How cells respond to DNA breaks in mitosis

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Abstract

DNA double-strand breaks (DSBs) are highly toxic lesions that can lead to chromosomal instability if they are not repaired correctly. DSBs are especially dangerous in mitosis when cells go through the complex process of equal chromosome segregation into daughter cells. When cells encounter DSBs in interphase they are able to arrest the cell cycle until the breaks are repaired before entering mitosis. However, when DSBs occur during mitosis, cells no longer arrest but prioritize completion of cell division over repair of DNA damage. This review focusses on recent progress in our understanding of the mechanisms that allow mitotic cells to postpone DSB repair without accumulating massive chromosomal instability. Additionally, we review possible physiological consequences of failed DSB responses in mitosis.

Differential responses to DNA breaks in interphase and mitosis

Cells must maintain genome stability during cycles of cell division in order to pass on their hereditary material intact to the next generation [1]. DNA is vulnerable to endogenous and exogenous sources of damage, so cells have therefore evolved a complex network of biochemical pathways to counteract these threats, collectively called the DNA damage response (DDR). Activation of the DDR involves recognition and repair of DNA lesions, modulation of chromatin structure and transcription, and **cell-cycle checkpoint** induction [2]. Cell fate upon genotoxic stress in multicellular organisms is also ultimately controlled by the DDR, which determines whether cells re-enter the cell cycle, or permanently exit it (**senescence**) or undergo programmed cell death (**apoptosis**).

The vast majority of human cell-based DDR studies have been performed on asynchronous cell populations, with any influence of cell cycle usually studied in the context of comparing how cells respond to DNA damage in specific stages of **interphase** (G1, S, G2). Far less is understood about how cells cope with genotoxic stress during mitosis, when chromatin becomes dramatically condensed, the **nuclear envelope** (NE) separating nucleus and cytoplasm has broken down, and cells are coordinating the complex process of equal **chromosome segregation** into daughter cells [3]. Our lack of understanding is in part due to the difficulties of studying a highly dynamic process that can be completed in less than an hour [4], with only a small percentage of cells in an asynchronous population at any one time attempting it. In this review, we describe recent advances in our understanding of how cells respond to DNA breaks in mitosis, highlight the differences in the cellular responses to DNA breaks in interphase and mitosis, and identify key questions that remain to be addressed.

DNA damage checkpoint signalling in interphase and mitosis

Many types of DNA damage can be repaired rapidly without the need for cells to activate cell-cycle checkpoints or sustain other global signalling responses. However, some lesions such as DNA double-strand breaks (DSBs) are highly toxic and can be particularly challenging for cells to repair accurately without causing genome instability [1], especially if left unrepaired when cells attempt chromosome segregation during mitosis. The DDR is initiated by three structurally related protein kinases: ATM, ATR and DNA-PKcs [5]. ATM and DNA-PKcs are primarily activated by DSBs, whereas ATR is activated by DNA replication stress or lesions that are processed to produce single-stranded DNA (ssDNA). The primary role of DNA-PKcs is probably limited to DSB repair in the vicinity of the lesion, whereas ATR and ATM activate both local and global cellular responses to DNA damage, in part by phosphorylating and

activating other kinases such as CHK1 and CHK2, respectively [5]. However, it is important to note that ATM, ATR and DNA-PKcs have a range of overlapping substrates and can substitute for each other to some extent.

All three DDR kinases are recruited and activated by a discrete set of co-factors. ATM is recruited and activated by the MRE11-RAD50-NBS1 (MRN) complex, which can recognise and bridge broken double-stranded DNA (dsDNA) ends [6,7]; DNA-PKcs is recruited and activated at dsDNA ends by Ku, a heterodimeric complex consisting of two subunits, Ku70 and Ku80 [8]; and ATR has a stable binding partner, ATRIP [9], which binds to the heterotrimeric RPA complex when it is bound to ssDNA [10]. ATM and DNA-PKcs are activated by binding to their co-factors at DSB ends, but ATR requires additional stimulation by either TOPBP1 or ETAA1, which both contain an ATR-activation domain [11-13]. ETAA1 is recruited directly to RPA-coated ssDNA where it can activate ATR kinase activity, whereas the factors and mechanisms controlling TOPBP1 recruitment are not yet clear but may involve multiple, possibly redundant mechanisms [14-16].

In response to DNA damage, ATM and ATR activate cell-cycle checkpoints to arrest cells at the G1/S or G2/M boundaries [17]. In contrast, S phase checkpoint signalling limits DNA replication origin firing, stabilises stalled replication forks and increases nucleotide supply to slow S phase progression and maintain the fidelity of DNA replication rather than causing cell-cycle arrest [18]. While there are differences in how these checkpoints are activated, they all share essentially the same output, i.e. inhibition of the cyclin-dependent kinase (CDK) activity that drives cell-cycle progression [5]. This is achieved rapidly by ATM/ATR-dependent activation of checkpoint kinases that inactivate the CDC25 family of phosphatases, which counteract inhibitory phosphorylation of CDKs by the WEE1 kinase. A slower, transcription-dependent inhibition of CDKs by p21 is also activated by ATM- and ATR-dependent phosphorylation of p53, which is particularly important for the G1/S checkpoint.

What about DNA damage checkpoint signalling in mitosis? Once cells have moved beyond prophase, there is no DNA damage checkpoint until daughter cells re-enter G1 in the next cell cycle [19]. While high levels of DNA damage can delay the metaphase-to-anaphase transition, this is due to defects in microtubule attachment to **kinetochores** and an active **spindle assembly checkpoint**, rather than direct signalling from DNA lesions [20].

It is therefore clear that cells at some point become committed to mitosis and prioritize completion of cell division over repair of DNA damage. There are several mechanisms that prevent inhibition of CDK1 by DNA damage signalling in mitosis. Firstly, little or no

transcription occurs during mitosis except possibly at specific genes and regions such as **centromeres** [21-23], so mechanisms such as p21 induction and subsequent inhibition of CDKs are disabled. Another major mechanism involves ubiquitylation and proteasomal degradation of key cell cycle and DNA damage checkpoint **mediators**. WEE1 is phosphorylated by CDK1 and PLK1 at the onset of mitosis, which creates phospho-degrons that are recognized by the SCF-βTrCP ubiquitin ligase, resulting in WEE1 degradation [24]. Claspin, a protein required for CHK1 activation [25], is also phosphorylated by PLK1 and degraded by SCF-βTrCP [26-28]. Finally, CHK2 is phosphorylated and inhibited by PLK1 upon mitotic entry, which prevents CHK2 activation rather than promoting its degradation [29]. ATM-dependent phosphorylation of CHK2 is also reduced during mitosis, which may further limit CHK2 kinase activity [29,30]. This may be required because aberrant ATM-CHK2 signalling during mitosis can result in stabilisation of kinetochore–microtubule attachments to chromosomes, thereby increasing the frequency of lagging chromosomes during anaphase [31]. Taken together, CDK1-Cyclin B activity is thus maximised at the end of prophase through multiple mechanisms and can no longer be inhibited by ATM or ATR signalling until mitosis is complete.

Interestingly, there is a short time frame during the early stages of mitosis between antephase (defined as the period after centrosome separation occurs but before chromosomes condense) and late prophase, when even a small amount of DNA damage triggers cells to decondense their chromosomes and to return to antephase [19], where they permanently exit the cell cycle [32]. The DNA damage threshold for re-entering the cell cycle is lower than for the G2/M checkpoint, with more cells becoming senescent when irradiated in antephase compared to G2 at similar doses of genotoxic stress [32]. This response is ATM-dependent [33], and is mechanistically distinct from the previously described "antephase checkpoint" [34], which is reversible and activated by **mitotic spindle** poisons, and depends on CHFR and p38 MAP kinases but not ATM/ATR signaling [33-36].

DSB repair and the chromatin response to DNA breaks in interphase and mitosis

In interphase, most DSBs are repaired via the non-homologous end-joining (NHEJ) or homologous recombination (HR) pathways [37]. NHEJ re-ligates DNA ends with limited or no DNA end-processing, and is the predominant DSB repair mechanism cells use throughout interphase except at collapsed DNA replication forks, where NHEJ is highly toxic so HR pathways must be used instead [38,39]. HR pathways rely on the presence of a homologous

donor template for repair and accurate regeneration of the sequence surrounding the break site. In order to avoid loss of heterozygosity, HR is restricted to S and G2 phases of the cell cycle where the sister chromatid is used as template for repair synthesis [37]. **DNA-end resection** to produce ssDNA is a prerequisite for HR, and the extent to which it is allowed to proceed is one of the factors that determines DSB repair pathway choice. Not surprisingly, DNA-end resection is a tightly regulated process that is restricted to S and G2 phases of the cell cycle due to CDK-dependent phosphorylation of CtIP, a factor that initiates resection at DSB ends in cooperation with the MRN complex [40-45].

DSB repair pathway choice is not only controlled at DSB ends by the extent of DNA-end resection, but also at the level of chromatin modifications in regions flanking the DSB site[37]. Chromatin is extensively modified by post-translational modifications in large regions spanning several hundred kilobases of DNA flanking both sides of a DSB. These regions form cytologically discernible subnuclear structures that are referred to as nuclear foci, DNA damage foci, or **ionizing radiation** (IR)-induced foci (IRIF; [40,41]). The key regulators of IRIF formation are histone proteins that form the core of the nucleosome, the organizational unit of eukaryotic genomes. In response to DSBs, the histone H2A variant H2AX, a component of the nucleosome core structure that comprises 10–15% of total cellular H2A in higher organisms, is rapidly phosphorylated by ATM and DNA-PKcs on a conserved serine residue (Ser139) at its C-terminus. Phosphorylated H2AX (termed γH2AX; [42]) "spreads" over large chromatin domains throughout the cell cycle but is strictly confined to the damaged chromosome and does not involve neighboring undamaged chromosomes [43].

The phosphorylated H2AX C-terminus serves as a chromatin mark that flags regions in the genome that contain DNA breaks. MDC1, a large protein that belongs to the mediator group of DDR factors, specifically binds to the phosphorylated H2AX C-terminus and is the predominant γH2AX recognition factor in mammalian cells [44]. Upon phosphorylation by ATM, MDC1 recruits and activates the ubiquitin ligase RNF8 via direct interaction [45-47]. RNF8 ubiquitylates the linker histone H1 in chromatin regions flanking DSBs, thereby generating an additional histone mark that recruits and activates another ubiquitin ligase, RNF168 [48]. RNF168 mono-ubiquitylates H2A-type histones on Lys15 [49,50], thus generating a binding site for 53BP1, a mediator protein that limits DNA-end resection to promote the fidelity of DSB repair [51,52]. 53BP1 binds to ubiquitylated H2A-type histones via a ubiquitin-dependent recruitment (UDR) motif ensuring its specific binding to damaged chromatin [51]. In addition, 53BP1 contains a Tudor domain that binds specifically to histone H4 when it is di-methylated on Lys20 [53], thus making 53BP1 a bivalent histone mark reader

[54]. Upon accumulation around DSBs, 53BP1 recruits various effector proteins, including RIF1 [55-58], PTIP [59], and the recently discovered shieldin-CST complex that limits end resection to promote the fidelity of DSB repair by NHEJ and HR over mutagenic pathways such as single-strand annealing [52,60-70] (**Figure 1A**). Recent studies revealed that an additional function of 53BP1 and RIF1 (but not shieldin) is to stabilize three-dimensional chromatin topology at DSB sites to safeguard epigenetic integrity [71].

In mitosis, H2AX is also phosphorylated at sites of DSBs and is readily recognized by MDC1 [30]. However, part of the signaling cascade downstream of MDC1 that regulates DSB repair pathway choice is interrupted in mitotic cells. For example, RNF8, RNF168, 53BP1 and BRCA1 are not recruited during mitosis [29,30,72,73]. It was proposed that two roadblocks inhibit recruitment of these proteins: first, a CDK1-dependent inhibition of the RNF8-MDC1 interaction blocks the initiation of chromatin ubiquitylation [74]; and second, the phosphorylation of 53BP1 on two residues in the UDR motif by mitotic kinases that inhibits its interaction with ubiquitylated H2A/H2AX, thus blocking 53BP1 accumulation on damaged chromatin [74,75]. As a result, chromatin ubiquitylation is abrogated throughout most of mitosis, but can occur in telophase [76]. 53BP1 recruitment on the other hand is dependent on the removal of the inhibitory UDR phosphorylations by protein phosphatase 4C (PP4C) and does not occur until cells enter G1 [75,76]. The striking inhibition of the chromatin response to DSBs in mitosis thus raises the question of how DNA breaks are dealt with in this phase of the cell cycle (**Figure 1B**).

Are DSBs repaired in mitosis?

The absence of 53BP1 recruitment to sites of DSBs in mitosis removes a major DNA-end resection roadblock that could in theory allow hyper-resection in mitosis. However, this is not observed in mitotic cells [77,78], possibly due to the fact that chromosomes are too highly condensed for enzymes involved in long-range resection to overcome. In addition to lack of DNA-end resection, key HR factors such as BRCA1 and RAD51 are also not recruited to mitotic DSB sites [30,79]. The bulk of evidence therefore suggests that DSB repair by HR is inactive in mitosis. A lack of RPA-coated ssDNA in mitosis might also explain why ATR is not recruited to DSBs during mitosis, although ATR could be activated in this cell-cycle phase by other mechanisms [80].

The situation is less clear when it comes to NHEJ: while early studies suggested that DSB repair is universally inhibited during mitosis [19,81], and that therefore DSBs are also not repaired by NHEJ until the following G1 phase, the absence of NHEJ during mitosis has never

been demonstrated directly. In fact, there is circumstantial evidence arguing that although chromatin responses are attenuated during mitosis (see above), the DSB repair machinery itself may remain active to some extent. For example, Ku foci still form on mitotic chromatin [82,83], although it is unclear if they are resolved during mitosis or in the following G1. Given that expression of 53BP1 mutants that cannot be phosphorylated by mitotic kinases promote **sister-telomere** fusions [74], it seems likely that at least some NHEJ activity is present in mitotic cells. On the other hand, there is evidence that XRCC4 is phosphorylated by mitotic kinases and that this attenuates its localization at DSB sites [84]. Thus, it is still unclear to what extent the core NHEJ machinery is inhibited during mitosis.

Regardless, in the absence of efficient DSB repair, broken chromosomes represent a major threat for mitotic cells since broken chromatid fragments lacking a centromere (so-called acentric fragments) would be unable to segregate properly without the existence of compensatory mechanisms. A simple such mechanism would be tethering of broken chromosome ends until they can be repaired in the following G1 phase. Evidence for the existence of a tethering mechanism has been described in *Drosophila melanogaster*, where acentric chromatid fragments segregate efficiently to opposite poles because of a DNA tether that is covered with the mitotic kinases Polo (PLK1 in vertebrates), Aurora B and BubR1 [85] (**Figure 2A**). One consequence of BubR1 localization on these DNA tethers is the local inhibition of the **anaphase-promoting complex/cyclosome** (APC/C) complex, which may be required for proper transmission of broken chromosomes in *Drosophila* [86]. It is currently not clear if DSBs are similarly stabilized during mitosis in mammalian cells, but inhibition of the APC/C is the outcome of the spindle checkpoint, and it is known that in human cells this checkpoint is not activated by DSBs. However, recent evidence suggests that tethering of acentric chromatid fragments during mitosis may at least exist in mammalian cells [15]. As described above, the chromatin response to DSBs is only partially disrupted in mitosis, such that the upstream events such as H2AX phosphorylation and MDC1 recruitment still occur, and the kinase activities of ATM and DNA-PKcs are important for cell survival in response to mitotic DNA breaks [30]. It was recently shown that a major role of the chromatin response to DSBs during mitosis is the recruitment of the mediator protein TOPBP1 [15]. TOPBP1 is a versatile protein with multiple roles in DNA replication, DNA repair and transcription [16]. In interphase cells, TOPBP1 is recruited to DSBs via interaction with 53BP1 and other factors [87], but in mitosis, it is recruited to sites of DSBs via direct phosphorylation-dependent interaction with MDC1. Disruption of this interaction results in chromosomal instability that likely originates from errors in mitosis [15]. Interestingly, TOPBP1 accumulates at sites of

DSBs in mitosis in filamentous assemblies that frequently bridge two MDC1 IRIF either within (intra) or occasionally between (inter) chromosomes, indicating that they may represent structures that tether broken chromosomes [15] (**Figure 2B**). Whether or not these tethers are related to the BubR1-coated DNA tethers observed in *Drosophila* is currently not clear, but it is interesting to note that *Drosophila* strains lacking the MDC1 orthologue *MU2* show high frequencies of terminal deficiencies (i.e. chromosomes that have lost their tip regions) [88], which would be an expected outcome of defective tethering of acentric fragments in mitosis and are also observed in human cells lacking MDC1-TOPBP1 interaction [15].

Consequences of defective responses to DNA breaks during mitosis

If a DSB that occurs in mitosis fails to be repaired or tethered, the affected chromosome breaks into two pieces: an acentric chromosome fragment and a centric chromosome fragment, each containing a telomere on one end, and free DNA end on the other (**Figure 3**). Since the acentric fragment is not able to interact with the mitotic spindle, it lingers near the equatorial plate during anaphase. These acentric fragments usually randomly segregate into the cytoplasm of one of the daughter cells and are converted into micronuclei (see below).

The centric fragment on the other hand can attach to the spindle and thus be properly segregated into one of the daughter cells, but its free DNA end can potentially fuse to another centric fragment from a different chromosome or to an intact chromosome with a deprotected telomere in the subsequent G1, leading to the formation of a **dicentric chromosome** [89,90]. These dicentric chromosomes are sources of so-called breakage-fusion-breakage (BFB) cycles, first described by Barbara McClintock in 1941 [91]. BFB cycles are a major source of chromosomal instability in cancer [92].

Micronuclei on the other hand are associated with heavily deregulated DNA metabolism and can thus give rise to a unique form of chromosomal instability termed chromothripsis [93], where large numbers of clustered chromosomal rearrangements occur in a confined genomic region typically only on one chromosome. Chromothripsis can occur when a micronucleus is reincorporated into the nucleus in a subsequent mitosis [94]. Thus, failed responses to DSBs in mitosis can critically contribute to the formation of the kind of chromosomal instability frequently observed in cancers (**Figure 3**).

It is well established that genotoxic stress leads to inflammatory responses. For example, exposure of cells to DSB-inducing agents leads to the expression of **type I interferons**, which activates p53 and induces senescence [95]. It was originally suggested that DNA fragments leaking from the site of DNA damage in the nucleus into the cytosol are recognized by cyclic

GMP-AMP synthase (cGAS), an important sensor of cytosolic DNA that activates innate immunity by engaging the stimulator of interferon genes (STING) cascade [96,97]. However, two recent studies identified micronuclei as the main source of immune stimulatory cytosolic DNA [98,99]. Even though micronuclei are structurally comparable to primary nuclei, their NE is unstable, resulting in frequent NE breakdown [100], which leads to the exposure of fragmented DNA from within the micronuclei to cGAS and activates an inflammatory response (**Figure 3**). Thus, a failed response to DNA breaks in mitosis may have physiological consequences beyond accumulation of chromosomal instability and may contribute to shape the immune microenvironment of tumors.

Under-replicated DNA as an endogenous source of mitotic DSBs

Due to the relative amount of time cells spend in mitosis compared to interphase, the majority of DSBs that cells acquire from exogenous sources would be expected to occur and be repaired in interphase. Clearly however, the fact that mitosis-specific DSB responses exist and are apparently conserved from some single-celled organisms to humans [15], means that DSBs must occur to a significant extent during cell division and be recognised for maintenance of genome stability. What might the source of such DSBs be? Previous work indicates that at least some of the DSBs that occur during mitosis are introduced deliberately by cells as a result of unfinished DNA replication in the previous S phase.

Under-replicated DNA often arises from specific regions of the genome called **common fragile sites** (CFSs), which are replicated late in S phase and are sensitive to breakage under conditions of replication stress such as aphidicolin treatment (when CFSs are said to be "expressed"; [101]). CFS expression is an active process, promoted by RAD52 and a large protein complex containing SLX1-SLX4, MUS81-ERCC1 and XPF-ERCC1 (SMX) nucleases [102-105]. Under-replicated regions that have been processed by SMX can still be replicated even though cells have passed through S phase, via a break-induced conservative DNA replication pathway called **MiDAS** (mitotic DNA synthesis) that requires TOPBP1 [106], POLD3 (a non-catalytic subunit of DNA polymerase δ) [104,107], and prior disassembly of the S phase replisome by the ubiquitin ligase TRAIP [108-110].

Concluding remarks

The key features of the specialized response to DNA breaks in mitosis can be summarized as follows. Upon entering prophase, cells do not arrest the cell cycle in the presence of DSBs and

instead prioritize completion of cell division over repair of DNA breaks. The chromatin response to DSBs is truncated in mitosis. Even though H2AX is phosphorylated in mitosis and recognized by MDC1, recruitment of key downstream factors that regulate DSB repair pathway choice such as 53BP1 and BRCA1 is blocked. Also, DNA-end resection, a pre-requisite for DSB repair by HR, is limited. Instead, cells utilize a pathway to tether broken chromosomes that involves recruitment of TOPBP1 by direct interaction with MDC1, which is critical for suppression of chromosomal instability during mitosis. A failed DSB response in mitosis results in micronuclei formation, which can lead to chromothripsis and activation of innate immune responses via the cGAS-STING pathway.

Despite recent insights, one key unanswered question (**see Outstanding questions box**) is if DSBs that occur during mitosis are repaired or tethered until NHEJ is reactivated in the following G1. Indirect evidence exists for both options and it is formally possible (perhaps even likely) that DSBs in mitosis can be either tethered or repaired, depending on the context of when and where they occur and whether they have compatible ends for repair. TOPBP1 has emerged as a key factor in the response to mitotic DSBs. Therefore, it will be essential to define the molecular mechanisms by which it suppresses chromosomal instability in mitosis. This will include the detailed characterization of how its recruitment to DSBs in mitosis is regulated, how TOPBP1 bridges form, as well as the identification of putative downstream factors that are regulated by the MDC1-TOPBP1 complex. A deeper mechanistic understanding of how cells deal with DNA breaks in mitosis may lead to new ways to exploit these phenomena for anti-cancer therapies.

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Figure legends

Figure 1: The response to DNA double-strand breaks at damaged chromatin regions is re-wired in mitosis.

(A) In response to a DNA double-strand break in interphase cells, the histone H2A variant H2AX is phosphorylated in chromatin flanking the break by the kinases ATM and DNA-PK. The phosphorylated H2AX (γH2AX) mark is read by MDC1, which recruits the ubiquitin (Ub) ligase RNF8, thereby launching a ubiquitylation signalling cascade on damaged chromatin resulting in recruitment of BRCA1, 53BP1 and its downstream effectors TOPBP1, RIF1 and shieldin/CST.

(B) In mitosis, H2AX is still phosphorylated and MDC1 is recruited but CDK1-dependent phosphorylation of RNF8 inhibits its interaction with MDC1 and leads to the abrogation of the chromatin ubiquitylation cascade. In addition, mitotic kinases prevent 53BP1 recruitment by phosphorylating it in its ubiquitin-dependent chromatin interaction domain. TOPBP1, which is recruited by 53BP1 in G1, is directly recruited by MDC1 in mitosis to tether broken chromosome ends until DNA repair is reactivated in the following interphase.

Figure 2: Potential DNA end-tethering mechanisms in *Drosophila* and mammalian cells.

(A) DSBs induced by the I-Cre1 endonuclease in the rDNA repeats of chromosome X in *Drosophila* cells produce acentric fragments that efficiently segregate poleward due to DNA tethers that connect them to the centric fragments. These DNA tethers are decorated with the mitotic kinases BubR1, Polo and Aurora-B, as well as INCENP. Downregulation of BubR1 and Polo function results in acentric segregation defects.

(B) IR-induced DSBs in prometaphase chromosomes in human cells lead to γH2AX formation and recruitment of MDC1. Through direct phosphorylation-dependent interaction, MDC1 mediates the accumulation of filamentous TOPBP1 assemblies at sites of mitotic DSBs. These TOPBP1 filaments can bridge two MDC1 IRIF and may thus represent tethering structures. Disruption of MDC1-TOPBP1 complex formation leads to defective TOPBP1 recruitment in mitosis and chromosomal instability.

Figure 3: Physiological consequences of a failed response to DNA breaks in mitosis.

If DSBs are not tethered or repaired in mitosis, acentric fragments are not segregated and can form micronuclei. DNA metabolism is heavily disturbed in micronuclei,

which results in chromatin fragmentation. If a micronucleus is re-incorporated in the main nucleus, it may induce chromothripsis. In addition, the nuclear envelopes of micronuclei are unstable, which allows exposure of fragmented chromatin to cGAS, a cytosolic sensor of DNA. This leads to an inflammatory response via activation of STING-dependent type I interferon production. Centric fragments from unrepaired DSBs during mitosis are properly segregated but end up with terminal deficiencies or form dicentric chromosomes, which in subsequent cell cycles, lead to chromosome bridges in anaphase and BFB cycles.

Glossary box

Interphase: the segment of the cell cycle that lies between two cell divisions. In interphase the chromatin is replicated and the cell prepares for cell division. It is sub-divided in synthesis phase (S phase) and two gap phases (G1 and G2).

Senescence: irreversible cell-cycle arrest characterized by alterations in gene expression patterns and genome organization.

Apoptosis: a highly regulated and tightly controlled form of programmed cell death that occurs in multicellular organisms, mediated in response to DNA damage by the tumour suppressor p53.

Nuclear envelope: two lipid bilayer membranes surrounding the cell nucleus and encasing the genetic material. Nuclear pores in the nuclear envelope allow exchange of materials between the cytosol and the nucleus.

Cell-cycle checkpoints: control mechanisms in eukaryotic cells that block or slow cell-cycle progression to ensure proper cell division in response to stress. DNA damage checkpoints are the G1/S checkpoint, the intra-S phase checkpoint and the G2/M checkpoint.

Chromosome segregation: a mitotic process in which the two sister chromatids separate from each other and migrate to opposite poles of the nucleus.

Centromere: a specialized DNA sequence on chromosomes where the sister chromatids are paired together. During mitosis, spindle fibres attach to the centromere via the kinetochore.

Kinetochore: a disc-shaped protein structure associated with centromeres where the spindle fibres attach during cell division to pull sister chromatids apart.

Spindle assembly checkpoint: a cell-cycle checkpoint that prevents separation of the sister chromatids until each chromosome is properly attached to the spindle apparatus.

DNA-end resection: nucleolytic processing of DNA ends at sites of DSBs that generates 3' single-stranded DNA overhangs necessary to induce the process of homologous recombination.

Ionizing radiation: types of radiation (including X-rays or gamma rays) that carry sufficient energy to detach electrons from molecules, thus ionizing them.

Mediator: a protein that mediates the interaction between two proteins that are unable to interact directly with each other.

Sister telomeres: telomeres at the tips of the paired sister chromatids. In early phases of mitosis, sister telomeres are located in close proximity to each other.

Anaphase-promoting complex/cyclosome (APC/C): a multi-subunit E3 ubiquitin ligase complex that targets various cell-cycle proteins for degradation by the proteasome and thus mainly regulates metaphase to anaphase transition.

Mitotic spindle: a cytoskeletal structure mostly composed of microtubules that forms during mitosis to separate sister chromatids.

Dicentric chromosome: abnormal chromosome that contains two centromeres. They can form by fusion of two chromosome segments that each contain a centromere or by intra-chromosomal recombination and give rise to anaphase chromosome bridges and breakage-fusion-breakage cycles.

Type I interferons: a group of signalling proteins of the innate immune system mainly important for fighting viral infections. Type I interferons bind to the specific cell surface receptor IFN-α/β.

Common fragile sites (CFSs): regions of chromosomes that preferentially acquire gaps or breaks after replication stress. A commonly used inducer of CFSs is aphidicolin, which inhibits DNA polymerase α.

MiDAS: a mitotic DNA synthesis pathway activated by DNA replication stress, required if cells leave S phase before finishing DNA replication.

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690

Highlights

- DNA double-strand breaks (DSBs) are one of the most toxic types of DNA lesions. Although much is known about how cells respond to DSBs in interphase, far less is understood about how they respond to such lesions during mitosis.

- The major DSB repair pathways, non-homologous end-joining and homologous recombination, are inhibited during mitosis, and there is no DNA damage checkpoint after late prophase until cells re-enter G1 when mitosis is complete.

- DSBs that occur during mitosis must be stabilized until the next G1 phase in order to avoid mis-segregation of acentric fragments and chromosomal instability.

- The chromatin response to DSBs is rewired during mitosis. Chromatin ubiquitylation is blocked and 53BP1, BRCA1 and their downstream effectors are not recruited. Instead, TOPBP1 is recruited via direct binding to MDC1 and this mechanism is required to maintain chromosomal stability.

- Micronuclei from a failed DSB response during mitosis can induce chromothripsis and trigger an inflammatory response by activating cGAS-STING signalling.

Outstanding questions

- Can breaks acquired during mitosis be repaired by an end-joining mechanism or is DSB repair completely blocked in mitosis? Could tethering of chromosome fragments during mitosis be a compensatory mechanism to prevent mis-segregation of acentric fragments and micronuclei formation?

- Are the mitotic kinases BubR1, PLK1 and Aurora B also implicated in tethering of acentric chromosome fragments in mammalian cells, similar to their *Drosophila* orthologues? And are these proteins recruited to sites of DSBs by γH2AX-MDC1-TOPBP1 in mitosis?

- What is the molecular function of TOPBP1 during mitosis? TOPBP1 is a classical adaptor protein that mediates protein-protein interactions. What protein interactions are mediated by TOPBP1 in mitosis and how are filamentous TOPBP1 tethers assembled and regulated?

- What is the physiological source of mitotic DSBs? Are most DSBs encountered during mitosis a result of problems inherited from the previous S phase?

- How important is the response to DSBs in mitotic cells for multicellular organisms? Are specific tissues or stages of development affected more if DSBs acquired during mitosis cannot be stabilized and/or repaired?

Figure 1 (original)

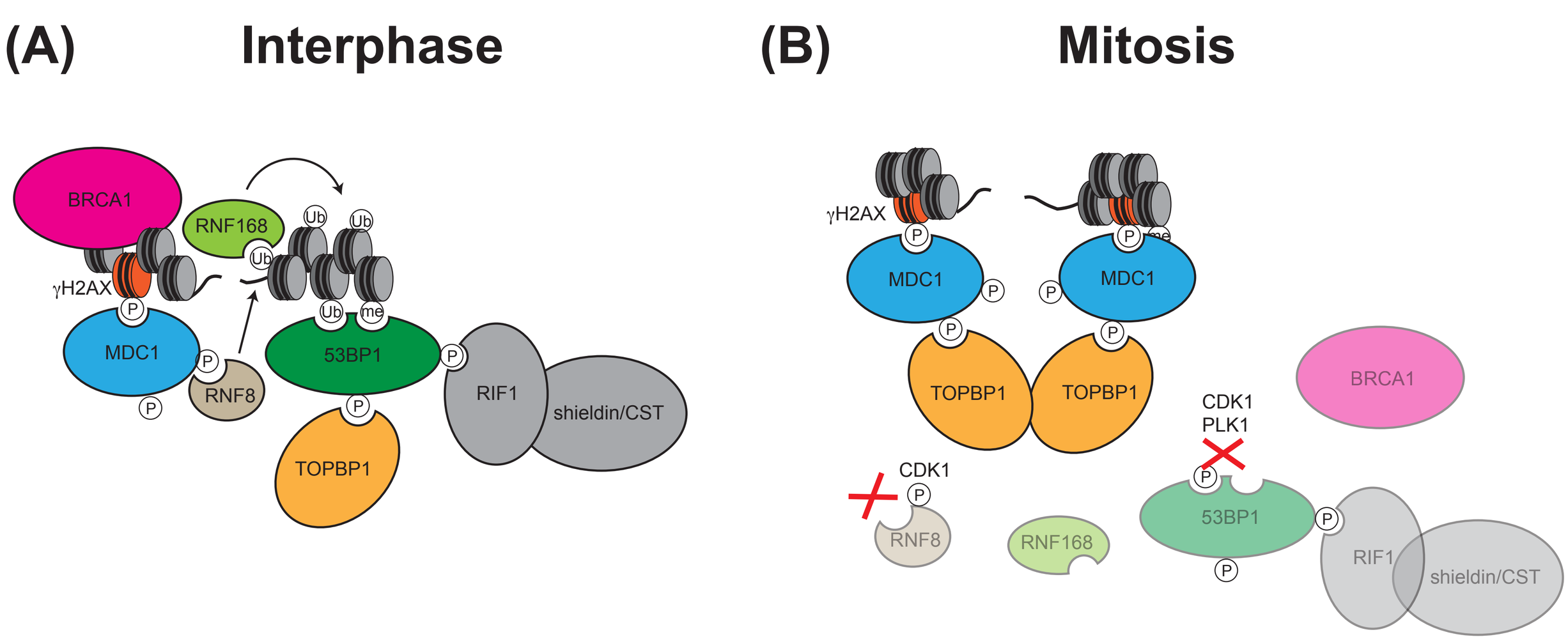

Figure 2 (original)

(A) Fruit fly

Metaphase

I-CreI-induced
DNA breaks in
rDNA repeats

D. melanogaster
Chromosome X

Anaphase

spindle
microtubules

BubR1
Aurora-B
POLO
INCENP
DNA tether

(B) Human

Metaphase

IR-induced
chromosome
break

Anaphase

spindle
microtubules

MDC1
P
TOPBP1
TOPBP1
P
MDC1

Figure 3 (original)