Available online at www.sciencedirect.com

ScienceDirect

Current Opinion in Cell Biology

Cell cycle control of DNA joint molecule resolution

Philipp Wild and Joao Matos

The establishment of stable interactions between chromosomes underpins vital cellular processes such as recombinational DNA repair and bipolar chromosome segregation. On the other hand, timely disengagement of persistent connections is necessary to assure efficient partitioning of the replicated genome prior to cell division. Whereas great progress has been made in defining how cohesin-mediated chromosomal interactions are disengaged as cells prepare to undergo chromosome segregation, little is known about the metabolism of DNA joint molecules (JMs), generated during the repair of chromosomal lesions. Recent work on Mus81 and Yen1/GEN1, two conserved structure-selective endonucleases, revealed unforeseen links between JM-processing and cell cycle progression. Cell cycle kinases and phosphatases control Mus81 and Yen1/GEN1 to restrain deleterious JM-processing during S-phase, while safeguarding chromosome segregation during mitosis.

Address
Institute of Biochemistry, HPM D6.5 – ETH Zürich, Otto-Stern-Weg 3, 8093 Zürich, Switzerland

Corresponding author: Matos, Joao (joao.matos@bc.biol.ethz.ch)

Current Opinion in Cell Biology 2016, **40**:74–80

This review comes from a themed issue on **Cell nucleus**

Edited by **Ulrike Kutay** and **Orna Cohen-Fix**

http://dx.doi.org/10.1016/j.ceb.2016.02.018

Introduction

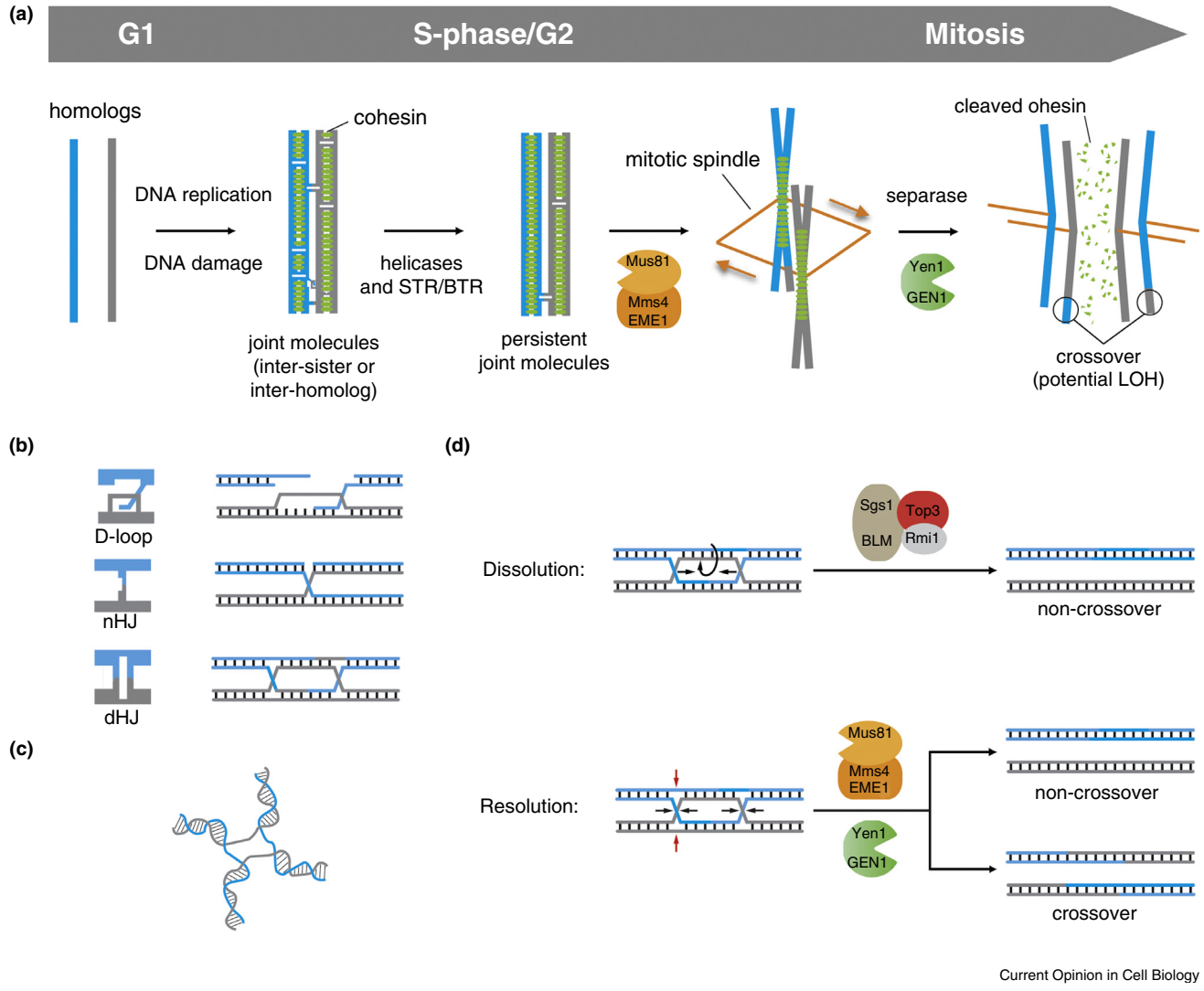
As the genome is recurrently exposed to endogenous and exogenous stresses, accurate inheritance of genetic information requires continuous repair of DNA lesions [1,2]. Homologous recombination (HR), the error-free DNA double-strand break (DSB) repair pathway, utilizes the intact sister chromatid (or on rare instances the homologous chromosome) as a template to synthesize the missing DNA sequence and re-join the broken ends [3,4]. This process, however, entails the formation of stable DNA connections between chromosomal arms, which need to be disengaged prior to cell division. Therefore, while contributing to genome stability, recombinational DNA repair promotes formation of dangerous intermediates, which require especial attention from cells.

To separate DNA joint molecules (JMs) that form during HR, proliferating cells (i.e. cells that give rise to progeny through mitotic division) are endowed with various JM-processing enzymes. Anti-recombinogenic helicases such as Mph1/FANCM, Srs2 and RTEL1 disengage the majority of early JMs resulting in the repair of DSBs without the reciprocal exchange of flanking DNA sequences (non-crossover) [5–7] (Fig. 1A and B). If left unprocessed, early JM intermediates mature into four-way junctions — also known as Holliday junctions (HJs) — in which sister chromatids (or homologous chromosomes) become covalently linked [8,9]. Because of their stability in connecting the two DNA duplexes, HJs are arguably the most dangerous of all recombination intermediates (Fig. 1A–C). Interestingly, HR-mediated DSB repair is not the only source of HJs. Four-way DNA junctions that resemble canonical HJs can also arise, for instance, upon replication stress and replication fork reversal [10].

To ensure robust processing of late JMs, eukaryotic cells rely on at least three genetically and biochemically distinct pathways: the STR/BTR complex (yeast Sgs1-Top3-Rmi1, human BLM-TOPOIIIα-RMI1-RMI2), the heterodimeric structure-selective endonuclease Mus81 (Mus81-Mms4 in budding yeast, Mus81-Eme1 in fission yeast, MUS81-EME1 and MUS81-EME2 in human cells) and the HJ resolvase Yen1/GEN1 [11–15]. STR/BTR migrates and decatenates double Holliday junctions (dHJs) by a mechanism termed 'dissolution'. Mus81 and Yen1/GEN1 cut individual HJs through nucleolytic 'resolution' (Fig. 1D) [16]. It is important to point out that the functions of Mus81 and Yen1/GEN1 are not limited to HJ processing. Both nucleases are thought to cleave, for example, HJ precursors, such as nicked HJs [12,17–19]. Furthermore, at least in mammalian cells, HJ incision by MUS81 requires pre-nicking of the opposite strand by the structure-selective endonuclease SLX1-SLX4 [20–25].

Besides covalently connecting recombining DNA duplexes, HJs entail a second feature that cannot be underestimated by cells: their processing can lead to the incidence of reciprocal genetic exchanges (crossovers). Hence, if the template used for repair is the homologous chromosome, instead of the sister chromatid, loss of heterozygosity (LOH) can ensue (Fig. 1A). To suppress crossovers (COs), and the potential for LOH, proliferating cells dissolve most dHJs using the STR/BTR pathway, which leads to formation of non-crossover (NCO) recombinants, exclusively [5,11]. Mus81 and Yen1/GEN1, which resolve HJs to generate both COs and NCOs, also contribute to JM processing. However,

Fig. 1



Processing of DNA joint molecules (JMs) during the cell cycle. Repair of unscheduled chromosomal lesions by homologous recombination leads to formation of DNA-based chromosomal connections between sister chromatids and, occasionally, homologous chromosomes. **(a)** During S-phase and G2, anti-recombinogenic helicases disengage the majority of early JMs by destabilizing displacement loop (D-loop) structures. Structures that mature to form double Holliday junctions (dHJs) are 'dissolved' by the STR/BTR complex, to generate non-crossover (NCO) recombinants. Up-regulation of Mus81-Mms4/EME1 and Yen1/GEN1 nuclease activities during mitosis ensures the 'resolution' of persistent JMs that escape the STR/BTR complex. These include dHJs but also single HJs and nicked HJs. Processing of late JMs by Mus81-Mms4/EME1 and Yen1/GEN1 can lead to formation of crossovers (COs) and drive loss of heterozygosity (LOH). **(b)** Depiction of key DNA JM intermediate structures that link recombining sister chromatids or homologous chromosomes. For easier visualization only inter-homolog JMs (blue-grey) are shown. **(c)** Sketch of a single Holliday junction connecting two DNA duplexes. **(d)** Illustration of the outcome of STR/BTR-dependent 'dissolution' and Mus81-Mms4/EME1- and Yen1/GEN1-dependent 'resolution' pathways. Convergent branch migration of two HJs catalyzed by the Sgs1/BLM helicase and subsequent Top3-dependent dissolution of the hemicatenane results in the formation of NCO recombinants. Conversely, nucleolytic resolution by Mus81-Mms4/EME1 or Yen1/GEN1 yields NCOs and COs with equal probability. Cleavage of the crossing strands (black arrows) prevents formation of COs. Processing of the non-crossing strands (red arrows) promotes formation of COs. The depicted NCO arises from cleavage of the crossing strands in both HJs. Cleavage of the non-crossing strands in one of the HJs (left) and cleavage of the crossing strands in the other HJ gives rise to the portrayed CO. For simplicity, genetic exchange without CO formation is not shown.

both enzymes appear to function as a backup to STR/BTR, or whenever JMs contain single HJs, which require nucleolytic resolution [17,26••,27,28,29]. It is important to mention that HR drives genetic exchange and the creation of new parental alleles in germ cells undergoing meiosis. To this end, cells modify JM processing significantly [30]. For example, during meiosis, the mismatch repair factors Mlh1, Mlh3 and Exo1 act in a fourth pathway of HJ processing,