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Recombination-mediated genome rearrangements

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Abstract

Homologous recombination (HR) is a universal DNA double-strand break (DSB) repair pathway that uses an intact DNA molecule as a template. Signature HR reactions are homology search and DNA strand invasion catalyzed by the prototypical RecA-ssDNA filament (Rad51 and Dmc1 in eukaryotes), which produces heteroduplex DNA-containing joint molecules (JMs). These reactions uniquely infringe on the DNA strands association established at replication, on the basis of substantial sequence similarity. For that reason, and despite the high fidelity of its templated nature, DSB repair by HR authorizes the alteration of genome structure, guided by repetitive DNA elements. The resulting structural variations (SVs) can involve vast genomic regions, potentially affecting multiple coding sequences and regulatory elements at once, with possible pathological consequences. Here, we discuss recent advances in our understanding of genetic and molecular vulnerabilities of HR leading to SVs, and of the various fidelity-enforcing factors acting across scales on the balancing act of this complex pathway. An emphasis is put on extra-chromosomal DNAs, both product of, and substrate for HR-mediated chromosomal rearrangements.

Main text

The core HR steps are (1) homology sampling by a conserved RecA/Rad51-ssDNA nucleoprotein filament (NPF) that registers an intact identical (homologous) or similar (homeologous) dsDNA molecule in the genome; (2) DNA strand invasion, which results in the formation of a joint-molecule (JM) called a displacement (D)-loop containing heteroduplex DNA (hDNA), a displaced ssDNA, and flanking strand exchange junctions; and (3) DNA synthesis from the hDNA 3' extremity, which restores the DNA sequence disrupted by the DSB and authorizes re-joining with the second DSB end for completion of repair (**Figure 1**). The pathway has two main types of vulnerabilities: one is genetic, as the presence of dispersed DNA repeats can confound the homology search process into recombining at a non-allelic (*i.e.* ectopic) loci; the other pertains to the processing of JMs, which can fail to restore the initial configuration of the damaged molecule and/or of its donor (**Figure 1**). Unless otherwise stated, we will discuss these generally conserved mechanisms and regulations in mitotic *S. cerevisiae* cells, where the mechanistic understanding of HR with respect to genomic stability is most advanced.

The genetic challenge of HR: overlooking the overwhelming.

Despite their relatively low abundance in *S. cerevisiae*, dispersed repeats are expected to be frequently exposed by resection, embedded within the NPF, and used to query the genome [1]. Dispersed DNA repeats greatly outnumber allelic targets, yet the HR machinery manages to quasi-exclusively template repair accurately from the allelic locus (**Figure 2A**). The stringency of the donor selection process presumably results from two rounds of homology assessments [2], first at the level of dsDNA sampling by the NPF, and then at the nascent D-loop level. Biochemical studies with bacterial RecA and eukaryotic Rad51 revealed that dsDNA sampling operates *via* multiple short and unstable probing attempts along the NPF [3,4]. A conserved recognition threshold of 8 matched nucleotides, although insufficient to ensure uniqueness, weeds out most heterologies [5–7]. DNA strand invasion can convert these matched synaptic complexes into a D-loop JM (**Figure 1**). The majority of D-loops are reversed before DNA synthesis can be initiated [1,8–12]. This reversal is promoted by conserved helicase/topoisomerases (see below), whose defects greatly elevate repeat-mediated SVs [13]. We proposed that the D-loop reversal/extension competition sets a kinetic proofreading scheme that amplifies donor-specific advantages for re-invasion, against disruption, and/or for DNA synthesis initiation, based on homology length, positioning relative to the DSB end, overall sequence similarity, or a combination thereof [1] (**Figure 2**). For instance, mismatch repair proteins notoriously stimulate disruption of homeology-containing D-loops in a process coined heteroduplex rejection, *de facto* biasing donor usage on the basis of sequence similarity. Interestingly, checkpoint regulations tie together long-range resection and JM metabolism [14], presumably increasing the stringency of donor selection with the risk of exposing a repeat in the resection span. These observations suggest that HR steps regulation may be choreographed in broad phases of varying fidelity at increasing time post-DSB formation, up to checkpoint adaptation.

These molecular-scale transactions are subordinate to the collision probabilities between the NPF and potential donors. Consequently, factors dictating spatial chromatin organization and locus confinement are also expected to influence NPF encounters with ectopic donors, and thus the occurrence of repeat-mediated SVs [15]. In *S. cerevisiae*, ectopic repair success correlates with the contact frequency imposed by the Rab1 organization of chromosomes, defined by passive nuclear anchors at centromeres and telomeres and limited

chromatin mobility [16–18]. Alternatively, repeat-rich heterochromatin in various eukaryotes segregates from euchromatin into HP1-mediated B compartments that are refractory to HR [19–21], similar to the ribosomal DNA repeat-containing nucleolus in *S. cerevisiae* [22]. These two compartmentalization mechanisms both occlude potential ectopic donors for the NPF.

On the contrary, tethering the DSB region to specific dsDNA molecules may promote their disproportionate sampling and usage. For instance, the competitive repair for a-to-alpha mating-type switching in *S. cerevisiae* is regulated by a specialized *MAT*-donor tethering mechanism [23,24]. Cohesin (and related complexes) plays a more general dsDNA tethering role, both in *trans* by ensuring sister chromatid cohesion and in *cis* through bi-directional translocation on a single DNA molecule, in a process referred to as “loop extrusion” [25–27]. In addition to the promiscuous and transient interactions at the cohesin level, the reiterated threadings delineated by *cis* boundaries maintains domains of preferential interactions along and between chromosomes [28]. While sister chromatid cohesion has long been recognized as promoting sister usage [29–31], the involvement of cohesin-mediated chromatin folding in regulating general DSB repair has only recently begun to be investigated [32–34]. Notably, the DSB region was recently shown to block cohesin translocation in both *S. cerevisiae* and human cells, causing side-specific contact enrichment between the DSB region and the flanking chromatin [32,33]. Functionally, this DSB-cohesin configuration as well as the more general context of preferential interaction domains (*e.g.* TADs in mammals and individualized chromosomes in *S. cerevisiae*), regulates histone modifications by a DSB-localized kinase [32], and homology sampling by the NPF [33] (depicted in **Figure 2B**). Notably, it biases homology sampling in *cis* in a side-specific manner over the loop expansion span, which is tuned by cohesin regulators [33]. In a related fashion, it regulates RAG-mediated selection of *cis* recombining partners for the assembly of antigen receptor genes [35–37]. Cohesin correspondingly traps the NPF intra-chromosomally, limiting its ability to sample dsDNA in *trans* [33], consistent with its role in inhibiting DSB mobility [18]. It may act similarly in inhibiting NHEJ-mediated translocations [38], as cohesin accumulates at both HR- and NHEJ-prone DSBs in human cells [32]. Since sister chromatids mainly interact at the level of loop bases in human cells [39], having the DSB placed at the cohesin level may also stimulate sister sampling, conjointly with sister chromatid cohesion (**Figure 2B**). These multi-pronged regulations of NPF-dsDNA collision probabilities likely mitigate the risk for HR-mediated SV formation, or their extent thereof.

Clearly, the efficiency and clairvoyance of homology search in a repeat-loaded genome results from molecular processes (*i.e.* dsDNA sampling and JM metabolism) dynamically adjusted over time and nested into higher-order organizational chromatin contexts. Much work remains to quantitatively define their relative contributions, how their relationships are spatiotemporally adjusted and adapted at different stages of the life cycle (*i.e.* mitosis *vs.* meiosis) [40,41], and ultimately predict the consequences of specific molecular and organizational dysregulations on HR efficiency and fidelity.

DNA joint molecules: structural liabilities for HR fidelity

The second main HR vulnerability lies in the structure of the D-loop, a central JM intermediate of the pathway substrate for three main types of enzymatic activities: structure selective endonucleases (SSEs) that recognize and cleave JM junctions [42]; DNA polymerases that perform displacement DNA synthesis [43]; and DNA helicases/topoisomerases that take hDNA apart (**Figure 3A**). These activities (or absence thereof) funnel HR into one of its various, more or less conservative subpathways (**Figures 1 and 3**). In the

Synthesis-Dependent Strand Annealing (SDSA) pathway predominantly used for double-ended DSB repair, disruption of the extended D-loop and Rad52/Rad59-mediated annealing to the opposite DSB end ensures limited DNA synthesis and preserve the integrity of the dsDNA donor [44]. Failure to do so, for instance in the case of single-ended DSBs, leads to the low-fidelity long-range DNA synthesis prone to template switching known as Break-Induced Replication (BIR; reviewed in [45]) (**Figure 3B**). In addition to *trans*-acting D-loop disruption activities, recent work revealed multiple *cis*-acting obstacles potentially inhibiting BIR completion, such as head-on PolII-mediated transcription and interstitial telomeric sequences [46,47], in addition to centromeres [44,48]. These obstacles may restrain BIR within telomere-proximal regions [49] but likely increase the risk of causing complex SVs via template-switch (**Figure 3B**).

BIR generates potentially complex SVs while leaving the dsDNA template intact. Differently, endonucleolytic processing of JMs by SSEs can generate SVs by providing opportunities for covalent rejoining between invading and donor dsDNA (**Figure 3C**). The impact on the repair outcome depends on the JM processed, which can occur in the absence of DNA synthesis at the level of D-loops (half-crossover) and generate additional DSBs upon processing of multi-invasion JMs, in a mechanism referred to as multi-invasion-induced rearrangements (MIR) [50,51]. Certain combinations of DNA strand cleavage may also disrupt the sister or allelic donor, thus complicating or eliminating opportunities for faithful repair. Overlaid controls on SSEs, including nuclear exclusion for Yen1/GEN1, restrict their activity to mitosis for the resolution of dead-end JMs [42,52]. Consequently, JM persistence up to mitotic entry or nuclear envelope defects may be a time window at risk of forming HR-mediated SVs. Intriguingly, HR-mediated loss-of-heterozygosity or SV frequently co-occur in *S. cerevisiae*, suggesting the existence of transient phases of non-conservative processing of HR intermediates [53]. The fact that HR takes place in metaphase-arrested cells in *S. cerevisiae*, and that co-occurring LOH and SVs also frequently coincided with aneuploidies [54] suggests that they originated from premature mitotic entry, possibly exposing unresolved JMs to activated SSEs. Such premature entry and associated unleashing of SSEs may underlie the genomic plasticity of asexual human pathogens exhibiting notable DNA damage checkpoint defects [55].

Of note, the low-fidelity subpathways of HR (*i.e.* crossover, BIR, half-crossover, MIR) enacted at an allelic site are inconsequential for overall genome structure while their occurrence at an ectopic site will necessarily results in SV formation. Consequently, HR-induced genomic instability results from combined defects of donor selection and of conservative JM processing. It highlights the importance of D-loop reversal, involved at both levels, in ensuring the safe deployment of HR.

The disloyal competition of extrachromosomal DNA repeats

Vast swaths of eukaryotic genomes are made of repeated sequences (*e.g.* $\approx 6\%$ in *S. cerevisiae*; $\approx 45\%$ in humans), long recognized as a mediator of SVs whose recurrence scales with repeat length [13,56–61]. DNA repeats can also exist extra-chromosomally, either in the form of retro-transcribed cDNA [62] or as DNA circles [63] (**Figure 1**). The contribution of these potentially massive sources of repeated template to HR-mediated SV, as well as the delineation of cellular mechanisms and contexts regulating their occurrence and usage are incompletely understood. For instance, environment-induced episodes of retrotransposon de-repression or retroviral infection may lower the fidelity of HR repair by stimulating random cDNA production, counteracted by RNaseH enzymes [62]. Perhaps more stably, DNA circles (referred to as ecDNA, eccDNA, double-minutes, t-circles or episomes depending on length, origin or rebranding, see

[63]) can provide a transient and massive excess of repair template in dividing cells. Although the role of these circles has long been recognized in the acquisition of resistance to chemotherapeutic drugs or antibiotics in many systems, recent experimental evidence of their coincidence with bursts of genomic instability in cancer cells [64,65] raises exciting mechanistic questions: are they byproducts or active components of the abrupt mutational episodes at play in cancer, and whose mechanisms remain obscure ([64], and see [66] for a historical perspective).

The relationship between DNA circles and HR is twofold. First, they can possibly arise from ectopic intra-chromatid crossover if the initiating lesion occurred within a repeat, or from D-loop cleavage or MIR if a repeat was present in the resection span (**Figure 4A** and [67]). Accordingly, DNA circles involving repeated DNA elements form abundantly in a resection-, HR- and SSE-dependent fashion in *S. cerevisiae* [22,68,69]. Crossovers produce circles without copy number gain, as they leave a reciprocal deletion (*i.e.* the episome model), limiting their potential to drive gene amplification at the subsequent cell division. Differently, D-loop cleavage can yield copy number gains in addition to the circle, upon sealing of the as-yet-unrepaired initiating lesion (**Figure 4A**). These circles may in turn provide a new, larger repeated template for HR repair prone to generate tandem amplification by crossover or rolling circle mechanisms upon chromosomal damage (**Figure 4B**). Such circle-based amplifications are observed with t-circles in ALT cells [70], and possibly at play during chromothripsis [65]. Alternatively, large palindromic circles can originate from ligation of neo-synthesized DNA strands in regressed replication forks [71]. Such circles could produce the puzzling DUP-TRP/INV-DUP rearrangements observed in various pathologies [57,72], upon reintegration in the genome by HR [71].

Where do these circles localize once formed? Some may partition based on their structure or genetic content, with HP1-mediated B compartmentalization presumably maintaining repeat-rich circles out of reach from HR in most eukaryotes. Some others may freely diffuse in the nucleoplasm and circumvent the spatial restrictions imposed on homology search by static anchors or active threaders (*e.g.* cohesin). This lack of spatial constraint will provide substrate for copying and/or integration of seemingly distant chromosomal regions at break sites in transient episodes of genomic instability [64]. Finally, the lack of a centromere makes them prone to missegregate at mitosis, leading to uneven distribution of amplified genetic material [73] and prone to further rearrangements in micronuclei [65].

Where have the repeat-mediated rearrangements been for the last decade?

Analysis of SV junction sequences is the prime basis from which the mutational mechanism is being inferred. Oddly, the predominant role played by non-allelic HR in natural and pathological human variations (*e.g.* BRCA1 rearrangements, see [74]; [57]) has been relayed to a second order phenomenon since the replacement of long-range PCR and targeted sequencing by high-throughput short-read sequencing. Increased coverage, longer reads and improved mapping pipelines progressively brings repeat-mediated SV back from the bin [75,76]. These novel generation studies put the spotlight back onto homology-directed repair in shaping somatic diversity in humans [75], and will be instrumental in better defining the contribution of HR to genomic instability phenomena of relevance for human health.

Figure legends

Figure 1: Overview of homologous recombination and its vulnerabilities. The D-loop intermediate is a liability both in the donor selection process and for the repair outcome. DSB: double-strand break. NPF: (RecA/Rad51) nucleoprotein filament.

Figure 2: Spatial and molecular gatekeepers of the homology search process. (A) Multiple overlaid mechanisms at the NPF and JM levels result in biasing homology search away from dispersed repeats and toward the sister chromatid, the most conservative repair template. (B) Putative mechanisms by which cohesin regulates homology search during HR.

Figure 3: The D-loop, a pivotal JM intermediate at risk of misprocessing. (A) D-loop junctions are substrates for three main types of proteins (nucleases, helicases and polymerases) conserved in eukaryotes. (B) Mutagenic HR outcome caused by unrestricted D-loop extension. (C) Mutagenic or non-conservative repair outcomes caused by endonucleolytic processing of various types of JMs.

Figure 4: DNA circles: product of, and substrate for HR-mediated amplification. (A) Mechanisms of circle formation upon dHJ-mediated crossover or D-loop cleavage (MIR not shown, see [67]). The repeat does not need to be present at the DSB in the D-loop cleavage model, greatly expanding the number of lesions conducive to this type of rearrangements. Furthermore, D-loop cleavage forms a circle without fixing the initial damage, providing opportunities for greater amplification of the inter-repeat region than the crossover model. (B) DNA circles provide an expanded sequence space for both DNA damage and ectopic repair, potentially leading to massive amplification from a single damage.

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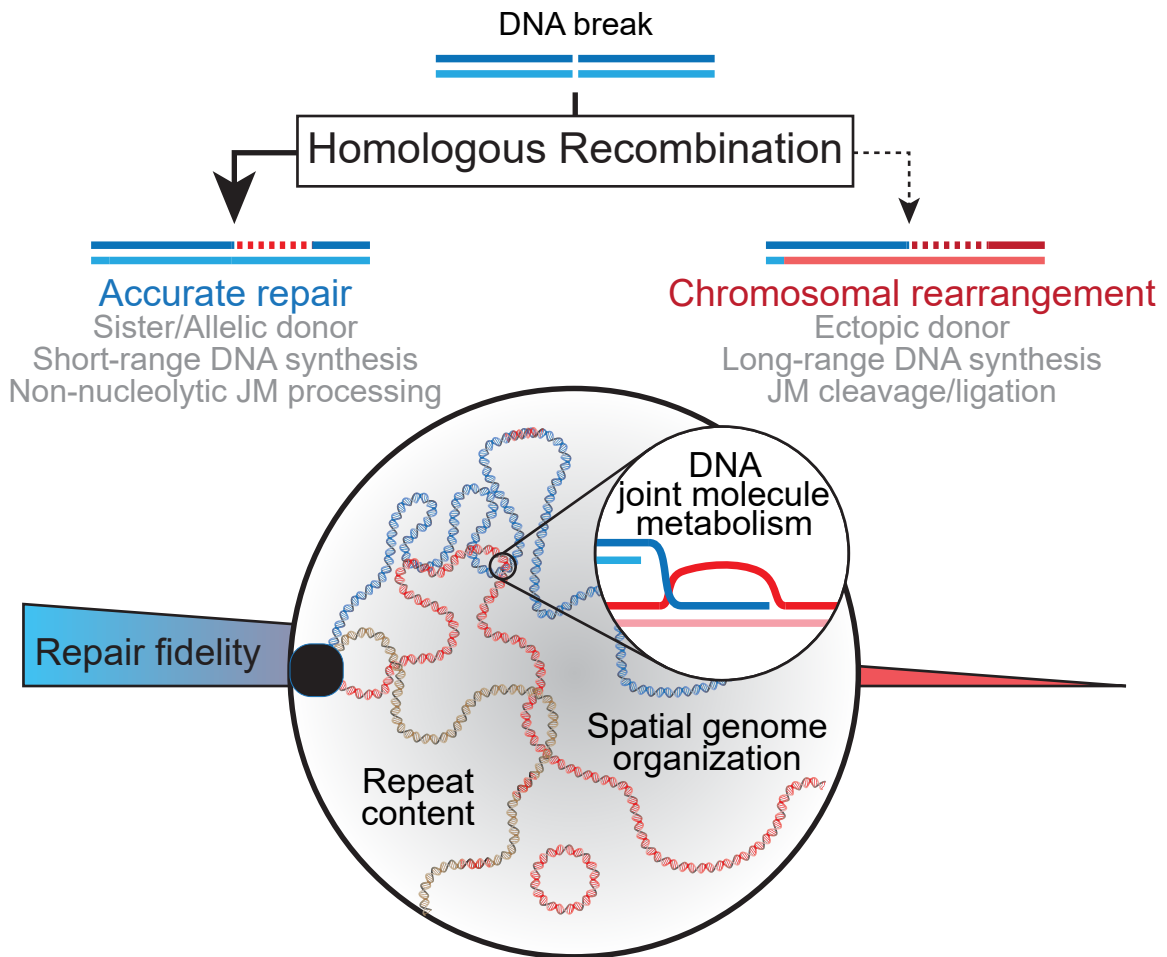
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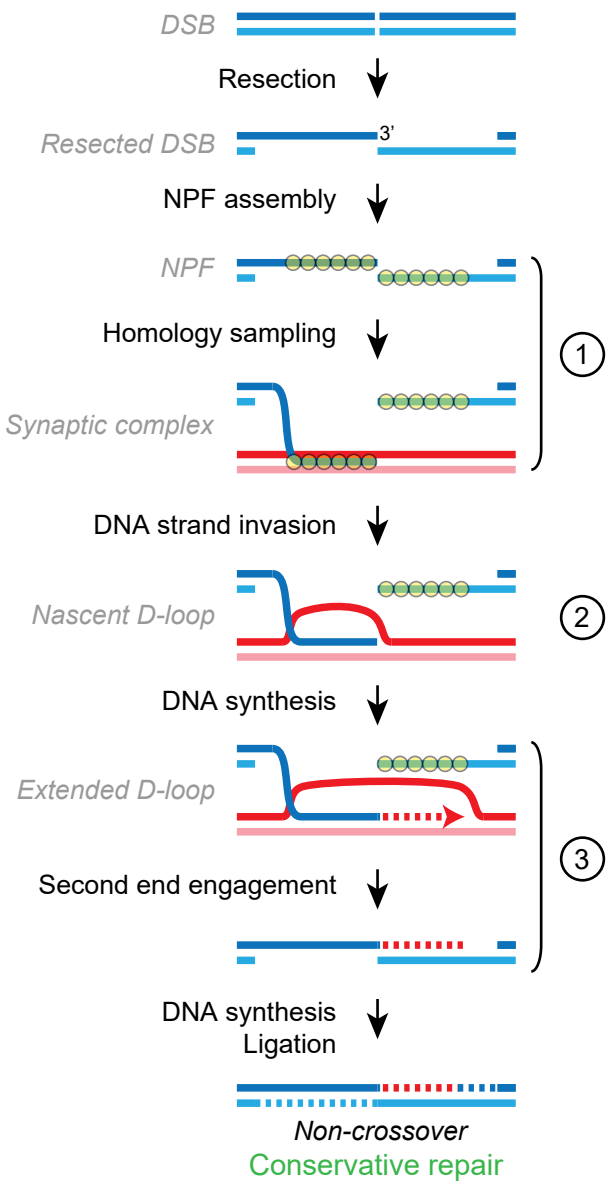
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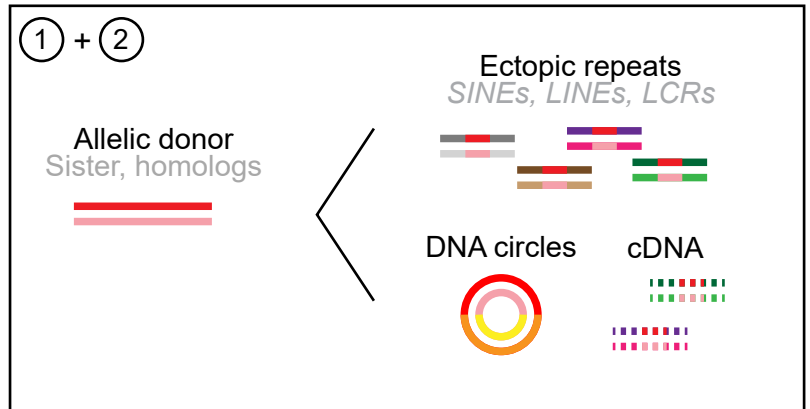
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Genetic challenge: excess repeats



Structural vulnerabilities: JM features

