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# Cell cycle control of DNA joint molecule resolution

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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.

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## Introduction

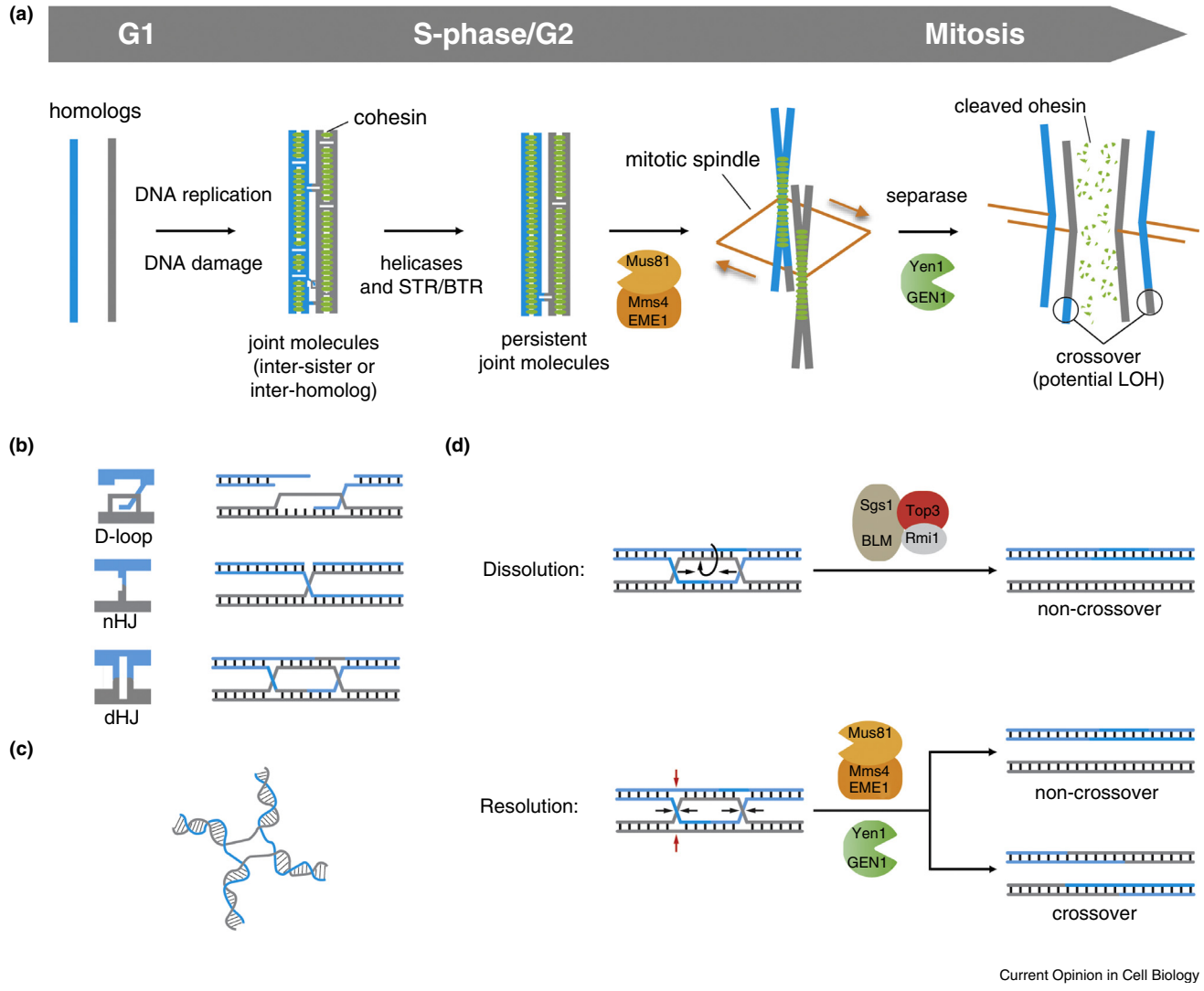
As the genome is recurrently exposed to endogenous and exogenous stresses, accurate inheritance of genetic information requires continuous repair of DNA lesions [1,2]. Homologous recombination (HR), the error-free DNA double-strand break (DSB) repair pathway, utilizes the intact sister chromatid (or 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,

which channels a substantial fraction of JMs to strictly generate COs [31].

So how do proliferating cells configure STR/BTR, Mus81 and Yen1/GEN1 to ensure efficient DNA repair, while minimizing the occurrence of COs? In recent years, accumulating evidence from different organisms revealed that pathway usage is tightly controlled by cell cycle kinases and phosphatases, and is thus coordinated with progression through the cell cycle. This review focuses on how budding yeast cells wire Mus81-Mms4 and Yen1 activation to cell cycle progression, which precludes toxic processing of recombination intermediates during S-phase and safeguards resolution of persistent DNA JMs during mitosis.

## Cell cycle regulation of DNA joint molecule processing

Budding yeast cells exhibit temporal separation in the formation of CO and NCO recombinants upon DSB repair [5]. In proliferating cells, STR dissolves most dHJs at early stages of the cell cycle, to generate NCO recombinants. In *STR* mutants, however, JMs persist until later stages of the cell cycle when they are processed to generate both COs and NCOs [5,26••]. Since Mus81 and Yen1 functionally overlap with STR and can process HJs to generate a mixture of COs and NCOs [28,29,32], a model was put forward in which both nucleases resolve JMs that escape STR-mediated HJ dissolution. These would occasionally include dHJs, but also single HJs and nicked HJs (Fig. 1A and B).

How cells establish such temporal separation in pathway usage has been a subject of intense research. Consequently, the last four years have brought us considerable progress in the delineation of the cellular mechanism used to provide STR the leading role in JM processing. While it remains unclear whether the function of STR is cell cycle-regulated, evidence for stage-specific activation of Mus81 and Yen1/GEN1 has emerged from different organisms, including *S. cerevisiae*, *S. pombe* and human cells in culture [20,33••,34•,35,36•,37•,38••,39••]. As the mechanistic details of Mus81 and Yen1/GEN1 regulation have been reviewed elsewhere [40,41], we will only discuss the key points here.

## Control of Mus81 and Yen1 nucleases by cell cycle kinases and phosphatases

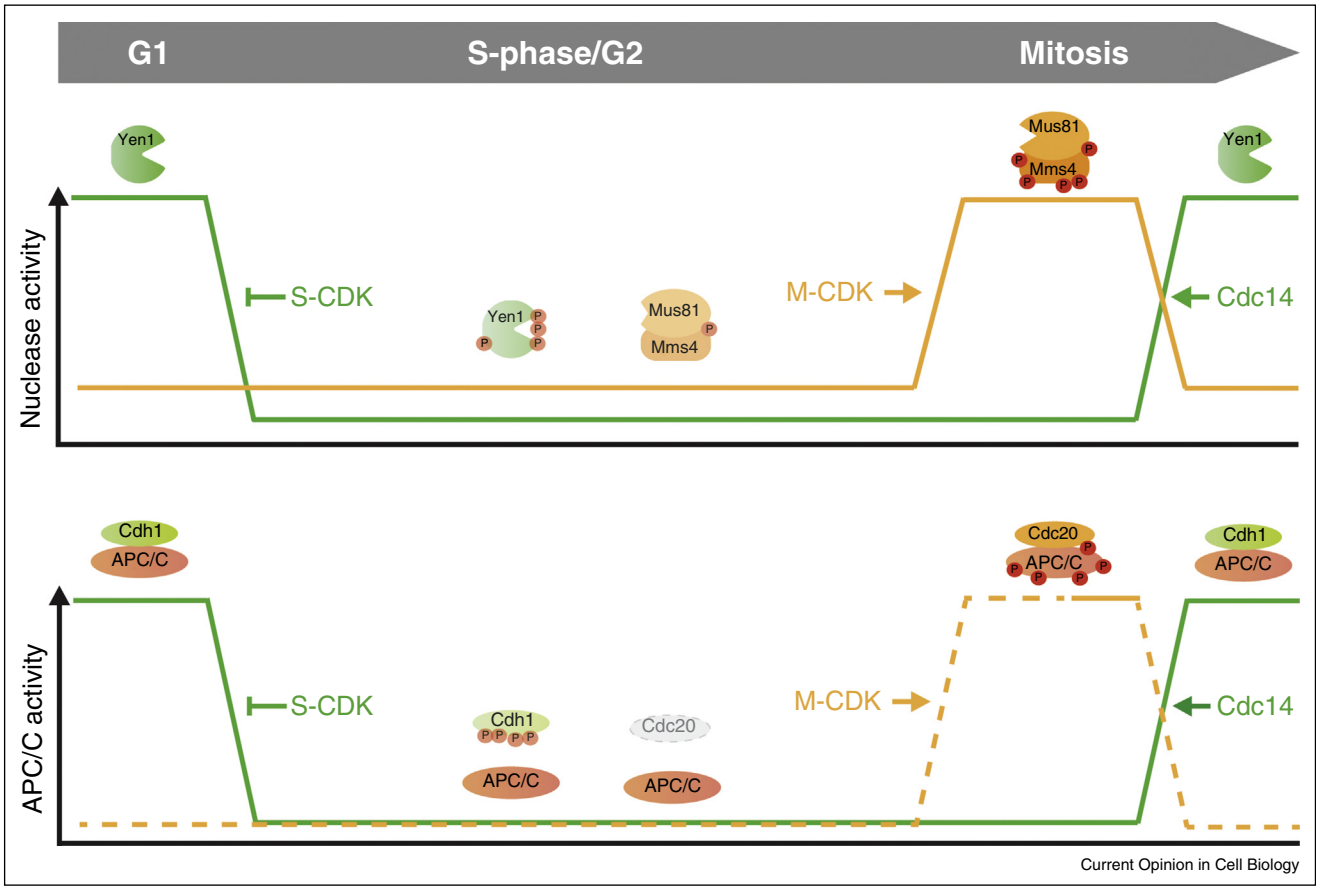
Several studies have provided complementary insight into the elegant modes of regulation of the Mus81 and Yen1 nucleases [34•,37•,39••,42••]. Both enzymes are regulated by cell cycle stage-specific phosphorylation events that impose temporal control on their actions and lead to their sequential activation. Mus81-Mms4 activity is regulated by cell cycle stage-specific phosphorylation events that render the nuclease most active at the $G_2$/M transition [42••] (Fig. 2). This cyclic pattern of activation is reminiscent of the oscillating activities of cyclin-dependent kinases (CDKs), which drive orderly progression through the cell cycle by changing the properties of a plethora of substrates [43]. Indeed, M-phase CDK (Cdc28 in budding yeast) collaborates with a second cell cycle kinase, Polo-like kinase Cdc5, in modifying Mus81-Mms4 at the onset of mitosis [34•,36•,37•,39••,42••,44]. More specifically, multiple phosphorylation events on the non-catalytic subunit Mms4 trigger hyperactivation of the Mus81-Mms4 nuclease complex. Precisely how phosphorylation enhances Mus81 activity and function remains to be elucidated.

Yen1 function is similarly under the control of cell cycle stage-specific modifications, which impinge on its catalytic activity as well as on its subcellular localization (Fig. 2A). However, contrary to Mus81, extensive phosphorylation of Yen1 by S-phase CDKs down-regulates its activity and, alongside, directs Yen1 away from its nuclear substrates, into the cytoplasm. At the onset of anaphase, the cell cycle phosphatase Cdc14 promotes the reverse reaction allowing Yen1 to re-shuttle into the nucleus (Fig. 2A) [33••,38••,42••]. Mechanistically, on the one hand, phosphorylation lowers the affinity of Yen1 to its negatively charged DNA substrate, on the other hand, renders its nuclear localization signal nonfunctional, thus impairing its nuclear accumulation [33••,38••,45].

Collectively, the work described above began to elucidate how cells adjust the usage of JM-processing enzymes. To prioritize STR-mediated dissolution, cells temporally and spatially restrain Mus81-Mms4 and Yen1 functions. This generates a wave of JM dissolution during S-phase that is followed by two consecutive waves of JM resolution during mitosis, reducing enzyme–substrate competition. Such pattern of regulation reduces the occurrence of mitotic COs, limiting the potential for LOH, while also ensuring the resolution of persistent JMs, which could interfere with chromosome segregation and lead to aneuploidy.

As predicted from the model described in Fig. 1, mutations that cause premature Mus81-Mms4 or Yen1 activation induce pathway competition and trigger a significant increase in mitotic COs and LOH [33••,34•,38••,39••]. However, this is not the only phenotype associated with untimely nuclease activation. Interestingly, premature activation of Mus81-Mms4 and Yen1 during S-phase appears to be deleterious to DNA replication and repair [33••,36•,39••]. Thus, it is possible that the tight control of Mus81-Mms4 and Yen1 function also relates to their limited substrate specificity. Since both nucleases are proficient in cleaving model replication forks and other branched DNA intermediates, it is likely that the restraint of their activities during S-phase prevents the toxic processing of vital DNA replication and repair intermediates.

**Fig. 2**




Activity profiles of *S. cerevisiae* Mus81-Mms4 and Yen1 nucleases and APC/C ubiquitin ligase during the cell cycle. The activities of Mus81-Mms4 and Yen1 are regulated by cell cycle stage-specific phosphorylation events. Mus81-Mms4, which is partly active during G1 and S-phase/G2, becomes fully activated at the G2/M transition, upon M-CDK- and Cdc5-mediated phosphorylation. Yen1 is kept in an inactive state by S-CDK-mediated phosphorylation until metaphase. Cyclin degradation and release of Cdc14 phosphatase from the nucleolus promotes rapid Yen1 dephosphorylation and its concomitant activation at anaphase. Yen1 remains active until the G1/S-phase transition of the subsequent cell cycle. Analogous to Mus81-Mms4 and Yen1 regulation, the APC/C complex is positively and negatively regulated by CDK-dependent phosphorylation events. M-phase CDK and Cdc5 promote APC/C activation at the G2/M transition. APC/C activation also requires association to Cdc20, which is negatively regulated by the spindle assembly checkpoint (SAC) (orange dashed line depicts phosphorylated APC/C that is prevented from interacting with Cdc20 by SAC). APC/C$^{Cdc20}$ triggers the metaphase-anaphase transition. Decreasing cyclin levels and Cdc14 activation lead to replacement of Cdc20 by Cdh1, which maintains the activity of APC/C high until S-CDK activity rises at the G1/S transition of the following cell cycle.

## Wiring DNA joint molecule resolution to cell cycle progression: parallels between Mus81/Yen1 and APC/C$^{Cdc20/Cdh1}$ regulation

One remarkable feature of the consecutive but temporally separable waves of JM processing by Mus81-Mms4 and Yen1 is that they peak at metaphase and anaphase, respectively. As such, both nucleases complement each other in resolving JMs at the onset and throughout all stages of mitosis (Fig. 2) [42••]. As pointed out earlier, this is achieved through a common regulator, CDK, which activates Mus81 and inactivates Yen1, creating two interlinked waves of JM resolution that are absent during S-phase while covering all stages of mitosis, as well as G1 (Fig. 2). This strategy used to reduce JM resolution during S-phase while enhancing it at all other stages of the cell cycle may appear complex at a first glance. However, activation of Mus81-Mms4 and Yen1 shares remarkable similarities to the regulation of one of the most prominent CDK substrates required for the segregation of chromosomes, the anaphase-promoting complex/cyclosome (APC/C) [46].

The ubiquitin ligase APC/C is indispensable for the rapid and irreversible metaphase to anaphase transition, as well as for mitotic exit [46,47]. The metaphase-anaphase transition is achieved by ubiquitin-dependent proteasomal

degradation of securin, which inhibits the protease separase that upon cleavage of cohesin ultimately triggers sister chromatid segregation. Mitotic exit relies on cyclin B degradation, which results in M-phase CDK inactivation [48–51]. Since the APC/C plays a crucial role in genome stability by facilitating accurate chromosome segregation, it comes as no surprise that its activity is subject to a complex interplay of regulatory events. In fact, the APC/C functions during mitosis and $G_1$ while it is kept inactive during S-phase (Fig. 2). Analogous to Mus81 regulation, cyclin B-CDK and Cdc5-mediated phosphorylation contribute to APC/C activation during mitosis [52–54]. These events precede association of the APC/C with one of its co-activators, Cdc20, which functions as a substrate receptor and stimulates the catalytic activity of the ligase. $APC/C^{Cdc20}$ subsequently targets securin and cyclin B for degradation, in a process that is tightly controlled by the spindle assembly checkpoint (SAC) [47]. This mode of regulation is strikingly reminiscent of the CDK- and Cdc5-mediated activation of Mus81 nuclease at the $G_2$/M transition, which similarly promotes disjoining of chromosome–chromosome linkages (Fig. 2).

Anaphase and mitotic exit are characterized by a decline in CDK activity that is accompanied by activation of cell cycle phosphatases such as Cdc14. This leads to the replacement of Cdc20 by Cdh1, a co-activator of the APC/C that is negatively regulated by CDK [46]. This switch is mirrored by the inactivation of Mus81 at anaphase and concomitant activation of Yen1 through declining CDK-mediated inhibition, as well as actively promoted dephosphorylation by Cdc14 [33••,38••]. While Yen1 ensures the elimination of any remaining recombination intermediates, $APC/C^{Cdh1}$ targets — among others — Cdc20 itself leading to exit from mitosis. Yen1 and $APC/C^{Cdh1}$ remain active in $G_1$ until entry into S phase, at which stage S-phase CDK inhibits both enzymes. The $APC/C^{Cdh1}$ complex ensures that cyclins are kept at low levels providing the cell with sufficient time before engaging in another cell division cycle; the role of active, nuclear Yen1 in $G_1$ is currently unknown, yet it may be required to resolve repair intermediates deriving from spontaneous DNA lesions.

The overall emerging picture is that Mus81, Yen1, $APC/C^{Cdc20}$ and $APC/C^{Cdh1}$ activities are dampened during S-phase. This allows cells to accumulate securin and establish cohesin-based chromosomal connections and prevents Mus81 and Yen1 from prematurely resolving DNA-based chromosomal connections. Hence, cells appear to utilize a common strategy to coordinate the establishment and disengagement of protein-based and DNA-based chromosomal connections with cell cycle progression and cell division.

## Conclusions and outlook

Cell cycle regulation of Mus81-Mms4 and Yen1 appears to serve the simple need of yeast cells to moderate JM resolution specifically during S-phase. This prevents competition with STR, which processes JMs without the danger of eliciting reciprocal genetic exchanges (COs). At the same time, it also ensures that structures that are refractory to STR, such as single HJs or nicked HJs are processed in time for chromosome segregation and cell division (Fig. 1).

Despite the recent advances, many questions concerning the regulation of JM processing remain open. We will only enumerate a few of them: (1) is the restraint of Mus81-Mms4 and Yen1 activities during S-phase important to prevent toxic processing of vital DNA replication and repair intermediates? Since both nucleases are capable of processing a variety of branched DNA structures *in vitro*, their late activation may contribute to genome stability in yet unanticipated ways; (2) how does phosphorylation regulate Mus81-Mms4 and Yen1 nucleases at the molecular level? While some insight has already been obtained for Yen1, it remains unclear how phosphorylation of Mms4 may enhance the activity of Mus81. To fully answer this question, a structural biology approach will be required. Another interesting aspect of Mus81-Mms4 phosphorylation is that it also regulates interaction with the scaffold proteins Dpb11/TOPBP1 and Slx4, which coordinate the cellular response to replication fork stalling [36•,55]. How phosphorylation regulates the formation of the Mus81-Mms4-Dpb11-Slx4 complex is an interesting research topic; it remains to be investigated whether in addition to phosphorylation also other posttranslational modifications, such as sumoylation, may play a role in regulating Mus81-Mms4 function [56,57]; (3) is Mus81-Mms4 and Yen1 regulation important during meiosis? While initial work demonstrated that both enzymes have important roles and are tightly regulated during meiosis, it remains unclear whether regulation of their activities is important for successful haploidization; (4) do other organisms control Mus81 and Yen1/GEN1 activities? How? While initial work implies that fission yeast as well as human cells tightly regulate Mus81 and Yen1/GEN1 (absent in fission yeast) [20,35], the precise mechanisms remain poorly understood. Furthermore, important functions for Mus81 and GEN1 orthologs have been identified in a variety of other organism, including worms and flies [58,59]. It will be interesting to understand whether such organisms employ similar strategies to yeast in establishing a hierarchy in pathway usage.

In summary, while much insight has been gained in recent years, how and why cells confine the activity of JM processing enzymes to defined temporal windows is only starting to be elucidated. Future work holds the promise of uncovering an even more elaborate regulatory network with important functions for maintenance of genome stability.

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