred to as non-allelic homologous recombination – NAHR) is the DSBR model. It entails the formation of a two-ended DSB within a repeated sequence, dual mismatched invasion/annealing of an ectopic repeat for formation of a dHJ intermediate and its resolution as a CO. Depending on the respective location and orientation of the damaged and donor repeats involved and the respective planes of dHJ cleavage, the NAHR event will lead to a balanced translocation, an interstitial inversion, an interstitial deletion associated or not with a balanced duplication, an isodicentric chromosome, or a circular chromosome (see one example in Figure 5A). Additional pathways can generate a subset or additional repeat-mediated rearrangements from a single DSB: SSA generates deletion in the absence of DNA strand invasion, while BIR involves a single DSB end and causes rearrangements associated with copy number gain and/or extensive gene conversion, and unbalanced translocations (reviewed in Refs. [58,59]).

Several lines of evidence strongly suggest that some of the ectopic recombination events underlying these rearrangements occur in the germline during the first meiotic division.[55] In particular, several disease-causing rearrangements were unambiguously shown to arise during human meiosis and not in somatic tissues.[60,61] Furthermore, modification of the recognition sequence of the mammalian meiotic DSB-licensing methyltransferase PRDM9[62,63] altered the frequency of these rearrangements.[64] Consistently, the frequency of repeat-mediated rearrangements increases with the proximity to, and the density of,

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PRDM9 binding-sites,[61,65,66] unlike mitotic NAHR hotspots.[67] Surprisingly, PRDM9 binding-sites were found enriched not within the repeat, but at a distance of up to 2 kb,[65] which corresponds to the span of meiotic DSB resection in mammals.[68] This observation is supported by a controlled genetic system in *S. cerevisiae* in which rearrangements mediated by dispersed Ty elements occurred more frequently with DSBs induced near, rather than within the Ty.[69] Radiation-induced breaks show a similar propensity to induce HR between non-allelic repetitive sequences.[70] In addition, somatic mosaicism events provide compelling evidence for mitotic cases of NAHR in humans.[65,67] Since MIR inherently exploits homologies distant from the DSB site to generate rearrangements between repeated sequences, we suggest that MIR is a mechanism underlying NAHR in yeast and human somatic cells and meiosis. Depending on the repeatlocation, orientation, and whether invasion occurs on the same or different chromatids, MIR can lead to the various types of the aforementioned rearrangements (Figure 5B, C and Figure S2, Supporting Information).

Relative to the DSBR model, MIR increases the window in which a DSB must fall to induce chromosomal rearrangements: while DSBs must occur within the repeat and preferentially away from the edges in the DSBR model, MIR can also occur with DSBs formed in the vicinity provided that resection exposes the flanking repeats. This increased sequence space for NAHR of MIR relative to the DSBR thus depends both on the length of the repeat and the resection tract (Figure 5D), akin to SSA.[58,71] Meiotic resection tracts hover around 1 kb in yeast and mammalian meiosis[15,52,68] and up to 40 kb and more in mitotically dividing yeast cells[72,73] causing large NAHR events.[28,69] While this gain in sequence space may be small for large segmental duplications, it should become significant for short dispersed repeats. Moreover, MIR decreases the number of concomitant homeologous invasions required for NAHR from two to one (the second invasion exploits extensive homologies at an allelic position; compare Figure 5A and B). Third, MIR generates additional single-ended DSBs, near or within a repeated sequence, potentially leading to additional rearrangements (Figure 5B and C). Fourth, while Figure 5B and Figure S2, Supporting Information, depict MIR-based NAHR with one of four possible orientations of the repeats, we note that, contrary to the DSBR model, there is no mechanistic constraint on the orientation of the repeats for the production of all types of rearrangements.

These features of MIR may underlie both simple and complex NAHR-mediated rearrangements between *Alu* elements in human[74–76] (reviewed by Kim et al.[57]) and the higher probability of DSBs within *Alu* repeats.[77] Notably, given their limited length (≈300 bp) and sequence similarity (71%),[78] *Alu* elements are poor substrates for CO formation by DSBR, as established with model sequences in budding yeast.[79–81] These inhibitory effects presumably result from a requirement for concomitant invasion of, or annealing to, the same donor by both ends of the DSB to form the dHJ intermediate (each being targeted for heteroduplex rejection by MMR[82]) and/or the negative effect of resection tracts distant from the homologous region that could also inhibit dHJ formation.[83] In contrast, the two homeologous invasions during NAHR by MIR are successive (Figure 5B) and their processing HJ-free, thus relieving major kinetic constraints on the process.

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4. Conclusions and Perspectives

A risk inherent to the homology search process during HR is the formation of MI, a joint molecule that threatens the integrity of the donors upon processing by SSEs in mechanisms collectively referred to as MIR. Depending on the respective location of the invading and donor molecules, MIR can lead to rearrangements of various types and magnitude, accompanied or not by additional single-ended DSBs. Hence, MIR provides a novel framework to interpret simple as well as complex genomic rearrangements involving repeated sequences. We also suggest that MI joint molecules are intermediates of physiological HR such as inter-homolog CO formation during meiosis. While we delineated two general mechanisms for MIR, many aspects remain unknown: i) the exact structure of the MI joint molecule; ii) the identity of the nucleases, their exact cleavage sites during MIR, and the involvement of branch migration; iii) the physiological and pathological contexts prone to MIR; and iv) the identification of a mutational signature unique to MIR. It will be of particular interest to demonstrate MI joint molecules and MIR in human cells and their involvement in the formation of pathological rearrangements.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

BIR	break-induced replication
CO	crossover
dHJ	double Holliday junction
DSB	double-strand break
DSBR	double-strand break repair
HR	homologous recombination
JM	joint molecule
mcJM	multi-chromatid joint molecule
MI	multi-invasion
MIR	MI-induced rearrangement

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NAHR	non-allelic homologous recombination
SDSA	synthesis-dependent strand annealing
ssDNA	single-stranded DNA
SSE	structure-selective endonuclease

References

[1]. Bell JC, Kowalczykowski SC, Annu. Rev. Biochem 2016, 85, 193. [PubMed: 27088880]

[2]. Forget AL, Kowalczykowski SC, Nature 2012, 482, 423. [PubMed: 22318518]

[3]. Wright WD, Heyer WD, Mol. Cell 2014, 53, 420. [PubMed: 24486020]

[4]. Piazza A, Wright WD, Heyer W-D, Cell 2017, 170, 760. [PubMed: 28781165]

[5]. Saini N, Ramakrishnan S, Elango R, Ayyar S, Zhang Y, Deem A, Ira G, Haber JE, Lobachev KS, Malkova A, Nature 2013, 502, 389. [PubMed: 24025772]

[6]. Stafa A, Donnianni RA, Timashev LA, Lam AF, Symington LS, Genetics 2014, 196, 1017. [PubMed: 24496010]

[7]. Elango R, Sheng Z, Jackson J, DeCata J, Ibrahim Y, Pham NT, Liang DH, Sakofsky CJ, Vindigni A, Lobachev KS, Ira G, Malkova A, Nat. Commun 2017, 8, 1790. [PubMed: 29176630]

[8]. Schwartz EK, Heyer WD, Chromosoma 2011, 120, 109. [PubMed: 21369956]

[9]. Ehmsen KT, Heyer WD, Nucl. Acids Res 2008, 36, 2182. [PubMed: 18281703]

[10]. Ehmsen KT, Heyer WD, Nucl. Acids Res 2009, 37, 2026. [PubMed: 19211663]

[11]. Ranjha L, Anand R, Cejka P, J. Biol. Chem 2014, 289, 5674. [PubMed: 24443562]

[12]. Rogacheva MV, Manhart CM, Chen C, Guarne A, Surtees J, Alani E, J. Biol. Chem 2014, 289, 5664. [PubMed: 24403070]

[13]. Claeys Bouuaert C, Keeney S, PLoS Genet 2017, 13, e1006722. [PubMed: 28505149]

[14]. Su XA, Freudenreich CH, Proc. Natl. Acad. Sci. USA 2017, 114, E8392. [PubMed: 28923949]

[15]. Zakharyevich K, Ma YM, Tang SM, Hwang PYH, Boiteux S, Hunter N, Mol. Cell 2010, 40, 1001. [PubMed: 21172664]

[16]. Zakharyevich K, Tang SM, Ma YM, Hunter N, Cell 2012, 149, 334. [PubMed: 22500800]

[17]. Nishant KT, Plys AJ, Alani E, Genetics 2008, 179, 747. [PubMed: 18505871]

[18]. Schärer OD, Cold Spring Harbor Perspect. Biol 2013, 5, a012609.

[19]. Prado F, BioEssays 2014, 36, 451. [PubMed: 24615940]

[20]. West SC, Blanco MG, Chan YW, Matos J, Sarbajna S, Wyatt HD, Cold Spring Harbor Symp. Quant. Biol 2015, 80, 103. [PubMed: 26370409]

[21]. Mehta A, Haber JE, Cold Spring Harbor Perspec. Biol 2014, 6, a016428.

[22]. Szostak JW, Orr-Weaver TL, Rothstein RJ, Stahl FW, Cell 1983, 33, 25. [PubMed: 6380756]

[23]. Schwacha A, Kleckner N, Cell 1995, 83, 783. [PubMed: 8521495]

[24]. Bzymek M, Thayer NH, Oh SD, Kleckner N, Hunter N, Nature 2010, 464, 937. [PubMed: 20348905]

[25]. Sun H, Treco D, Szostak JW, Cell 1991, 64, 1155. [PubMed: 2004421]

[26]. Martini E, Borde V, Legendre M, Audic S, Regnault B, Soubigou G, Dujon B, Llorente B, PLoS Genet 2011, 7, e1002305. [PubMed: 21980306]

[27]. Marsolier-Kergoat M-C, Khan MM, Schott J, Zhu X, Llorente B, bioRxiv 2017.

[28]. Yin Y, Dominska M, Yim E, Petes TD, Elife 2017, 6, e28069. [PubMed: 28714850]

[29]. Andersen SL, Zhang A, Dominska M, Moriel-Carretero M, Herrera-Moyano E, Aguilera A, Petes TD, PLoS Genet 2016, 12, e1005938. [PubMed: 26968037]

[30]. Cole F, Baudat F, Grey C, Keeney S, de Massy B, Jasin M, Nat. Genet 2014, 46, 1072. [PubMed: 25151354]

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[31]. Oh SD, Lao JP, Hwang PYH, Taylor AF, Smith GR, Hunter N, Cell 2007, 130, 259. [PubMed: 17662941]

[32]. Ip SCY, Rass U, Blanco MG, Flynn HR, Skehel JM, West SC, Nature 2008, 456, 357. [PubMed: 19020614]

[33]. Schwartz EK, Wright WD, Ehmsen KT, Evans JE, Stahlberg H, Heyer WD, Mol. Cell. Biol 2012, 32, 3065. [PubMed: 22645308]

[34]. Wyatt HDM, West SC, Cold Spring Harbor Perspect. Biol 2014, 6, a023192.

[35]. Punatar RS, Martin MJ, Wyatt HDM, Chan YW, West SC, Proc. Natl. Acad. Sci. USA 2017, 114, 443. [PubMed: 28049850]

[36]. Dehe PM, Gaillard PHL, Nature Rev. Mol. Cell Biol 2017, 18, 315. [PubMed: 28327556]

[37]. Heyer WD, Ehmsen KT, Solinger JA, Trends Biochem. Sci 2003, 10, 548.

[38]. Osman F, Dixon J, Doe CL, Whitby MC, Mol. Cell 2003, 12, 761. [PubMed: 14527420]

[39]. Mazon G, Lam AF, Ho CK, Kupiec M, Symington LS, Nat. Struct. Mol. Biol 2012, 19, 964. [PubMed: 22885325]

[40]. Mazon G, Symington LS, Mol. Cell 2013, 52, 63. [PubMed: 24119400]

[41]. Jessop L, Lichten M, Mol. Cell 2008, 31, 313. [PubMed: 18691964]

[42]. De Muyt A, Jessop L, Kolar E, Sourirajan A, Chen JH, Dayani Y, Lichten M, Mol. Cell 2012, 46, 43. [PubMed: 22500736]

[43]. Tang S, Wu MKY, Zhang R, Hunter N, Mol. Cell 2015, 57, 607. [PubMed: 25699709]

[44]. Kaur H, De Muyt A, Lichten M, Mol. Cell 2015, 57, 583. [PubMed: 25699707]

[45]. Oh SD, Lao JP, Taylor AF, Smith GR, Hunter N, Mol. Cell 2008, 31, 324. [PubMed: 18691965]

[46]. Lisby M, Mortensen UH, Rothstein R, Nat. Cell Biol 2003, 5, 572. [PubMed: 12766777]

[47]. Brown MS, Grubb J, Zhang A, Rust MJ, Bishop DK, PLoS Genet. 2015, 11, e1005653. [PubMed: 26719980]

[48]. Storlazzi A, Gargano S, Ruprich-Robert G, Falque M, David M, Kleckner N, Zickler D, Cell 2010, 141, 94. [PubMed: 20371348]

[49]. Zickler D, Kleckner N, Cold Spring Harbor Perspect. Biol 2015, 7, 016626.

[50]. Hunter N, Kleckner N, Cell 2001, 106, 59. [PubMed: 11461702]

[51]. Kim KP, Weiner BM, Zhang LR, Jordan A, Dekker J, Kleckner N, Cell 2010, 143, 924. [PubMed: 21145459]

[52]. Mimitou EP, Yamada S, Keeney S, Science 2017, 355, 40. [PubMed: 28059759]

[53]. Bishop DK, Cell 1994, 79, 1081. [PubMed: 7528104]

[54]. Richard GF, Kerrest A, Dujon B, Microbiol. Mol. Bio. Rev 2008, 72, 686. [PubMed: 19052325]

[55]. Sasaki M, Lange J, Keeney S, Nat. Rev. Mol. Cell Biol 2010, 11, 182. [PubMed: 20164840]

[56]. Carvalho CM, Lupski JR, Nat. Rev. Genet 2016, 17, 224. [PubMed: 26924765]

[57]. Kim S, Cho CS, Han K, Lee J, Genom. Inform 2016, 14, 70.

[58]. Bhargava R, Onyango DO, Stark JM, Trends Genet 2016, 32, 566. [PubMed: 27450436]

[59]. Anand RP, Lovett ST, Haber JE, Cold Spring Harbor Perspect. Biol 2013, 5, a010397.

[60]. Turner DJ, Miretti M, Rajan D, Fiegler H, Carter NP, Blayney ML, Beck S, Hurles ME, Nat. Genet 2008, 40, 90. [PubMed: 18059269]

[61]. Lam KW, Jefrreys AJ, Proc. Natl. Acad. Sci. USA 2007, 104, 10950. [PubMed: 17573529]

[62]. Myers S, Freeman C, Auton A, Donnelly P, McVean G, Nat. Genet 2008, 40, 1124. [PubMed: 19165926]

[63]. Baudat F, Buard J, Grey C, Fledel-Alon A, Ober C, Przeworski M, Coop G, de Massy B, Science 2010, 327, 836. [PubMed: 20044539]

[64]. Berg IL, Neumann R, Lam KW, Sarbajna S, Odenthal-Hesse L, May CA, Jeffreys AJ, Nat. Genet 2010, 42, 859. [PubMed: 20818382]

[65]. Dittwald P, Gambin T, Szafranski P, Li J, Amato S, Divon MY, Rodriguez Rojas LX, Elton LE, Scott DA, Schaaf CP, Torres-Martinez W, Stevens AK, Rosenfeld JA, Agadi S, Francis D, Kang SH, Breman A, Lalani SR, Bacino CA, Bi W, Milosavljevic A, Beaudet AL, Patel A, Shaw CA,

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Lupski JR, Gambin A, Cheung SW, Stankiewicz P, Genome Res 2013, 23, 1395. [PubMed: 23657883]

[66]. Campbell IM, Gambin T, Dittwald P, Beck CR, Shuvarikov A, Hixson P, Patel A, Gambin A, Shaw CA, Rosenfeld JA, Stankiewicz P, BMC Biol 2014, 12, 74. [PubMed: 25246103]

[67]. Vogt J, Mussotter T, Bengesser K, Claes K, Hogel J, Chuzhanova N, Fu C, van den Ende J, Mautner VF, Cooper DN, Messiaen L, Kehrer-Sawatzki H, Hum. Mut 2012, 33, 1599. [PubMed: 22837079]

[68]. Lange J, Yamada S, Tischfield SE, Pan J, Kim S, Zhu X, Socci ND, Jasin M, Keeney S, Cell 2016, 167, 695. [PubMed: 27745971]

[69]. Hoang ML, Tan FJ, Lai DC, Celniker SE, Hoskins RA, Dunham MJ, Zheng YX, Koshland D, PLoS Genet 2010, 6, e1001228. [PubMed: 21151956]

[70]. Argueso JL, Westmoreland J, Mieczkowski PA, Gawel M, Petes TD, Resnick MA, Proc. Natl. Acad. Sci. USA 2008, 105, 11845. [PubMed: 18701715]

[71]. Pâques F, Haber JE, Microbiol. Mol. Biol. Rev 1999, 63, 349. [PubMed: 10357855]

[72]. Zhu Z, Chung WH, Shim EY, Lee SE, Ira G, Cell 2008, 134, 981. [PubMed: 18805091]

[73]. Mimitou EP, Symington LS, Nature 2008, 455, 770. [PubMed: 18806779]

[74]. Gu S, Yuan B, Campbell IM, Beck CR, Carvalho CM, Nagamani SC, Erez A, Patel A, Bacino CA, Shaw CA, Stankiewicz P, Cheung SW, Bi W, Lupski JR, Hum. Mol. Genet 2015, 24, 4061. [PubMed: 25908615]

[75]. Nazaryan-Petersen L, Bertelsen B, Bak M, Jonson L, Tommerup N, Hancks DC, Tumer Z, Hum. Mutat 2016, 37, 385. [PubMed: 26929209]

[76]. Bondurand N, Fouquet V, Baral V, Lecerf L, Loundon N, Goossens M, Duriez B, Labrune P, Pingault V, Eur. J. Hum. Genet 2012, 20, 990. [PubMed: 22378281]

[77]. Shaw CJ, Lupski JR, Hum. Genet 2005, 116, 1. [PubMed: 15526218]

[78]. Shen MR, Batzer MA, Deininger PL, J. Mol. Evol 1991, 33, 311. [PubMed: 1774786]

[79]. Jinks-Robertson S, Michelitch M, Ramcharan S, Mol. Cell. Biol 1993, 13, 3937. [PubMed: 8321201]

[80]. Inbar O, Kupiec M, Curr. Genet 2000, 38, 23. [PubMed: 10953878]

[81]. Welz-Voegele C, Jinks-Robertson S, Genetics 2008, 179, 1251. [PubMed: 18562664]

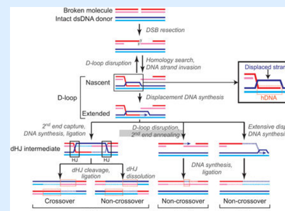
[82]. Spies M, Fishel R, Cold Spring Harbor Perspect. Biol 2014, 8, a022657.

[83]. Prado F, Aguilera A, Trends Genet 2003, 19, 428. [PubMed: 12902160]

Box 1.

Outline of recombinational DSB repair pathways

DSB repair by HR is initiated by nucleolytic processing of the DNA ends to expose long, 3′-protruding ssDNA molecules, onto which Rad51 and associated proteins assemble as a right-handed helical filament. This multivalent nucleo-protein filament undergoes a multiplexed genome-wide search for an identical (homologous) or near identical (homeologous) dsDNA molecule. DNA strand invasion of a dsDNA produces a joint molecule called a displacement loop (D-loop). A D-loop contains a heteroduplex DNA (hDNA, boxed in orange) region that consists in the base-pairing of the incoming strand to its complement in the dsDNA, and a displaced strand. Displacement DNA synthesis primed from the 3′ extremity extends the hDNA.Disruption of this extended D-loop and reanneling of the extended ssDNA to the complementary second end of the DSB funnels the DSB repair toward a non-crossover (NCO) outcome by Synthesis-Dependent Strand Annealing (SDSA). In situations where a second end is missing, extensive displacement (abbreviated displ. in drawing) DNA synthesis can ensue along the entire chromosome arm in a process known as Break-Induced Replication (BIR). It results in gene conversion of the distal extremity. Alternatively, the displaced strand generated upon D-loop extension can pair with the second end of the DSB, directing the intermediate toward the DSBR pathway. Following DNA synthesis and ligation, a double-Holliday Junctions (dHJ) intermediate is formed in which the incoming and donor dsDNA are topologically linked and delimited by two 4-ways junctions (HJ). The outcome of the endonucleolytic resolution of each HJ can be either a crossover (CO; reciprocal exchange of flanking DNA) or a non-crossover (see details in Figure 4A). dHJ dissolution leads to non-crossover only. The hDNA component of each repair outcome is boxed in orange. Dashed lines indicate recombination-associated DNA synthesis.



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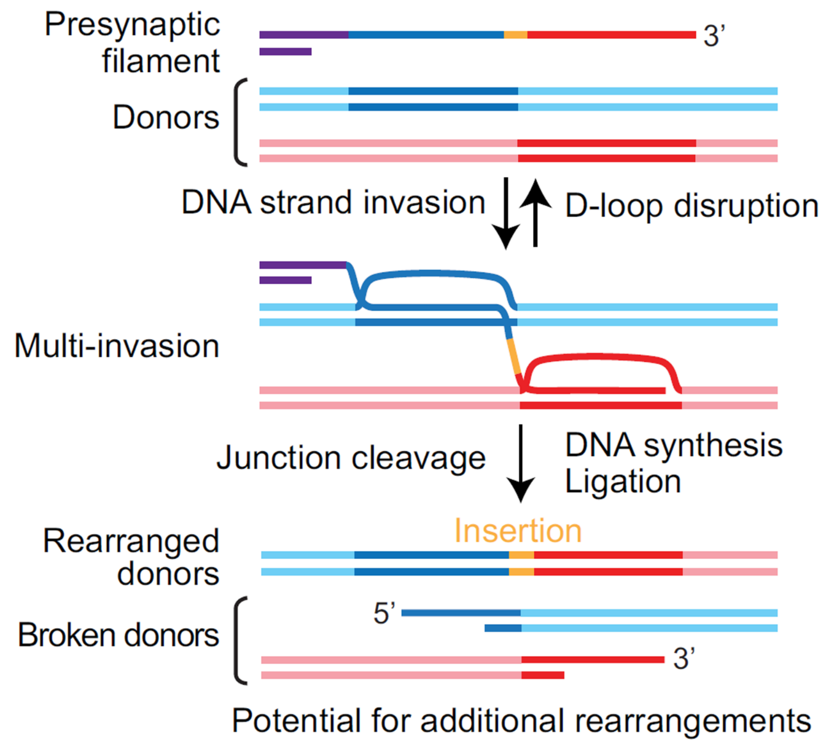

Figure 1.

Outline of multi-invasion-induced rearrangements and its regulation by *cis* and *trans*-acting factors.

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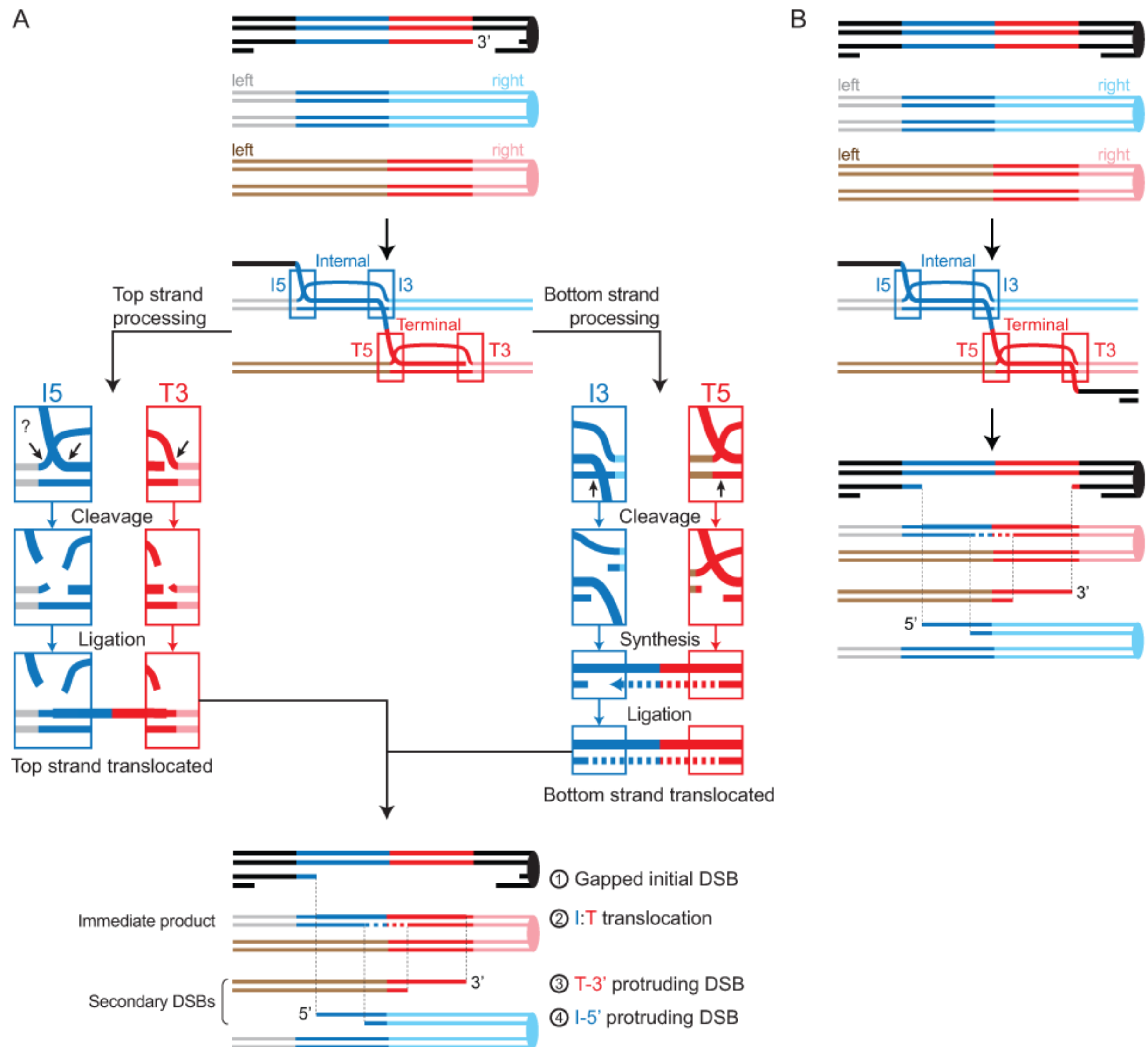

Figure 2.

MIR1 mechanism. A) Top and bottom strands processing results in a chromosomal translocation. Formation of each translocated DNA strand can be independent from each other. The only kinetic constraint of MIR1 is the cleavage of I5 prior to being reached by the synthesis initiated at T5. MIR1 leaves as immediate products: 1) the original DSB, which has lost its 3′ extremity involved in MIR and that can be repaired by HR using the homolog as a template; 2) the translocation chromatid; and 3) and 4) two oriented single-ended DSBs (the left side of the T donor with a 3′ protruding extremity and the right side of the I invasion with a 5′ protruding extremity). Both can be repaired with or without additional rearrangements, depending on their sequence context. The question mark indicates uncertainties regarding the nature and position of the cleavage events at I5. B) Outline of MIR1 initiated by a ssDNA gap. Dashed lines indicate recombination-associated DNA synthesis.

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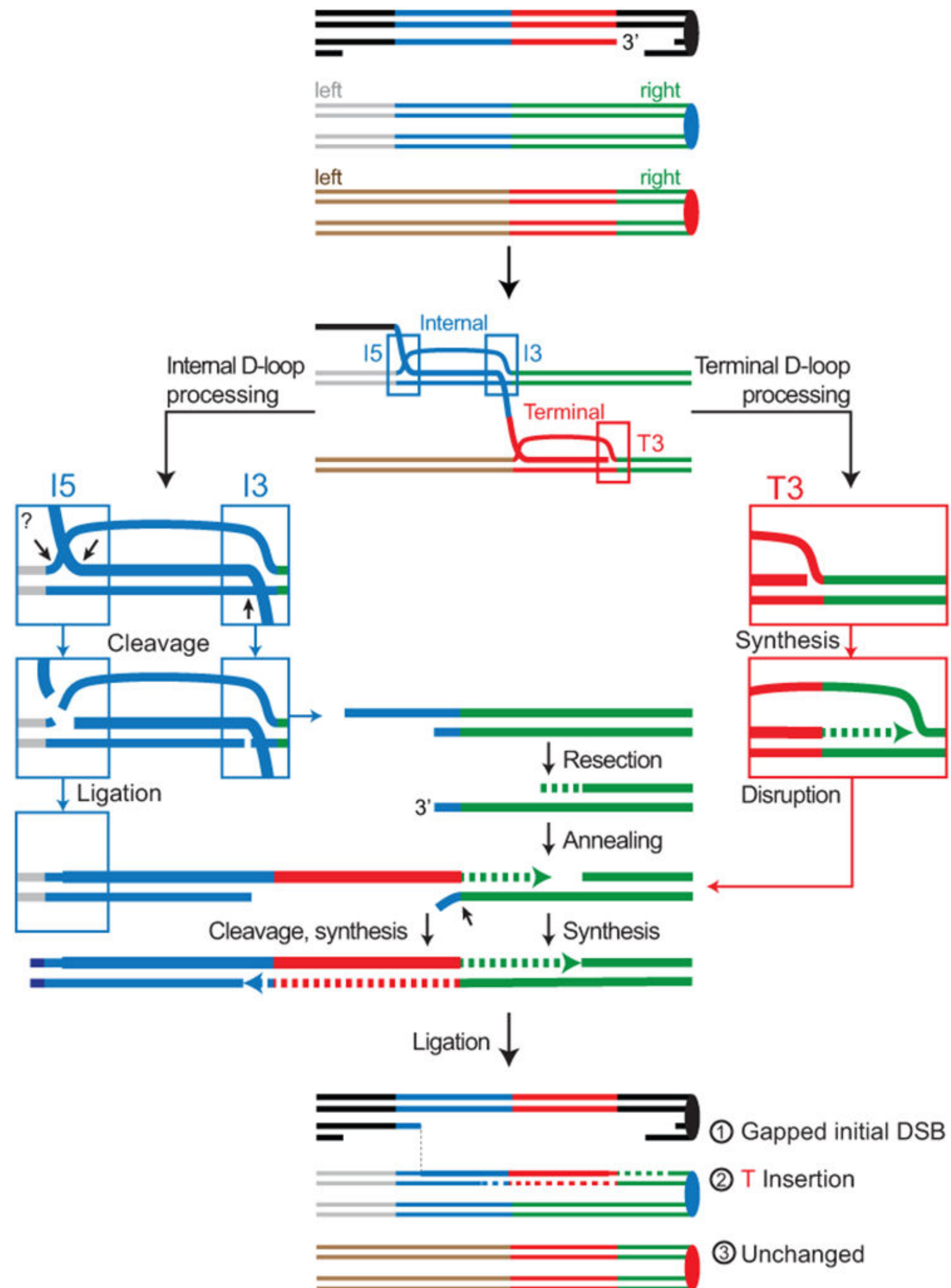

Figure 3.

MIR2 mechanism. The internal and distal donors must share downstream sequence homology (depicted in green). The endonucleolytic processing of the I invasion is identical to MIR1. The question mark indicates uncertainties regarding the nature and position of the cleavage events at I5. MIR2 produces a single, 5′ protruding one-ended DSB which upon resection is annealed to the displaced extended invading strand at the T donor. MIR2 leaves as immediate products: 1) the original DSB, which has lost its 3′ extremity involved in MIR and can be repaired by HR using the homolog as a template; 2) an apparent translocation which formally is an insertion. The chromosome with the terminal (red) donor is intact.

Hence, MIR2 does not propagate additional DSB. Dashed lines indicate recombination-associated DNA synthesis.

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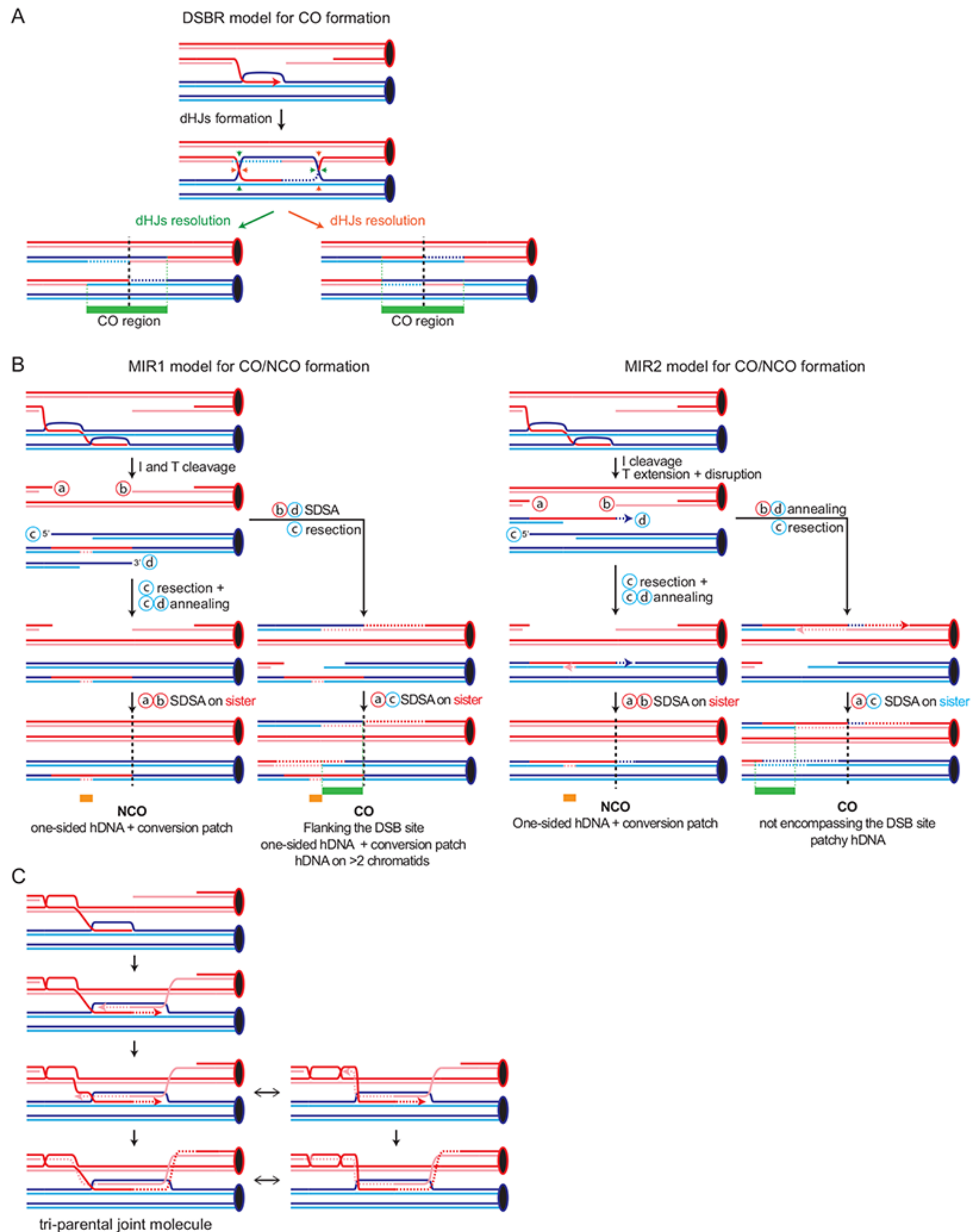

Figure 4.

MIR-based mechanisms for CO and mcJMs formation. A) The DSBR model for CO formation. Colored arrows indicate the two possible orientations for dHJ cleavage resulting in a CO. Dotted strands result from DNA synthesis. The green bars represent the CO region, which in both cases encompass the DSB site (dotted black line). B) Examples of MIR1- (left) and MIR2-based (right) mechanisms for NCO and CO formation upon inter-sister MI. The two initial DSB ends (a and b) and the two DSB ends generated upon MIR (c and d) are labeled. The particular outcome of their repair depends (i) on the relative timing of formation and processing of the “c” and “d” DSBs, and (ii) the specific template and repair

pathway employed. Additional examples of inter-homolog events and the various repair outcomes of downstream DSBs are depicted in Figure S1, Supporting Information. Orange bars denote gapped regions prone to gene conversion. Dotted strands result from DNA synthesis. The green bars represent the CO regions, which never encompass the DSB site (dotted black line). The outcome description follows the nomenclature of Ref. [21]. C) Example of tri-parental mcJMs formation from an inter-homolog MI precursor. The template switches can readily occur upon DNA strand exchange junctions migration towards the DNA synthesis direction. All strands are in the parental configurations. Dotted strands result from DNA synthesis. Double-headed arrows indicate equivalence of the intermediates.

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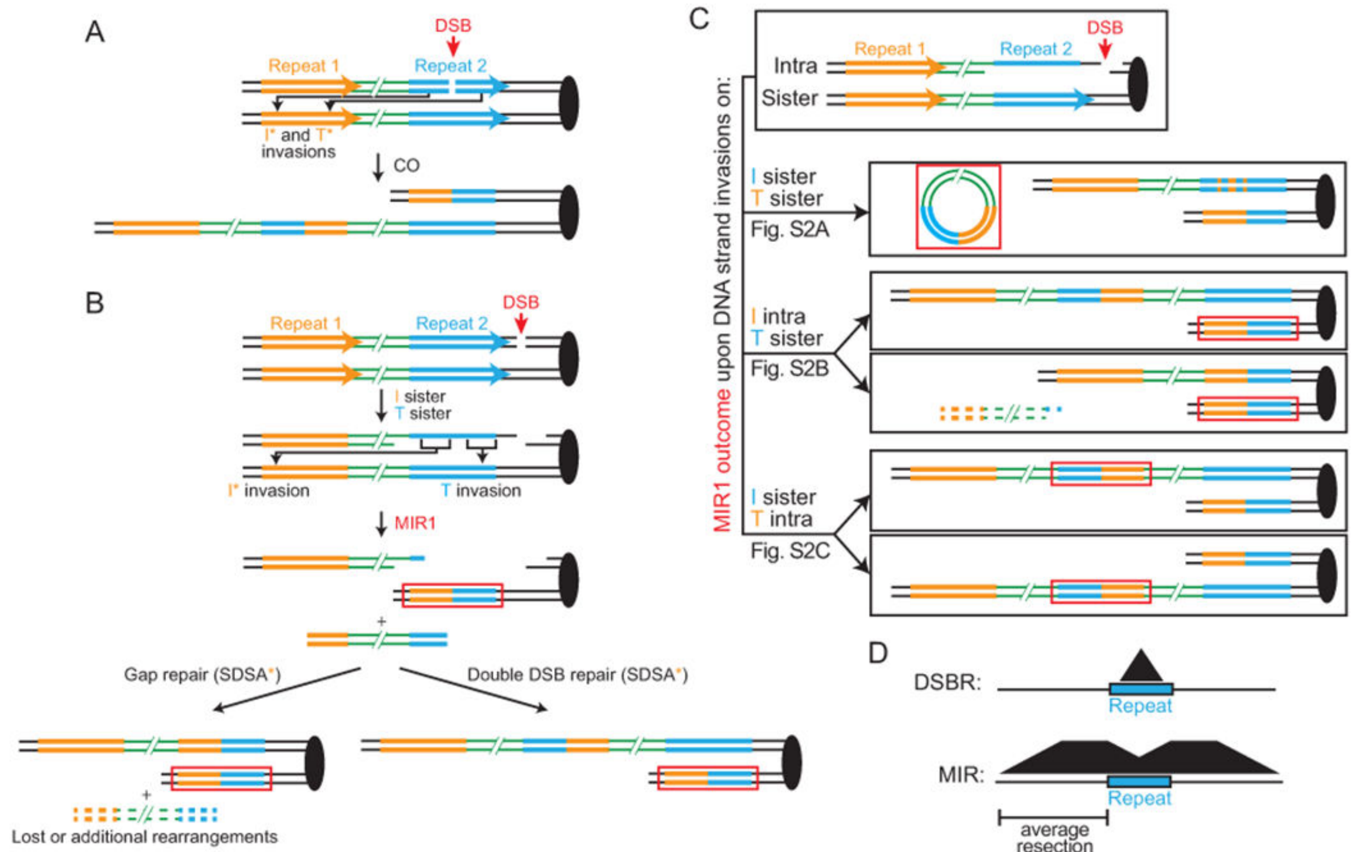

Figure 5.

MIR-based mechanisms for NAHR. A) DSBR model for NAHR. The DSB must occur within a repeat. * indicates homeologous invasions. B) Example of a MIR1-induced inter-chromatidal deletion event. In this scenario, the only homeologous invasion occurs internally, the distal invasion being allelic. The repair of the secondary DSBs will lead either to a loss of the intervening sequence (left) or a reciprocal duplication (right). The translocation produced by MIR is boxed in red. C) Other possible inter-chromatidal NAHR events depending on the repeats invaded and the pathway employed to repair the secondary DSBs. The translocations produced by MIR are boxed in red. For each outcome, a detailed mechanism is presented in Figure S2, Supporting Information. D) Schematic representation of the distribution around repeated regions of DSB sites prone to induce NAHR events according to the DSBR (top) and the MIR (bottom) models. For simplicity, heteroduplex regions are not drawn in this figure.