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RESEARCH HIGHLIGHT

The double life of Holliday junctions

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Homologous recombination (HR) is a process common to all organisms and involves the exchange of similar or identical DNA sequences. Such exchanges contribute to ensure genetic diversity in meiotic cells or to preserve genetic information during repair of damaged DNA in mitotic cells. The substrate utilized for DNA exchange in the two cell types is crucial to achieve these different aims.

Each round of DNA replication generates identical copies of chromosomes called sister chromatids. Diploid cells, such as mammals, have two homologue chromosomes, which are not identical, since they were inherited from each parent. In meiosis, HR preferentially involves homologs to produce new combination of DNA sequences in gametes. Conversely, sister chromatids are preferred over the homologs as substrates for mitotic recombination, which minimizes genetic alterations, such as loss of heterozigosity (LOH). LOH events increase in cancer and aging cells thus raising the interest in the dissection of the recombination mechanisms and in their regulatory circuits.

HR in meiosis involves the formation and resolution of four-way DNA structures, called Holliday junctions (HJs) (Figure 1A). These cruciform DNA structures were proposed as intermediates of recombination by Robin Holliday in 1964 to explain the phenomena of gene conversion, that is, the nonreciprocal transfer of information from one homolog to another [1]. In a single recombination event, gene conversion is frequently associated with crossovers (a reciprocal exchange of flanking markers). Both events can originate from the asymmetric cleavage of single HJs [1, 2] or even double HJs [3]. Double HJs and single HJs have been visualized in the yeasts *S. cerevisiae* and *S. pombe* during meiotic DSB repair [2, 4]. Yet, a key question is whether HJs are also the DNA intermediates used in DNA break repair during the mitotic cell cycle. In fact, mitotic crossovers are infrequent and there are alternative mechanisms to repair DNA lesions that do not require HJs formation. Alternatively, if HJs arise also during the mitotic cell cycle, then it is expected that they would be processed by symmetric cleavage or by "dissolution" to prevent crossover exchanges (Figure 1A). *In vitro* observations suggest that the Bloom RecQ helicase (Sgs1 in *S. cerevisiae*), together with the DNA topoisomerase (Top) III catalyze the "dissolution" of double HJs to non-crossovers [5]. The finding that DSB repair is accomplished with high crossover events in *sgs1* mutants both in mitosis and in meiosis [6, 7] supports the idea that this RecQ-dependent mechanism plays a crucial role in reducing DNA exchanges.

In a recent report, Bzymek *et al*. [8] provide the first physical evidence that cruciform structures resembling double HJs arise during mitotic DSB repair in eukaryotic cells. A single DSB was induced at a specific location into one of the homologs of a diploid *S. cerevisiae* strain. The repair intermediates were then analyzed by the two-dimensional gel electrophoresis (2D gel) technique, which allows the physical visualization of different types of branched DNA molecules. The DNA region analyzed also contains a restriction fragment length polymorphism so that the joint molecules that form between the two allelic "mom" and "dad" fragments have a different size compared to the junctions involving inter-sister species (just mom or dad molecules). Thus, in the 2D gel analysis, the three types of cruciforms can be separated and visualized with appropriate probes. The authors found that both inter-sister and inter-homologs DNA species accumulated during DSB repair in mitosis. Since the inter-homologue junctions were formed by fragments with a diverse size, Bzymek *et al*. could analyze the strand composition of the cruciform structures. They found that these junctions contained even, not odd, numbers of HJs, thus more likely two HJs. Notably, the region analyzed by Bzymek *et al*. is a meiotic hot spot of recombination, and using the same 2D gel approach, it was shown that double HJs accumulated during meiotic DNA break repair at the same region [4]. Thus, the double HJs are intermediates of DSB repair both in mitosis and meiosis. There are, however, important

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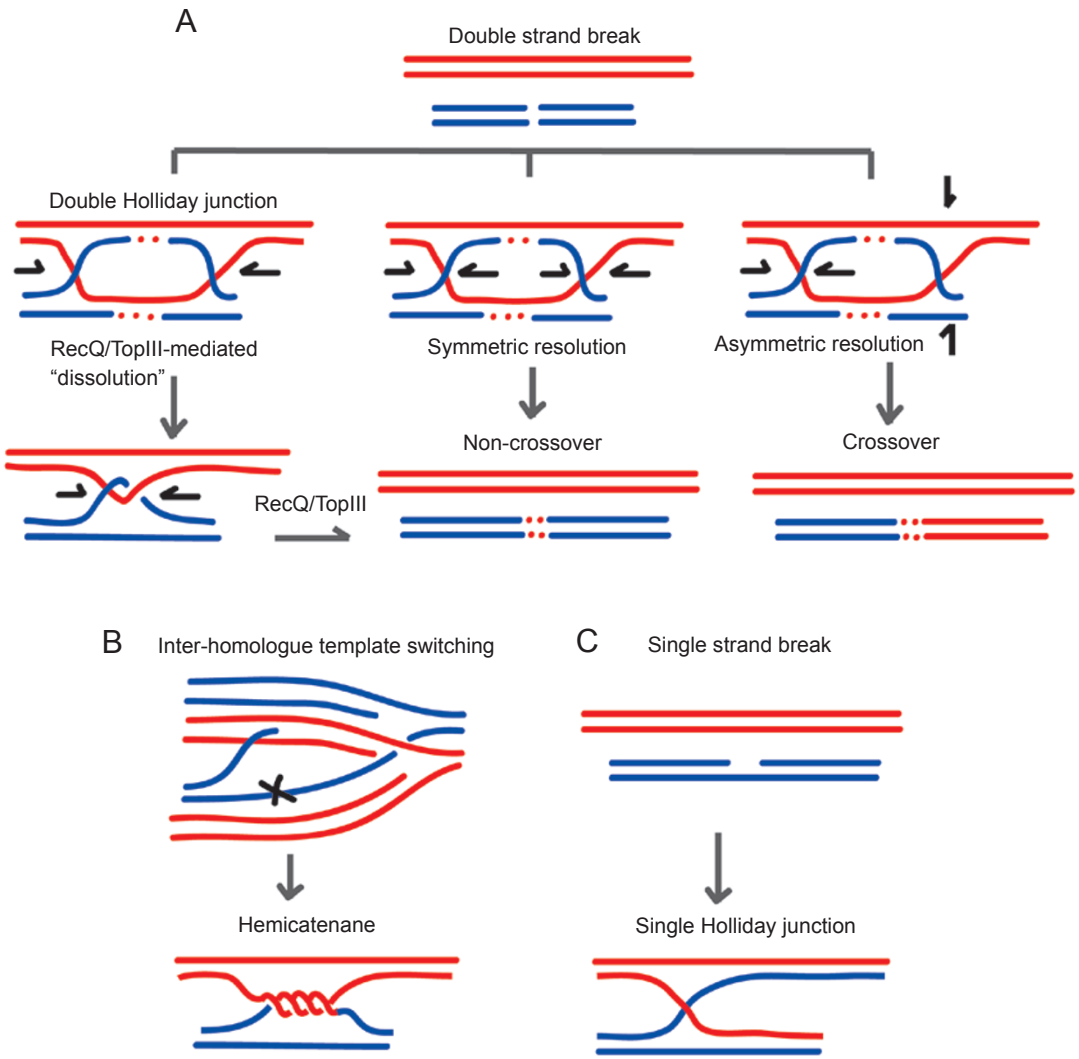

Figure 1 Inter-homologue mitotic junctions in recombinational repair. Bzymek *et al*. showed that double HJs are intermediates in mitotic DSB repair in diploid cells. Double HJs can be resolved by cutting in two diverse orientations producing non-crossovers and crossovers or they can be dissolved by RecQ/TopIII complex yielding exclusively non-crossovers **(A)**. Other types of inter-homologue joint molecules, such as hemicatenanes **(B)** and single HJs **(C)**, might originate, respectively, by template switching (black X symbol indicates damaged DNA) or during repair of single strand breaks.

differences in the behavior of inter-homologue junctions in the two contexts. First of all the accumulation of mitotic junctions is delayed compared to that of inter-sister structures, while both cruciform DNA species form with identical timing in meiotic recombination. This finding might suggest that, upon DNA breakage, homology search between sister chromatids is faster than homologs in mitosis, likely because sisters are connected by cohesion. In fact, while compelling data indicate that meiosis specific proteinaceous structures, called the synaptonemal complex, connects the homologs facilitating DNA exchange, there are contradictory observations that homologs pair in mitotic cell cycle [9]. Bzymek *et al*. also found that the levels of mitotic inter-homologue junctions are remarkably lower compared to those of meiotic junctions, although the rates of DSB formation were comparable in the two contexts. The inactivation of Sgs1 only slightly increases the peak level of mitotic inter-homologue joint molecules, although their resolution appears defective. These findings imply that double HJs are rare intermediates in mitotic DSB repair and most breaks are likely repaired by non-HJ pathway(s). When HJs form, they are dissolved by Sgs1/TopIII complex to prevent crossover outcomes. Yet, in the absence of Sgs1, the fraction of DSBs that are repaired with crossovers rises, but the pathway(s) responsible for the resolution of HJs remain(s) uncharacterized.

Other important questions remain

open. The nature of inter-sister chromatids junctions could not be defined in this study: are these DNA species double HJs or rather different cruciform molecules? Further, genetic observations seem to suggest that DSBs form rarely in mitosis and that DNA lesions accumulating during replication are probably one source for recombination events [10, 11]. Replication-dependent sister chromatid junctions were indeed observed in diverse organisms (see for example [12-14]) and recently interhomologue junctions were found to accumulate at damaged replication forks in *sgs1* mutants [15]. In some cases, these cruciform molecules resemble hemicatenanes, rather than HJs, and might originate during the bypass of DNA lesions through template switching. This recombination by-replication process was proposed to involve sister chromatids, but in theory, could take place also between homologs. Although double HJs are intermediates in DSB repair, it might be still possible that other types of junctions, including single HJs or hemicatenanes, are intermediates of recombinational repair in DNA replication (Figure 1B and 1C). Thus, the game may not be over yet.

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