Review Article

Processing DNA lesions during mitosis to prevent genomic instability

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Failure of cells to process toxic double-strand breaks (DSBs) constitutes a major intrinsic source of genome instability, a hallmark of cancer. In contrast with interphase of the cell cycle, canonical repair pathways in response to DSBs are inactivated in mitosis. Although cell cycle checkpoints prevent transmission of DNA lesions into mitosis under physiological condition, cancer cells frequently display mitotic DNA lesions. In this review, we aim to provide an overview of how mitotic cells process lesions that escape checkpoint surveillance. We outline mechanisms that regulate the mitotic DNA damage response and the different types of lesions that are carried over to mitosis, with a focus on joint DNA molecules arising from under-replication and persistent recombination intermediates, as well as DNA catenanes. Additionally, we discuss the processing pathways that resolve each of these lesions in mitosis. Finally, we address the acute and long-term consequences of unresolved mitotic lesions on cellular fate and genome stability.

Introduction

When cells encounter DNA damage, a series of signal transduction events occur, leading to the concerted recruitment and activation of various proteins at the DNA lesion, a process known as the DNA damage response (DDR) [1,2]. In the context of toxic DNA double-strand breaks (DSBs), two canonical DNA damage repair pathways are active during interphase: non-homologous end joining (NHEJ) and homologous recombination (HR). Whereas DNA ends are protected and rapidly ligated during NHEJ, HR involves DNA end resection to initiate strand invasion of the sister chromatid for templated repair [1].

In contrast with interphase, canonical DSB repair is inactivated in mitosis. Although upstream DDR signaling still occurs in mitosis, including H2AX phosphorylation and MDC1 recruitment, downstream signaling is inactivated upon mitotic entry [3,4]. The rewired response to DNA breaks during mitosis has been reviewed previously, but recent studies have shed new light on how DNA damage is processed in mitosis, including the involvement of alternative repair pathways in facilitating the resolution of mitotic DNA lesions [2,3,5]. In this review, we discuss how DNA lesions, predominantly originating during DNA replication, are transmitted into mitosis despite the presence of cell cycle checkpoints. Furthermore, we discuss how the mitotic context drives specific processing of these lesions. Specifically, mitotic kinases activate dedicated enzymes that act on DNA lesions, and ultimately ensure equal distribution of sister chromatids to each daughter cell. Finally, we discuss the acute and long-term consequences of unresolved mitotic lesions on cellular viability and genome integrity. Importantly, we focus on DNA lesions that arise from under-replicated DNA, unresolved HR intermediates and catenanes. We realize that a multitude of other DNA lesions exists, that may also be transmitted into mitosis [6,7], and may also be differentially repaired during mitosis.

Regulation of the mitotic DNA damage response

In addition to genomic location and chromatin state, cell cycle status greatly influences DNA repair pathway choice and the toxicity of the DNA lesions [8–11]. To prevent ongoing cell cycle progression in the presence of unrepaired DNA lesions, cells are equipped with DNA damage-induced cell cycle

checkpoints that can halt cell cycle progression until DNA lesions are resolved. The G1 checkpoint prevents damaged cells from initiating DNA replication. When DNA breaks arise during S-phase, the intra-S checkpoint is activated, which down-regulates CDK activity to restrain further firing of replication origins, thereby limiting overall replication (Figure 1). Subsequently, the G2/M checkpoint prevents cells from entering mitosis with DNA lesions [11].

In contrast with interphase, no cell cycle checkpoint is activated by DNA breaks during mitosis in mammalian cells. Instead, the spindle assembly checkpoint (SAC) functions to ensure faithful chromosomal segregation, suggesting a necessity to control accurate chromosome segregation and genomic stability over structural chromosome integrity [12]. Unless occurring in centromeric or telomeric regions, mitotic DNA breaks do not trigger cell cycle arrest [12–14], rendering cells more sensitive to DNA damage in mitosis [15,16]. Indeed, mitotic progression in the presence of DNA damage contributes to the cytotoxicity of many anti-cancer treatments [17–19].

The DDR kinases ATM and ATR play a key upstream role in damage-induced cell cycle arrest. ATM and ATR respond to different types of DNA lesions, but mediate converging downstream effects. In response to stretches of single-stranded DNA (ssDNA), for example at stalled replication forks, TOPBP1 in conjunction with the 9-1-1 complex activates ATR. Subsequently, ATR activates — among many other substrates — the CHK1 kinase, preventing mitotic entry and allowing time for cells to repair DNA lesions [20] (Figure 1). Conversely, the ATM kinase responds primarily to DSBs [21], and mediates cell cycle control through the downstream kinase CHK2 and p53, although the contribution of CHK2 in regulating cell cycle arrest has been challenged by several studies [22–24]. Interestingly, while these DDR kinases are triggered rapidly, certain thresholds for their activation have been described. Specifically, studies using *Xenopus laevis* egg extracts have
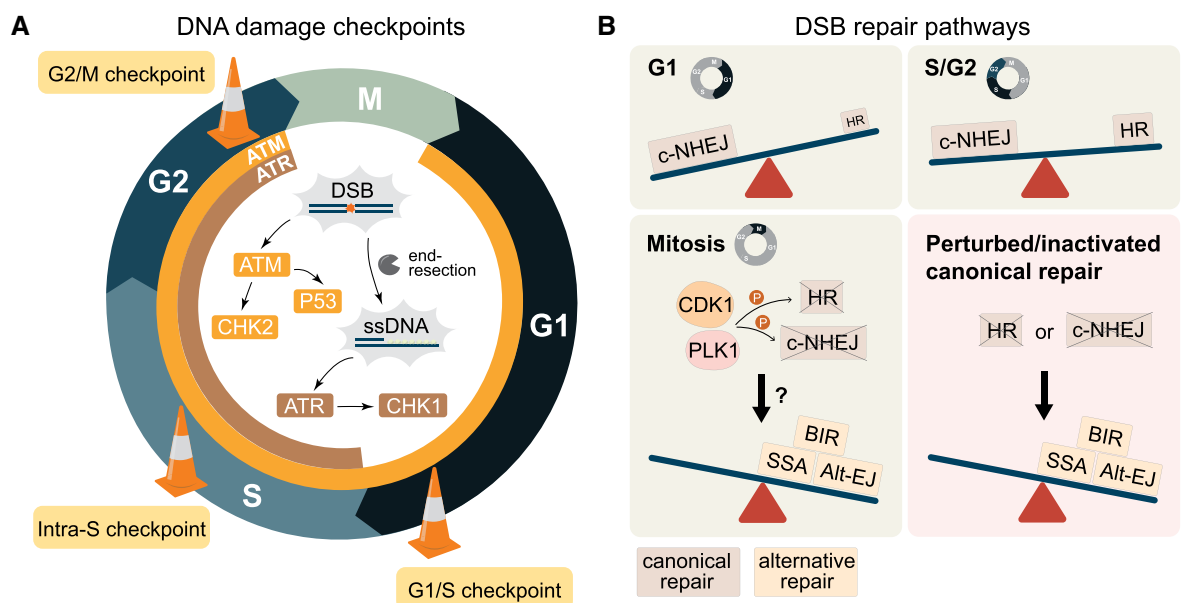
Figure 1. Regulation of DNA damage response throughout the cell cycle.
(**A**) Cells are equipped with checkpoints that regulate cell cycle progression upon DNA damage. ATR and ATM are key upstream checkpoint kinases that co-ordinate the DDR in response to single-strand DNA (ssDNA) and double-strand breaks (DSBs), respectively. Whereas ATM can be activated throughout interphase (orange line), ATR activation is restricted to S/G2 phase (brown line). Contrary to interphase, DNA damage does not halt cell cycle progression in mitosis. (**B**) In response to DSBs, cells utilize two canonical pathways to repair DSBs. Whereas canonical non-homologous end joining (c-NHEJ) is active throughout interphase, homologous recombination (HR) allows for templated repair sister chromatids become present in S/G2 phase. When these canonical pathways are not active due to genetic or experimental perturbations, alternative repair pathways, including break-induced replication (BIR), single-strand annealing (SSA), and alternative end joining (Alt-EJ), will be employed. The absence of canonical repair pathways is reminiscent of the mitotic state, in which mitotic kinases CDK1 and PLK1 inactivate many HR and c-NHEJ factors through phosphorylation.

identified that primed ssDNA gaps as small as ∼35 nucleotides can activate the ATR kinase, with larger ssDNA gaps strengthening the signaling [25]. Similarly, the ATM-dependent G2/M checkpoint was shown to only be robustly activated upon induction of 10–20 of DSBs [26]. Interestingly, a single DSB induced by a CRISPR/Cas9-based system was sufficient to delay cell cycle progression [27], although a possible cause of this delay could be the persistence of Cas9 to recut the gRNA site until erroneous repair has occurred. These findings suggest different requirements for cell cycle delay versus full arrest and illustrate context dependence on the source of DNA breaks and cell type. In line with this notion, 'leaky' checkpoints can explain the increased toxicity of low-dose radiation in cancer cells, and may contribute to the development of genomic instability in cancer [3,26,28,29].

Intriguingly, even under physiological circumstances, some DNA lesions go unnoticed by the G2/M checkpoint. For example, some replication-born lesions fail to trigger ATR, evading repair and allowing their propagation into mitosis. For instance, perturbed DNA replication upon PARP inhibition or overexpression of the *CCNE1* oncogene lead to DNA lesions that are transmitted into mitosis, and cause mitotic aberrations and genomic instability [17,30,31]. While the exact nature of these DNA lesions and the underlying mechanism for slippage of these lesions into mitosis remains unclear, it is tempting to speculate that perhaps the amount of ssDNA at the junction of a stalled replication fork is not sufficient to trigger the ATR checkpoint, or that no DSBs arise to activate ATM signaling.

During mitosis, the canonical NHEJ and HR DSB repair pathways are inactivated [2,3]. Specifically, phosphorylation of the NHEJ factors RNF8, RNF168, 53BP1, and XRCC4 by the mitotic kinases CDK1 and PLK1 inhibits their function (Figure 1). Likewise, key HR factors, including BRCA1 and RAD51 are not recruited to DNA breaks during mitosis [2,15,32]. The need to inactivate canonical DNA repair pathways in mitosis was underscored through forced recruitment of RNF8 and 53BP1 to DSBs, which leads to illegitimate usage of NHEJ at telomeres and ultimately telomere fusions [33]. Moreover, restoration of mitotic DSB repair through expression of phosphorylation-defective XRCC4 results in increased formation of anaphase bridges [32]. Whereas downstream NHEJ components are inactivated, upstream components of the DDR response, including the MRN complex, are still recruited to mitotic DSBs. As a consequence, mitotic DNA breaks activate ATM signaling, as well as subsequent phosphorylation of H2AX and recruitment of MDC1 [3,4]. Furthermore, the NHEJ kinase DNA-PK takes part in H2AX phosphorylation in response to mitotic DNA breaks [34]. Notably, resection of DSB was also reported to occur in mitotic *Xenopus* egg extract and human mitotic cells [35], suggesting that mitotic lesions are still actively processed during mitosis.

Beyond detection and initial processing, the DNA ends of DSBs were recently demonstrated to be tethered in mitosis to prevent mis-segregation of broken chromosomal arms. Mitotic DNA tethering involves MDC1, TOPBP1, and CIP2A [4,36,37], and possibly other components, since RPA3, Fancd2 and alternative end-joining DNA polymerase θ (Polθ) were shown to be recruited to mitotic DSBs in *Drosophila* papillar cells [38]. Moreover, loss of these factors resulted in mis-segregation of acentromeric DNA fragments, possibly reflecting a role in DNA tethering (Figures 1 and 2).

Incomplete cell cycle checkpoint control, and subsequent transmission of DNA damage into mitosis, is a common feature of cancer cells. It is, therefore, relevant to investigate which mechanisms respond to unresolved DNA lesions in mitosis, and whether these mechanisms impact on genome maintenance and cancer cell survival. Recent studies showed that POLθ, in conjunction with RAD52, repairs breaks originating from S-phase in HR-deficient cells at the onset of mitosis, suggesting that targeting mitotic DNA repair may potentiate the therapeutic effects of PARP inhibition, a clinically relevant treatment for HR-deficient tumors [39]. These findings provide early evidence that residual processing of mitotic DNA damage through alternative repair pathways occurs, and that targeting these processes may have therapeutic value.

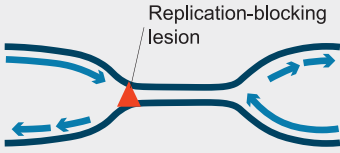# Endogenous DNA lesions in mitosis and their processing pathways

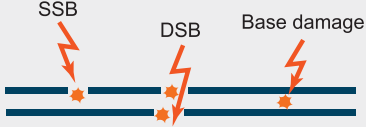Mitotic DNA lesions can originate from exogenous sources, including DNA-damaging treatments such as ionizing radiation, or in experimental settings using nuclease-mediated cleavage [40–44]. Under physiological conditions, however, mitotic DNA lesions predominantly result from endogenous factors (Table 1). In this section, we will discuss various sources of endogenous DNA lesions in mitosis along with the pathways that process them.
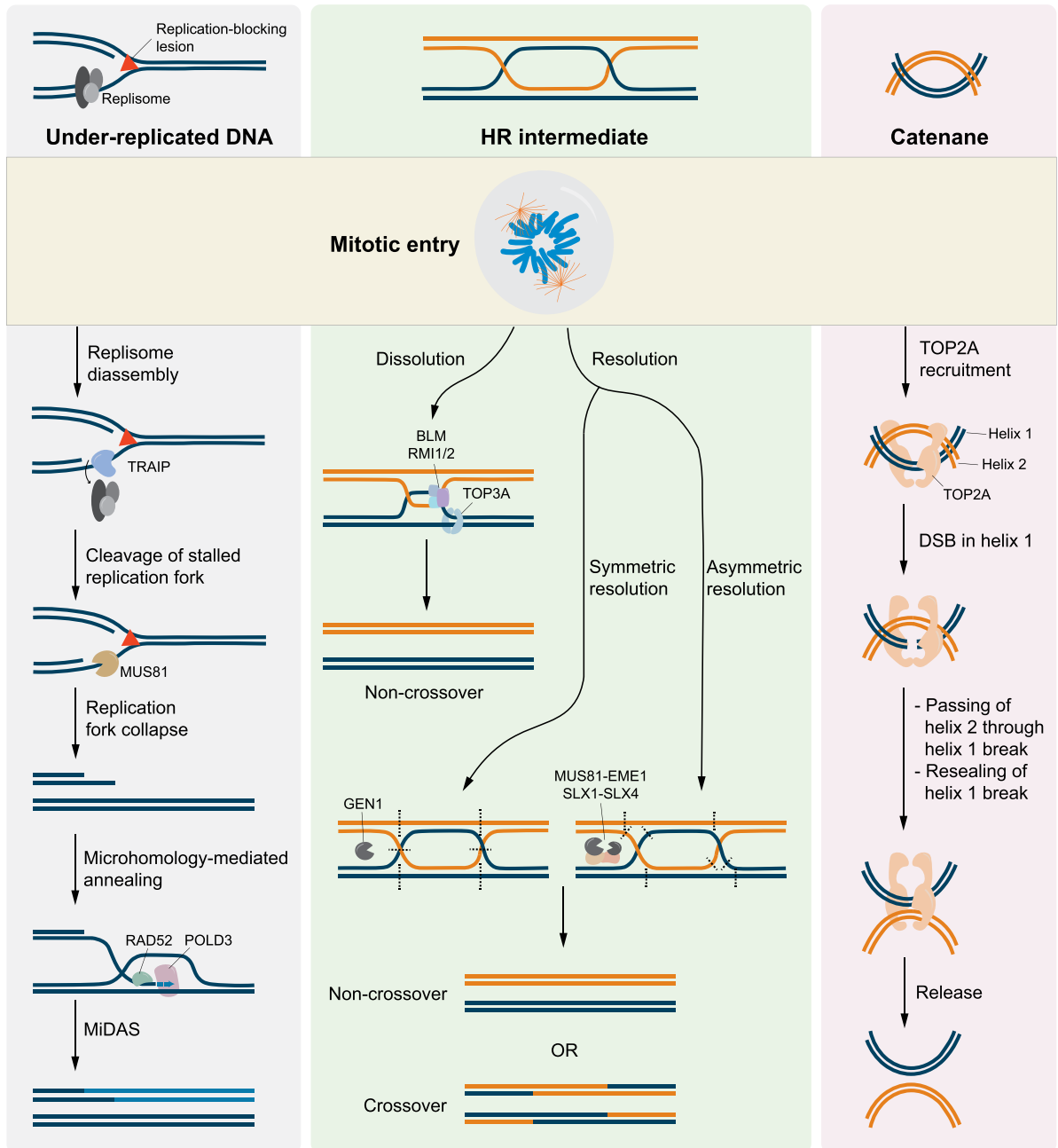
Figure 2. Processing of DNA lesions in mitosis.

DNA lesions that end up in mitosis are processed by distinct pathways. (*Left*) Under-replicated DNA originating from perturbed replication in S-phase are subjected to DNA synthesis in early mitosis (MiDAS), involving TRAIP-mediated disassembly of the replisome complex, cleavage of the stalled replication fork by the MUS81 endonuclease, RAD52-mediated homology search and POLD3-dependent DNA synthesis. (*Center*) Unresolved homologous recombination (HR) intermediates are processed by structure-specific nucleases upon mitotic entry. Dissolution via the BTR (BLM, TOP3A, RMI1/2) complex results in a non-crossover repair product, whereas resolution either via GEN1 or the MUS81–EME1–SLX1–SLX4 complex gives rise to a repair product with the possibility of crossover. Dotted lines indicate possible cleavage patterns by structure-specific nucleases. (*Right*) Intertwined DNA molecules in the form of catenanes are resolved by topoisomerase IIα (TOP2A) during the metaphase–anaphase transition.

Table 1. Different types of lesions in mitosis

Lesion type	How to study	References
Under-replicated DNA Replication-blocking lesion 	• Induction of replication stress and CFS instability with low/mild-dose of DNA polymerase inhibitor Aphidicolin	45
	• Slowing down of replication speed in BRCA2- and MCM-deficient cells	46
	• Oncogene overexpression to induce replication stress	30, 47, 48
HR intermediates 	• Labeled, branched DNA oligos resembling recombination intermediates incubated with purified protein of interest	49
	• BTR complex- and structure-specific nucleases-deficient cells to study resolution of Holiday junctions	50
Catenanes 	• Migration of catenated vs decatenated plasmids through agarose gel distinguishable by their size	51
	• Generation of a single catenane substrate using Tn3 site-specific recombinase	52, 53
	• Delaying decatenation at C-UFB using inhibitors of topoisomerase II (ICRF)	51
Nuclease-mediated breaks Cas9 I-SceI 	• CRISPR/Cas9-based DSB repair assay	40
	• I-SceI-based DSB repair pathway choice assay	41
	• FokI endonuclease fused to LacI to allow the formation of localized DSBs and the visualization of DNA repair factors at the lac operator repeats	42
Radiation-induced lesions SSB DSB Base damage 	• Laser microirradiation to visualize DNA damage site and recruitment of repair factors in nocodazole-arrested mitotic cells	35
	• Treatment of nocodazole-arrested cells with UV light and IR	43, 44
	• Ionizing radiation-induced foci (IRIF) in nocodazole-synchronized cells	44

Under-replicated DNA

To faithfully segregate sister chromatids during mitosis, DNA needs to be completely replicated. However, there are many processes that might interfere with the progression of replication forks. These processes are collectively termed 'replication stress' (RS), a phenomenon frequently observed in cancer [46,54,55]. Among other processes, replication can be perturbed by transcription occurring at nearby genomic regions, leading to collisions between the replication and transcription machineries [56]. Additionally, oncogene overexpression (e.g. *CCNE1*, *MYC*, and *RAS*), induces *de novo* firing of replication origins in gene-coding genomic loci [47]. Such unscheduled DNA synthesis exhausts the available pool of nucleotides and interferes with ongoing replication. Moreover, oncogene-induced *de novo* origin firing further increases collisions between replication and transcription machineries [57].

Failure to complete DNA replication is more likely to occur at difficult-to-replicate loci, including common fragile sites (CFSs) [58,59]. When cells are treated with replication inhibitors, such as aphidicolin [60], CFSs appear as gaps or breaks in metaphase spreads, referred to as 'CFS expression' [45,61]. CFSs have an AT-rich sequence composition, which makes them prone to the formation of secondary DNA structures, potentially hindering replication fork progression [62]. The majority of CFSs lie within large genes that require more than one cell cycle to be transcribed and are therefore more likely to encounter transcription–replication collisions [63–65]. In line with oncogene expression leading to perturbed replication, CFS expression and recurrent copy number alterations (CNAs) at CFSs has been linked to oncogene expression in cancers [48,58,59,66,67].

The majority of DNA replication occurs in S-phase, although replication of some genomic regions extends into G2 phase. Intriguingly, increasing evidence demonstrated that processing of late-stage replication intermediates occurs after cells enter mitosis [68,69]. Mitotic DNA synthesis (MiDAS) has been described as a last resort pathway to complete DNA replication early in mitosis, thereby preserving genomic integrity. MiDAS resembles break-induced replication (BIR) as it requires the POLD3 polymerase, the MUS81–EME1 endonuclease complex, and the RAD52 recombinase (Figure 2) [61,70,71]. Additionally, the TRAIP ubiquitin ligase, which drives replisome disassembly, is essential for the recruitment of MiDAS factors [72]. BIR is highly mutagenic [73], and it remains to be determined whether MiDAS is equally prone to induce mutations and whether it contributes to tumor mutational signatures. More recently, the concept of mitotic DNA replication has been challenged by the discovery that the commonly used CDK1 inhibitor RO-3306 non-specifically interferes with DNA synthesis. As a result, MiDAS detection in early mitosis may be a consequence of the off-target activity of RO-3306 [74], warranting the need to reassess experimental contexts to study MiDAS.

Fanconi anemia (FA) proteins FANCD2 and FANCI are recruited to CFSs in mitosis upon perturbed DNA replication [65]. Their role in resolving under-replicated DNA, however, is incompletely clear. During mitosis, FANCD2 localizes to adjacent foci on each sister chromatid [75]. FANCD2-positive lesions that remain unresolved in prophase persist during mitotic progression, where in anaphase they ultimately flank DAPI-positive chromatin bridges or DAPI-negative ultrafine DNA bridges (fragile site (FS)-UFBs, Figure 3) [75,76]. In cancer cells, FANCD2 was reported to be essential for MiDAS [77]. Additionally, FANCD2 has been reported to co-operate with the Bloom's syndrome helicase (BLM) to prevent chromosome mis-segregation upon mitotic transmission of RS-induced DNA lesions, pointing to a role in DNA repair beyond S-phase [45,78].
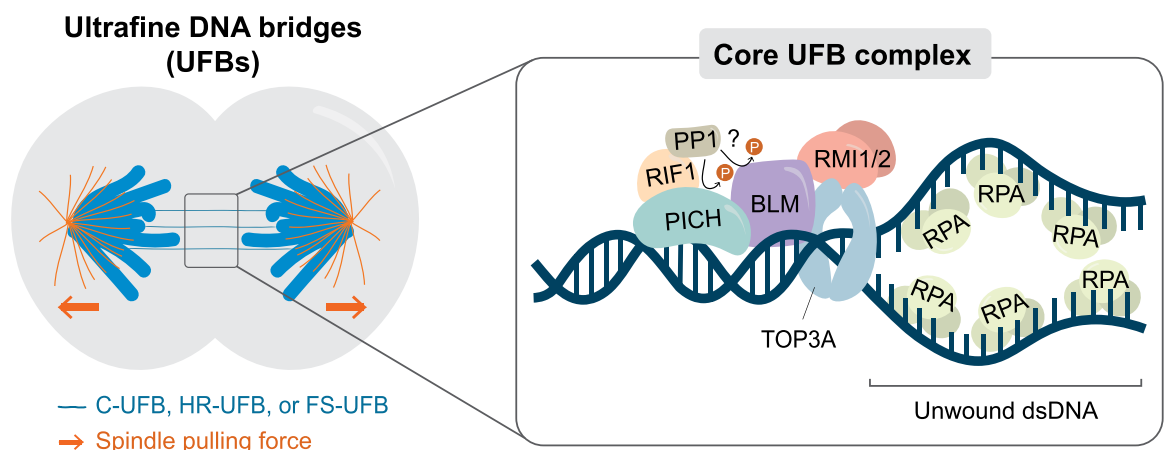
Figure 3. Unresolved lesions in mitosis are processed into ultrafine DNA bridges.
Failure to process joint DNA molecules in mitosis leads to persistent entangling of sister chromatids, generating ultrafine DNA bridges (UFBs) as cells progress into anaphase. Pulling force from mitotic spindle stretches the DNA, initiating binding of the PICH translocase to double-stranded DNA (dsDNA) regions of the UFB, and subsequent recruitment of the BTR (BLM, TOP3A, RMI1/2) complex and RIF1. RIF1 may interact with its effector protein phosphatase 1 (PP1), dephosphorylating PICH and BLM. The BLM helicase becomes activated and unwinds dsDNA into ssDNA, triggering localization of the RPA trimeric complex. Topoisomerase TOP3A may in turn decatenate ssDNA stretches to mediate resolution of UFBs. 'C-UFB' = centromeric UFB, 'HR-UFB' = homologous recombination UFB, 'FS-UFB' = fragile site UFB.

HR intermediates

HR involves strand invasion of a RAD51-coated DNA end into an undamaged sister chromatid, in order to allow DNA synthesis with the sister chromatid as a template. When there is second-end capture at the DSB site, these sister chromatids can form covalently linked four-way DNA junctions known as holliday junctions (HJs) (Figure 2) [79]. HJs represent joint DNA molecules that need to be removed prior to chromosome segregation during anaphase to ensure equal distribution of DNA to both daughter cells, which is required to prevent genomic instability.

HJs can be processed through 'dissolution' of double HJs or 'resolution' of both double and single HJs. Dissolution of double HJs involves the BTR complex, consisting of BLM, topoisomerase IIIα (TOP3α), RMI1, and RMI2, and it is the preferred mechanism for resolving double HJs because it generates non-crossover repair products (Figure 2) [49,79]. While it was long thought that HJs are resolved before the onset of mitosis, recent work showed that HJs can be resolved by BLM at the G2/M transition upon activation of BLM by CDK1 and PLK1 [80–82].

Resolution of both single and double HJs either results in non-crossover or crossover products. Crossovers are also known as sister chromatid exchanges (SCEs), and can be visualized via differential BrdU incorporation [50,80]. Resolution is conducted by the structure-specific nucleases MUS81–EME1, SLX1-SLX4, and GEN1 (Figure 2) [49,79]. The MUS81–EME1 and SLX1-SLX4 complexes have the highest activity in prometaphase of mitosis when these proteins associate with XPF to form the multimeric SMX complex [49,50]. The formation of the SMX complex is enhanced by phosphorylation of EME1 and SLX4 by the mitotic kinases CDK1 and PLK1 [49,50,83,84]. Upon mitotic activation, the SMX complex makes incisions on both sides of the HJs and ultimately generates an ssDNA overhang on one side of the DNA strand. This ssDNA overhang needs to be processed before the two DNA strands from the same sister chromatid can be ligated to generate an intact chromatid (Figure 2) [79].

In contrast with the SMX complex, GEN1 can cut the HJ without generating an ssDNA overhang. GEN1 is excluded from the nucleus during interphase, restricting the resolution of HJs by GEN1 until after nuclear envelope breakdown at onset of mitosis [68,85]. After its recruitment to HJs, GEN1 first makes a nick at one side of the HJ and will subsequently make a second nick at a symmetrical position on the other strand. These broken ends can be immediately ligated by a DNA ligase without any further processing. While this allows chromatid separation during mitosis, GEN1 utilization does result in SCEs (Figure 2) [79,86].

Elevated levels of SCEs are observed in cells with BLM-deficiency obtained from Bloom's syndrome patients, as these cells rely on resolution of HJs via the SMX complex or GEN1, instead of dissolution via the BTR complex. As a consequence, Bloom's syndrome patients develop cancer early in life due to genomic instability. Conversely, depletion of MUS81, SLX1, SLX4, or GEN1 results in a decrease in SCEs [50,80]. If HJs are not resolved by either the SMX complex or GEN1, they can end up as ultrafine bridges (UFBs), specifically HR-UFBs [68]. Interestingly, increased numbers of UFBs and SCEs were found in 53BP1-hypomorphic cells, which were suggested to originate from HR intermediates caused by the excessive level of HR in these cells [87].

Catenanes

Intertwined DNA molecules can also end up in mitosis as a consequence of normal DNA replication. During DNA replication both centromeres become topologically linked by double-strand catenanes (Figure 2, Table 1) [53]. These catenanes originate from S-phase and are carried over to mitosis to be resolved [81]. Apart from being intertwined DNA molecules that need to be untangled before anaphase can occur, catenanes may also be beneficial in supporting sister chromatid cohesion during early mitosis [88,89].

Topoisomerase IIα (TOP2α) resolves DNA catenanes during the metaphase–anaphase transition by generating a DSB that releases tension and uncouples the two sister chromatids [51,52,81]. TOP2α subsequently ligates the two broken ends to ensure untangling of the catenanes, and is essential for correct segregation of the sister chromatids at the beginning of anaphase (Figure 2) [90]. As expected, TOP2α inhibition impedes untangling of centromeric catenanes, leaving sister chromatids connected during anaphase and resulting in the formation of centromeric ultrafine DNA bridges (UFBs) (Figure 2) [45,68,78,87,91].

Consequences of unprocessed mitotic DNA damage
Ultrafine DNA bridges

When joint DNA molecules persist — either due to defective processing of catenanes, under-replicated DNA or HJs — UFBs emerge in anaphase. UFBs are DNA linkages undetected by conventional DNA dyes, and they

can only be visualized by immunofluorescence staining of UFB-localizing proteins including BLM, PLK1-interacting checkpoint helicase (PICH), Rap1-interacting factor (RIF1), and replication protein A (RPA) (Figure 4) [5,6,68]. As discussed previously, UFBs occur frequently at centromeres (C-UFBs) due to centromeric catenanes [81], at under-replicated DNA at fragile sites (FS-UFBs), or originate from unresolved HR products (HR-UFBs) [45,68,92]. Interestingly, these various kinds of UFBs arise during mitosis in unchallenged conditions, albeit to a different extent. This observation indicates that catenated DNA and covalently linked sister chromatids remain undetected by checkpoint signaling [6,7]. Apparently, these structures are not sensed by cell cycle checkpoints, perhaps because cells have evolved effective mechanisms to resolve these structures in mitosis.

PICH recruitment depends on tension and is recruited early to UFBs [93], which is required for both the recruitment of the BTR complex and RIF1 [6,7,81,82] (Figure 4). Both BLM and PICH are phosphorylated by CDK1 and PLK1 at the onset of mitosis [80–82]. The role of RIF1 at UFBs might be to recruit Protein Phosphatase 1, which can counteract PLK1 and CDK1 activity at UFBs (Figure 4) [94]. Conversely, BLM and PICH were suggested to function together with TOPIIIα and TOP2α to resolve C-UFBs during mitosis, in a process that requires topoisomerase-binding protein-1 (TOPBP1) [68,81]. Interestingly, CIP2A was recently shown to be essential for TOPBP1 recruitment to DNA breaks during mitosis, where it was demonstrated to
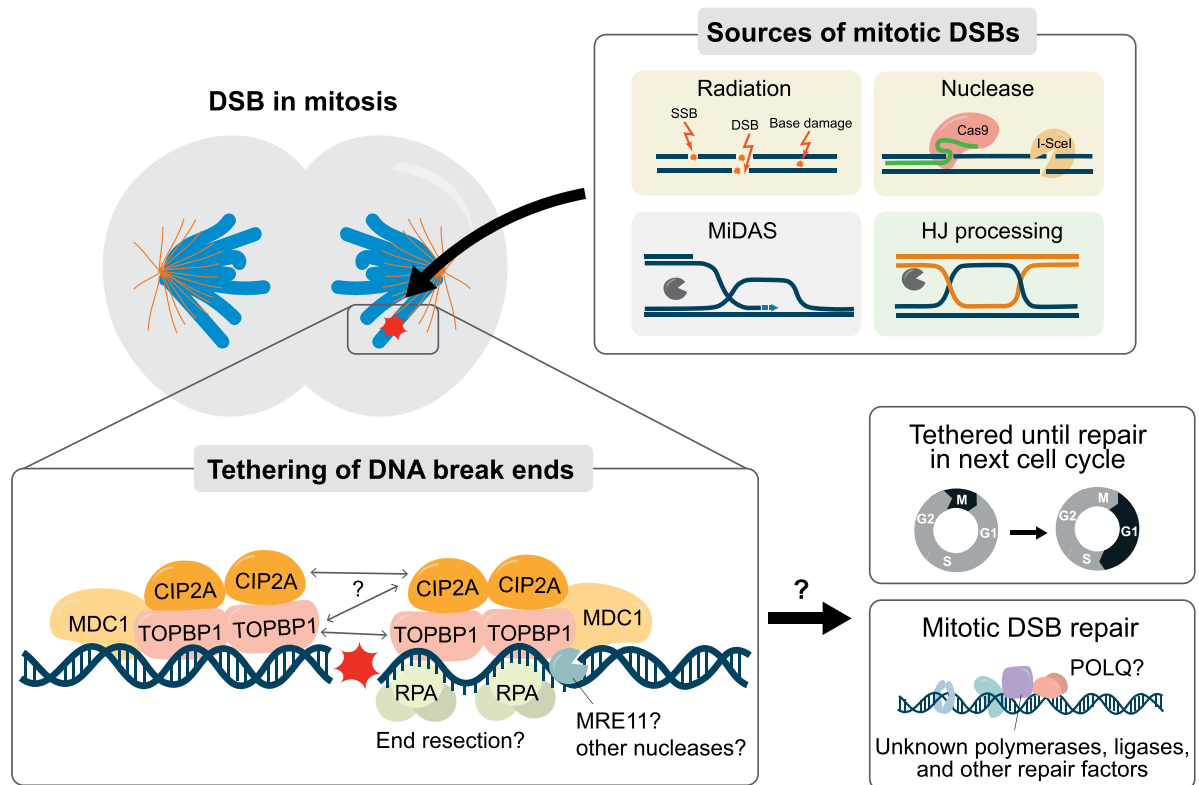
Figure 4. Tethering of DSB ends in mitosis.

(*Top right*) DSBs arising in mitosis can originate from ionizing radiation, experimental approaches using nuclease-mediated cleavage, as well as from mitotic processing of DNA lesions. (*Zoom in, bottom left*) At the damage site, recruitment of MDC1 mediates accumulation of TOPBP1 and CIP2A complexes, resulting in the tethering of two broken DNA ends in mitosis. TOPBP1 and CIP2A possibly form tethering complexes through interaction between its own homodimers and/or each other. Nucleases, including MRE11, may perform resection of broken DNA ends, allowing the loading of RPA onto ssDNA stretches, subsequently protecting them from nucleolytic degradation. Altogether, assembly of these factors forms a tethering structure that prevents the mis-segregation of broken, acentric, chromosomal arms, and formation of micronuclei. (*Bottom right*) DSB ends may remain tethered until cells progress to the next cell cycle in which canonical repair pathways are active. Alternatively, tethering may be an intermediate step prior to further processing by non-canonical repair factors activated in mitosis.

function in the tethering of DNA breaks (Figure 3) [4]. While not formally shown, CIP2A might also be required for TOPBP1 recruitment at UFBs. Taken together, UFB-localizing proteins clearly are required to resolve the joint molecule, resulting in a single DNA stretch. The ssDNA-binding protein RPA, detected at UFBs in later stages of anaphase, binds the unwound ssDNA to protect it from degradation, which is counteracted by RIF1 [5,6,20].

Contrary to other UFBs, FS-UFBs are marked by FANCD2 foci which flank BLM- and PICH-positive UFBs [45,78,92]. While the FA pathway was shown to be dispensable for the resolution of centromeric UFBs, it appears important for proper accumulation of BLM at non-centromeric UFBs [78]. In line with these observations, a functional FA pathway and proper BLM recruitment to non-centromeric UFBs was demonstrated to be required for UFB dissolution and for preventing chromosome mis-segregation [45,78].

While rare, unresolved UFBs can still be detected in telophase as nucleoplasmic bridges, and their frequency is increased upon depletion of UFB factors. Indeed, depletion of PICH, RIF1, or BLM causes an increase in number of bulky bridges in telophase [6]. Subsequently, impaired UFB resolution increases micronucleation and 53BP1 body formation in the following G1 phase [6]. As DNA damage in mitosis can have major consequences for daughter cells, UFB resolution acts as a last barrier to process DNA damage in mitosis.

Outcomes of mitotic DNA repair and consequences of unresolved mitotic DNA lesions

Entry into mitosis in the presence of unresolved DNA lesions may result in cell death, known as 'mitotic catastrophe' [95]. This phenomenon, at least in part, explains why therapeutically forcing cells into mitosis in the presence of unresolved DNA lesions could be utilized as anti-cancer strategies. This concept has been effectively demonstrated by targeting cell cycle checkpoint kinases, including Wee1, CHK1, and ATR, either alone or in the presence of DNA-damaging agents [17,30,96,97].

Intriguingly, even when cells are able to process DNA lesions during mitosis, mitotic DNA repair can still negatively impact genome integrity in several ways. For example, processing of under-replicated DNA by MiDAS may cause mutagenic scars. These scars could be similar to those generated from BIR, involving frameshifts and duplications, and are possibly caused by usage of low-fidelity polymerase POLD3 [73,98]. Alternatively, stalled replication forks may collapse and lead to different genomic scars if MiDAS is not functional. Specifically, exposed ssDNA becomes vulnerable to nuclease-mediated cleavage when the CMG replicative helicase is removed from stalled replication forks flanking an under-replicated locus [99]. Subsequent ligation of these cleaved DNA ends has been shown to cause deletions with microhomology at the break sites, reflecting the usage of POLθ [39,99,100]. Similar genomic scars were observed in BRCA-deficient tumors and HR-deficient cells, which experience perturbed replication and show increased numbers of mitotic DNA lesions [101]. Scars associated with BRCAness are also characterized by small tandem duplications, insertions, and deletions with flanking microhomology sequences [20]. These combined observations raise the question whether these genomic scars could result from mitotic processing of DNA lesions, rather than from aberrant repair in S and G2 phase of the cell cycle [20,58]. Notably, similar scars were observed at CFSs [59,62], and were linked to CNAs and recurrent breakpoints in cancer, underscoring the important role of resolving under-replicated DNA in tumor suppression [58,59,62].

When joint DNA molecules persist until late mitosis, they may rupture due to spindle-mediated tension and can be repaired by mutagenic repair through NHEJ in the next cell cycle [68]. For example, when the number of joint DNA molecules was elevated due to increased HR usage, tension-induced rupture was observed leading to persistent UFBs and ultimately DNA breakage [87]. Rupture of these UFBs may fuel breakage–fusion-bridge cycles that subsequently lead to both numerical chromosome alterations and structural rearrangements [87,102].

Finally, when DNA lesions are not properly repaired or remain tethered during mitosis, chromosome fragments can end up in the cytosol as micronuclei [4,103]. Lack of proper DNA metabolism components within micronuclei leads to perturbed replication and replication fork collapse, ultimately giving rise to chromosome shattering (i.e. chromothripsis) [104,105]. Mechanistically, micronuclei have been shown to contain high levels of RNA-DNA hybrids, which are processed by ADAR enzymes [106]. ADAR-mediated processing forms abasic sites and ssDNA nicks, which are converted into DSBs during replication. ADAR enzymes, which were described to be overexpressed in certain cancers, can therefore cause fragmentation of micronuclear chromosomes [106].

Combined, processing mitotic DNA lesions (or failure thereof) may lead to loss or gain of chromosome fragments. Single gains or losses can have major consequences when they affect tumor suppressors or oncogenes. This may promote malignant transformation and fuel genetic heterogeneity within tumors, and ultimately could accelerate tumor development or drive treatment failure [107]. Therefore, mitotic processing of DNA damage likely represents an important but understudied part of tumorigenesis.

To better understand the mitotic DDR and determine which genomic signatures arise when tumor cells utilize mitotic DDR, we need to uncover which DNA repair proteins are active during mitosis. Additionally, it is important to study the cellular consequences when DNA lesions are induced specifically in mitosis, and to pinpoint more precisely when the processing of DNA lesions occurs; at the end of G2, or during early or late mitosis. Such knowledge may be achieved by inactivating DNA repair proteins or disrupting repair complexes specifically during mitosis, using targeted degradation methods [108]. Examples hereof are POLθ and RAD52, which were recently shown to repair mitotic DNA breaks that originated from S-phase in HR-deficient cells [39]. These studies also suggested that targeting mitotic DNA repair could be a valuable tool to improve PARP inhibitor therapy for HR-deficient cancers [39]. Similarly, proteins like CIP2A and GEN1 that are excluded from the nucleus during interphase, may provide mitosis-specific targets to therapeutically target mitotic DNA processing [4,37,85]. Lastly, a significant step forward would be to determine the genomic scars associated with mitotic repair of DNA lesions. As a starting point, genomic scars induced by targeted depletion of mitotic DDR proteins (e.g. GEN1, CIP2A, RAD52, and POLθ) could provide a framework to build a mitotic mutational signature. Such a signature could be used to gain insight into the genetic consequences of (defective) mitotic repair. Also, genomic signatures associated with mitotic repair could be used to discover mitotic DDR proteins causing similar signatures and may be instrumental for the analysis of clinical samples to identify tumor types that are dependent on mitotic DDR for their survival.

Perspectives

- Transmission of unresolved DNA lesions into mitosis, a common feature of cancer cells, poses a cellular threat as most canonical repair pathways are inactive in mitosis. Insight in mitotic processing of DNA lesions is essential to understand its contribution to genome maintenance and tumor survival.

- While canonical DNA repair pathways are inactivated during mitosis, cells are equipped with various alternative repair systems that resolve joint DNA molecules and thereby prevent chromosome mis-segregation to maintain genomic stability.

- Focused analysis of DNA repair in specific cell cycle phases, particularly mitosis, will help to elucidate the extent to which mitotic DNA repair contributes to the landscape of mutational scars observed in cancers, and whether mitotic DNA repair is therapeutically actionable.

Competing Interests

The authors declare that there are no competing interests associated with the manuscript.

Abbreviations

BIR, break-induced replication; BLM, Bloom's syndrome helicase; CNAs, copy number alterations; DDR, DNA damage response; DSBs, double-strand breaks; FA, Fanconi anemia; HJs, holliday junctions; HR, homologous recombination; MiDAS, mitotic DNA synthesis; NHEJ, non-homologous end joining; PICH, PLK1-interacting checkpoint helicase; RIF1, Rap1-interacting factor; RPA, replication protein A; RS, replication stress; SCEs, sister chromatid exchanges.

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