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Review

So similar yet so different: The two ends of a double strand break

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ABSTRACT

Homologous recombination (HR) is essential for ensuring proper segregation of chromosomes in the first round of meiotic division. HR is also crucial for preserving genomic integrity of somatic cells due to its ability to rescue collapsed replication forks and eliminate deleterious DNA lesions, such as double-strand breaks (DSBs), inter-strand crosslinks, and single-strand DNA gaps. Here, we review the early steps of HR (homology search and strand exchange), focusing on the roles of the two ends of a DSB. A detailed overview of the basic HR machinery and its mechanism for template selection and capture of duplex DNA *via* strand exchange is provided. Roles of proteins involved in these steps are discussed in both mitotic and meiotic HR. Central to this review is the hypothesis, which suggests that in meiosis, HR begins with a symmetrical DSB, but the symmetry is quickly lost with the two ends assuming different roles; it argues that this disparity of the two ends is essential for regulation of HR in meiosis and successful production of haploid gametes. We also propose a possible evolutionary reason for the asymmetry of the ends in HR.

1. Conceptual overview

Homologous recombination (HR) is the process of repairing DNA lesions using homologous DNA sequences. It is a prominent feature of meiosis that allows homologous chromosomes to pair up and faithfully segregate into haploid gametes. HR in meiosis is tightly controlled since errors in chromosome segregation can lead to embryonic lethality and aneuploid progeny [1–3]. HR occurs in all domains of life (eukaryotes, prokaryotes and archaea) and involves proteins that share strong homology [4], therefore, it must have appeared in evolution before meiosis, which is only present in eukaryotes [5]. Presumably, the initial role of homologous recombination was to repair DSBs (double-strand breaks) that appear in DNA during mitotic cell cycle, either due to mutagens or during various DNA-related processes such as DNA replication [6,7].

Similar to how meiosis uses modified features of mitotic cell cycle [5], it also uses modified features of mitotic homologous recombination, so the overall pathways in mitosis and meiosis are similar (Fig. 1; roles of proteins will be discussed further). The dHJ pathway is capable of generating crossovers, while the synthesis-dependent strand annealing (SDSA) pathway always generates non-crossovers; both pathways involve both ends of the DSB and employ the homologous sequence (either on the sister chromatid or on the homologous chromosome, depending on the circumstances, which will be discussed below) as the template for DNA synthesis. Note that pathways that do not engage second DNA molecule and therefore lead to deletions, such as non-homologous end joining (NHEJ), microhomology-mediated end joining (MMEJ) and single-strand annealing (SSA) are not discussed here and therefore not shown. Break-induced replication (BIR), which uses only one end of the DSB is also not shown as it is discussed further (Fig. 2).

There are three fundamental differences, however, between homologous recombination in meiosis *vs* mitosis. The first fundamental difference is the source of DSBs: in mitosis, DSBs are an accident, while in meiosis, DSBs are intentional and form upon the action of a meiosis-specific protein Spo11 [8]. The second fundamental difference is partner choice (which molecule is used for D-loop formation): in mitosis, the preferred partner is sister chromatid, while in meiosis, it is the homologous chromosome. The third fundamental difference is abundance of products, namely CO (crossovers) and NCO (noncrossovers): the CO/NCO ratio is significantly greater in mitosis than in meiosis [9].

In this review, we entertain a hypothesis that explains how HR is regulated in meiosis, which is based on the notion that even though the two ends of the DSB are initially “equal,” their roles in the process of HR are very different: initially only one end engages with the homologous sequence (either sister chromatid or homologous chromosome). This end asymmetry hypothesis is mainly based on two groups of data [10,11]. While we believe that this hypothesis can explain a lot of puzzling observations about HR in meiosis, it has not been proven. Further experiments must be done to test if the two ends do, in fact,

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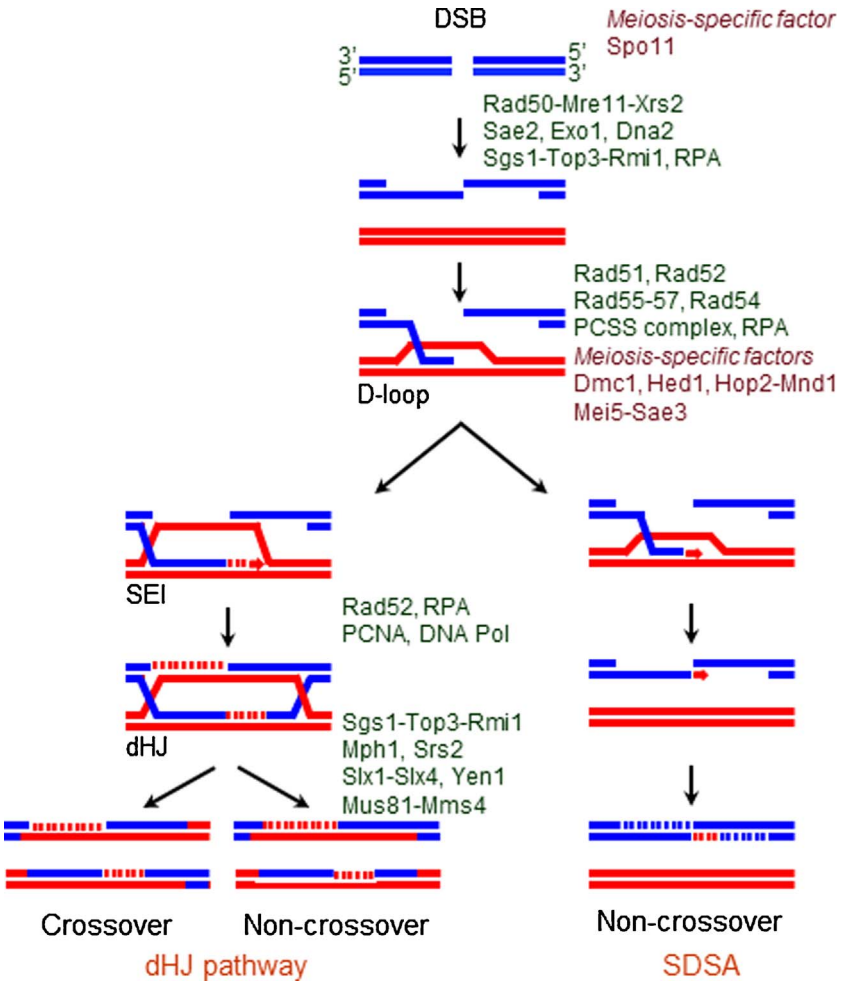

Fig. 1. Pathways of DSB repair by homologous recombination in mitosis and meiosis. ssDNA generated by multiple nucleases progresses for homology search and strand exchange catalyzed by Rad51 (and/or Dmc1) and various recombination mediators. After the D-loop is formed, the pathway bifurcates into the dHJ branch (capable of producing crossovers) and SDSA branch (which produces non-crossovers). DSB, double-strand break; D-loop, displacement loop; SEI, single-end invasion; dHJ, double-Holliday junction; SDSA, synthesis-dependent strand annealing. Dotted lines indicate newly synthesized DNA. Pathways that don't engage the second DNA molecule and therefore generate deletions around the DSB, such as non-homologous end joining (NHEJ), microhomology-mediated end joining (MMEJ) and single-strand annealing (SSA) are not shown. BIR is shown in Fig. 2.

have different roles in HR in meiosis, and this difference is essential for HR regulation.

The end asymmetry hypothesis proposes that while the "first end" searches for the homologous sequence and forms a D-loop, the "second end" passively waits. Most notably, the dHJ pathway begins with a symmetrical DSB (with both DNA strands broken, and in meiosis, with Spo11 attached to both sides of the break) and makes a double Holliday junction, which is also an inherently symmetrical structure. However, on the way from DSB to the double Holliday junction, the symmetry is transiently lost, when the "first end" searches for the homologous sequence and forms a displacement loop (D-loop), while the second end passively waits.

While it is not known what factors designate the "first" and "second" ends, perhaps there is an evolutionary reason for why the two ends might be unequal in the first place. As mentioned above, meiosis tends to modify features that already existed in mitosis as opposed to using completely new mechanisms. One of the most important instances when a DSB must be repaired in mitotic cycle is when it forms during DNA replication. Successful completion of DNA replication is required for cell division, yet replication fork often pauses and breaks [12]. This can lead to the formation of one-ended DSBs. A nick in the template strand leads to replication forks collapse and formation of a one-ended DSB (Fig. 2A). Replication fork stalling at an obstacle, such as a secondary structure in the template strand, can lead to fork regression; subsequent resolution of the Holliday junction can lead to the formation of a one-ended DSB (Fig. 2B) [13]. One-ended DSBs by definition must be repaired using only one end, since the other end is non-existent. Recombination-dependent replication (RDR) and BIR are the mechanisms responsible for repairing of one-ended DSBs in pro- and eukaryotic cells [14].

BIR is a pathway of HR in eukaryotes that repairs one-ended DSBs. It has been studied extensively in yeast [15] and has recently been implicated in restart of replication forks in mammalian cells [16]. Replication restart by BIR involves resection of the one-ended DSB that produces a 3′-overhang, invasion of the 3′-overhang into the sister chromatid to form a D-loop and subsequent re-establishment of the fork (Fig. 2C). While some models argue that a conventional replication fork is established, where leading and lagging strands are synthesized synchronously, recent findings in yeast support the idea that leading strand synthesis and simultaneous migration of the D-loop ("migrating bubble") lead to displacement of the newly synthesized leading strand, which is then used as a template for lagging strand synthesis (Fig. 2C, right panel). The result is *conservative* DNA replication, which explains high mutagenicity of BIR in eukaryotes [17]. In eukaryotes, if a replication fork has collapsed, eventually the neighboring fork will arrive, limiting the extent of BIR during replication fork restart [18].

In *E. coli*, PriA-directed fork restart rescues collapsed replication forks *via* RDR [19]. Broken replication forks are recovered *via* resection of the one-ended DSB that produces a 3′-overhang (mediated by RecBCD), invasion of the 3′-overhang into sister chromatid to form a D-loop (mediated by RecA) and subsequent re-establishment of a normal fork, mediated by PriA (Fig. 2C, left panel) [19]. Preferential orientation of chi sites (octonucleotide sequences that regulate activity of recombination proteins RecBCD that carry out end resection) relative to the direction of replication in the *E. coli* genome suggests that the primary role of RecBCD HR system in bacteria is to restart broken replication forks rather than repair DSBs that appear for other reasons [20]. While in eukaryotes, if a collapsed replication fork fails to restart,