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Holliday junction resolution: Regulation in space and time

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ABSTRACT

Holliday junctions (HJs) can be formed between sister chromatids or homologous chromosomes during the recombinational repair of DNA lesions. A variety of pathways act upon HJs to remove them from DNA, in events that are critical for appropriate chromosome segregation. Despite the identification and characterization of multiple enzymes involved in HJ processing, the cellular mechanisms that regulate and implement pathway usage have only just started to be delineated. A conserved network of core cell-cycle kinases and phosphatases modulate HJ metabolism by exerting spatial and temporal control over the activities of two structure-selective nucleases: yeast Mus81-Mms4 (human MUS81-EME1) and Yen1 (human GEN1). These regulatory cycles operate to establish the sequential activation of HJ processing enzymes, implementing a hierarchy in pathway usage that ensure the elimination of chromosomal interactions which would otherwise interfere with chromosome segregation. Mus81-Mms4/EME1 and Yen1/GEN1 emerge to define a special class of enzymes, evolved to satisfy the cellular need of safeguarding the completion of DNA repair when on the verge of chromosome segregation.

1. Introduction

Homologous recombination (HR) promotes the establishment of specialized chromosomal interactions that facilitate DNA repair and genome stability during mitotic proliferation and enable genome haploidization during meiosis. Therefore, the controlled engagement and disengagement of all such chromosomal connections is essential throughout the entire eukaryotic life cycle.

In mitotically proliferating cells, the repair of DNA lesions *via* HR involves pairing and strand-exchange between a damaged chromosome and its sister chromatid (and on rare occasions the homolog) leading to the formation of DNA joint molecules (JMs). JMs are usually disengaged at an early stage by anti-recombinogenic helicases such as Srs2, Mph1 or RTEL1 [1–4]. However, a small proportion mature to form repair intermediates in which the two recombining DNAs are covalently-linked by a four-way junction, known as a Holliday junction (HJ) [5–9]. On those rare occasions when recombination occurs between homologous chromosomes, rather than sister chromatids, it is important that the HJs are processed to give rise to non-crossover (NCO) recombinants, in order to avoid inter-homolog exchanges that can contribute to the onset of cancer by driving loss of heterozygosity of mutated tumor suppressor genes. This situation contrasts with that occurring in germ-line cells undergoing meiosis. Meiotic crossovers (COs) are required for the establishment of cohesin-mediated inter-homolog interactions, which are in most organisms indispensible for the bipolar orientation and segregation of recombined chromosomes of maternal and paternal origin [10,11].

In all cells, HJs provide a physical link between sister chromatids or homologous chromosomes, and a single unprocessed HJ could result in chromosome non-disjunction and aneuploidy, a feature commonly associated with cancer. Hence, despite being generated to help facilitate efficient DNA repair, HJs are perceived as toxic DNA structures as they could interfere with normal chromosome segregation. In the past three years, significant advances have been made that reshape our understanding of how and when these HR intermediates are processed. This review will focus on these new developments and on how they contribute to an updated model of HJ metabolism.

2. Holliday junction processing enzymes: a brief overview

Enzymes that specifically resolve HJs have been identified from a variety of organisms including bacteriophage, bacteria, archaea, yeast and humans. The prototypic Holliday junction resolvase, *E. coli* RuvC protein, interacts specifically with HJs and promotes

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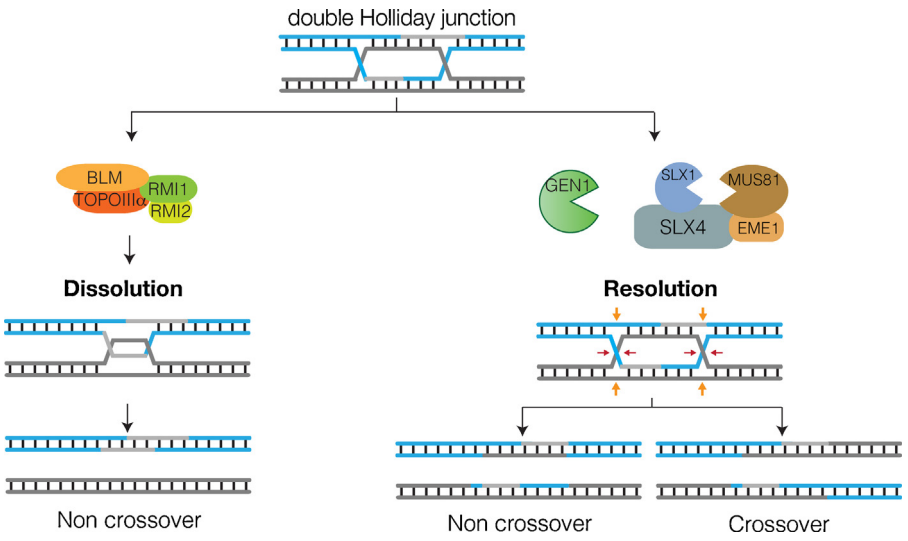

Fig. 1. DNA centric view showing three distinct pathways of Holliday junction processing. The BTR complex disengages dHJs, using the mechanism of "dissolution", to generate NCO recombinants. MUS81-EME1 and SLX1-SLX4 interact to form the SLX-MUS complex which resolves both single and double HJs by endonucleolytic cleavage to generate COs and NCOs. GEN1 provides a separate pathway of HJ resolution.

their cleavage by the introduction of symmetrically related nicks in strands located across the junction [12]. The resulting nicked duplex products can be readily ligated to restore the integrity of the DNA [13]. Although there are no sequence or structural homologies at the protein level, *S. cerevisiae* Yen1, and its human ortholog GEN1, carry out similar biochemical reactions to the bacterial protein [14,15]. However, despite the identification of Yen1/GEN1 as a HJ resolvase, the concept that a single and universal mechanism exists to process HJs has been challenged, leading to the discovery and characterization of several more enzymes capable of HJ processing. Firstly, budding yeast Mus81-Mms4 (human MUS81-EME1), an XPF-family heterodimeric endonuclease, was shown to process a broad range of branched DNA structures, including HJs [16,17]. However, its ability to cut intact HJs was found to be very limited in comparison with other structures, suggesting that a HJ precursor (such as a nicked HJ) might be its preferred DNA substrate [18–20]. To complicate the issue, a second heterodimeric structure-selective endonuclease, yeast Slx1-Slx4 (and its human ortholog SLX1-SLX4), was identified and shown to be capable of severing HJs [21–25]. In contrast to canonical HJ resolvases, however, SLX1-SLX4 is a promiscuous nuclease that nicks a wide variety of DNA secondary structures, and HJ cleavage results in the formation of products that can be only poorly ligated [23,26]. Yen1/GEN1, Mus81-Mms4/EME1 and Slx1-Slx4 all share the common property that HJ resolution gives rise to a mixture of COs and NCOs, depending on the orientation of cleavage (Fig. 1). It therefore seemed unlikely that these enzymes would be involved in the resolution of HJs in eukaryotic cells where non-crossover formation is favored.

Meanwhile, two remarkably different mechanisms of HJ processing were uncovered. The BLM helicase (Sgs1 in yeast), which is mutated in the cancer predisposition disorder Bloom's Syndrome (BS), is a component of the BLM-TopoIIIα-RMI1-RMI2 complex (BTR in humans, STR in yeast), which was shown to migrate and decatenate double Holliday junctions (dHJs) [27–29]. This reaction, which results exclusively in the formation of NCO recombinants, and is important in mitotic cells, is defined as HJ 'dissolution' rather than 'resolution' (Fig. 1). A second novel pathway of resolution was identified in meiotic cells, and this involves an as yet undefined biochemical mechanism that requires Mlh1, Mlh3 and Exo1 [30]. The primary products of this pathway are COs.

The diversity of enzymes and biochemical mechanisms currently implicated in HJ processing highlight the importance placed on ensuring the efficient disengagement of such structures. Furthermore, the possibility of generating specific recombination outcomes (CO *vs* NCO), depending on the pathway employed for HJ processing (*e.g.* resolution *vs* dissolution), begins to explain how mitotic and meiotic cells might process what appear to be related structures and yet generate different recombination outcomes. But in the same light, these studies led to a fundamental question – if cells contain such a range of enzymes that process HJs, how do they implement pathway usage?

3. Regulation of HJ resolvases: from yeast to man

Yeast cells exhibit a kinetic and genetic separation of CO and NCO recombinant formation during both mitotic and meiotic DSB repair. During mitotic proliferation, the majority of dHJs are processed at early stages of the cell cycle by STR-mediated dissolution, to generate NCOs. In *sgs1* mutants, however, JMs persist to a later stage of the cell cycle when they are processed by Mus81-Mms4 or Yen1, to generate a mixture of COs and NCOs [3,31–34] (Fig. 2). These observations led to the concept that the nucleases provide back-up pathways to STR-mediated HJ dissolution. In meiotic cells, most NCO recombinants arise prior to the formation of dHJs, whereas the COs arise at a later time in reactions that are dependent upon Ndt80-mediated transcription of the Polo kinase Cdc5 [35,36].

In human cells, the temporal separation of CO and NCO formation has yet to be demonstrated. However, as observed in yeast, BS cells that lack BLM display an increased frequency of CO formation, visualized as sister chromatid exchanges [37]. In these cells, CO formation is largely dependent on the actions of MUS81-EME1, SLX1-SLX4 and GEN1 [26,38]. These observations indicate that despite having a variety of enzymes capable of HJ resolution, pathway usage in mitotic cells is biased to favor STR/BTR-mediated dHJ dissolution and NCO formation. While it is possible that cells utilize more than one strategy to direct pathway usage and control the outcome of recombination, recent advances have uncovered a number of mechanisms by which cells control the actions of these structure-selective nucleases. Moreover, the realization that these enzymes are regulated now provides the key to understand several surprising differences between the genetics and biochemistry of HJ resolution.

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