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STATE-OF-THE-ART REVIEW

DNA damage response proteins regulating mitotic cell division: double agents preserving genome stability

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Keywords
DNA damage; mitosis; mitotic spindle; spindle checkpoint; Chk1; Chk2; ATM; ATR; MRN; BRCA

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The DNA damage response recognizes DNA lesions and coordinates a cell cycle arrest with the repair of the damaged DNA, or removal of the affected cells to prevent the passage of genetic alterations to the next generation. The mitotic cell division, on the other hand, is a series of processes that aims to accurately segregate the genomic material from the maternal to the two daughter cells. Despite their great importance in safeguarding genomic integrity, the DNA damage response and the mitotic cell division were long viewed as unrelated processes, mainly because animal cells that are irradiated during mitosis continue cell division without repairing the broken chromosomes. However, recent studies have demonstrated that DNA damage proteins play an important role in mitotic cell division. This is performed through regulation of the onset of mitosis, mitotic spindle formation, correction of misattached kinetochore–microtubules, spindle checkpoint signaling, or completion of cytokinesis (abscission), in the absence of DNA damage. In this review, we summarize the roles of DNA damage proteins in unperturbed mitosis, analyze the molecular mechanisms involved, and discuss the potential implications of these findings in cancer therapy.

An introduction to the DNA damage response and mitotic cell division

The DNA damage response is a complex network of signaling pathways that coordinates cellular reactions to DNA lesions. Such reactions range from cell cycle arrest and DNA damage repair to the induction of apoptosis or senescence. Defects in DNA damage signaling and repair can lead to genomic instability and are associated with tumorigenesis and human disorders [1]. The DNA damage response has been covered in several excellent reviews [1–4], and this review will only touch upon aspects that are relevant to mitotic cell division. Briefly, in vertebrate cells, the two main DNA damage signaling pathways consist of the ATM–Chk2 and ATR–Chk1 protein kinases. Ataxia-telangiectasia mutated (ATM) and ATM and Rad3 related (ATR) are members of the family of phosphoinositide-3-kinase-related kinases; keys to their substrates are the checkpoint effector kinases Chk1 and Chk2 that mediate a wide range of downstream responses. The ATM–Chk2 and ATR–Chk1 pathways respond to different DNA lesions. ATM is recruited to and activated primarily at DNA double-strand breaks via interaction with the Mre11-Rad50-Nbs1 (MRN) sensor complex [5,6]. In turn, active ATM acts locally to phosphorylate several substrates including the

Abbreviations
53BP1, p53 binding protein 1; AID, auxin-inducible degron; APC/C, anaphase-promoting complex/cyclosome; ATM, ataxia-telangiectasia mutated; ATR, ataxia-telangiectasia mutated and Rrad3-related; ATRIP, ATR-interacting protein; BRCA, breast cancer susceptibility gene; CPC, chromosomal passenger complex; CSF, cytostatic factor; ESCRT, endosomal sorting complex required for transport; FRET, fluorescence resonance energy transfer; GFP, green fluorescence protein; GTP, guanosine triphosphate; MDC1, mediator of DNA damage checkpoint 1; MMAP, MRN-associated protein; MRN, Mre11-Rad50-Nbs1; Plk1, Polo-like kinase 1.

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variant histone H2AX at serine 139 (γ-H2AX, a widely used marker of damaged DNA), the MRN complex itself and the downstream kinase Chk2 to induce Chk2 catalytic activity [7,8]. Once activated, Chk2 phosphorylates multiple targets inside the nucleus that are involved in cell cycle progression or apoptosis, including, for example, the p53 tumor suppressor protein and Cdc25 family phosphatases [9–12]. Other proteins recruited to sites of double-strand breaks include human mediator of DNA damage checkpoint 1 (MDC1), p53 binding protein 1 (53BP1), and breast cancer susceptibility gene 1 (BRCA1), which are ATM substrates and mediators in the DNA damage response [13].

ATR–Chk1 signaling on the other hand is activated most strongly when DNA replication is blocked, for example, after nucleotide depletion, inhibition of DNA polymerases, or UV-induced DNA lesions [3,14]. In these cases, DNA polymerases become uncoupled from the replicative helicase; as a result, tracts of single-strand DNA are generated and coated with replication protein A (RPA) [15]. ATR is recruited to such tracts through association of its partner ATR-interacting protein ATRIP with RPA and is activated by interaction with TopBP1 [16,17]. ATR is also activated in the presence of double-strand breaks when single-strand DNA is generated by nucleolytic strand resection [18]. Catalytically active ATR phosphorylates Chk1 at multiple sites including serines 317 and 345 within the C-terminal regulatory domain, and this step is essential for Chk1 biological activity [19,20]. In turn, active Chk1 dissociates from chromatin and phosphorylates several substrates [3,14]. Among others, Chk1 phosphorylates and inhibits Cdc25 family phosphatases and stimulates the activity of Wee1 to maintain high levels of inhibitory Cdk1 phosphorylation and prevent the onset of mitosis while DNA damage persists [12,21,22].

The mitotic cell division is one of the most dramatic cellular processes during which segregation of the replicated chromosomes (mitosis) is followed by cytoplasmic fission (cytokinesis) to give rise to two genetically identical daughter cells. Errors in mitotic cell division can lead to aneuploidy and chromosomal instability (i.e., continuous loss or gain of whole chromosomes or chromosome parts) that are associated with carcinogenesis [23–25]. During cell division, breakdown of the nuclear envelope enclosing condensed chromosomes is quickly followed by the assembly of the mitotic spindle, a microtubule-based apparatus that mediates chromosome alignment and segregation [26,27]. Furthermore, the mitotic spindle has a 'built-in' regulatory mechanism called the 'mitotic spindle checkpoint' (also known as 'spindle assembly checkpoint', SAC) that delays chromosome segregation until all sister kinetochores are stably attached to spindle microtubules to prevent erroneous chromosome segregation (reviewed in Refs [28–30]). After chromosome separation, cleavage furrow ingression in animal cells is mediated by a contractile actomyosin ring to generate a narrow intercellular canal that is later cleaved during abscission to release the two daughter cells [31,32].

The DNA damage response and the mitotic cell division were long viewed as unrelated processes with the DNA damage response preserving genome stability during interphase and the cell division machinery ensuring accurate distribution of the genetic material to the daughter cells. This was supported by early studies showing that vertebrate somatic cells irradiated in the nucleus during or after late prophase progress normally through mitosis thus exhibiting lack of an acute DNA damage-induced cell cycle arrest [33–35]. Furthermore, more recent studies have shown that mitotic cells can only mount a partial ('primary') DNA damage response in which γ-H2AX is phosphorylated and ATM, MRN, and MDC1 are recruited to double-strand break sites, whereas later ('secondary') DNA damage response cell signaling events such as recruitment of the E3 ubiquitin ligase RNF8, the nonhomologous end-joining factor 53BP1, or BRCA1 are suppressed [36–38]. As a result, cells 'mark' the damaged DNA for repair in the following G1 phase of the cell cycle but do not repair DNA lesions in mitosis, presumably to prevent telomere fusions and micronuclei formation [37]. However, in the last few years, several studies have reported a cross-talk between the DNA damage response and mitotic machineries to ensure optimal genome integrity in the absence of DNA damage. In this review, we summarize recent findings of DNA damage proteins regulating mitotic cell division in higher eukaryotic cells, analyze the molecular mechanisms involved, and discuss the potential relevance of these findings to cancer therapy.

Chk1 and other DNA damage response proteins in mitotic entry and mitotic progression

In eukaryotic cells, initiation of mitosis requires activation of Cdk1-cyclin B (Cyclin-dependent kinase 1) homologs. Cdk1-cyclin B is kept inactive during interphase through inhibitory phosphorylation of Cdk1 at the conserved sites threonine 14 (T14) and tyrosine 15 (Y15) by Myt1 and Wee1 kinases [39,40]. Just before mitosis, the activity of dual-specificity Cdc25 family

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phosphatases toward Cdk1-T14/Y15 exceeds that of the opposing kinases Myt1 and Wee1 resulting in Cdk1-cyclin B activation. Initial activation of the Cdk1-cyclin B complex occurs at the centrosome in mammalian cells; subsequently, active Cdk1-cyclin B accumulates to the nucleus to irreversibly commit the cell to mitosis [41,42]. Untimely activation of Cdk1-cyclin B can lead to premature entry to mitosis before the completion of S- or G2-phases and cell death by mitotic catastrophe [43].

Chk1 kinase is a master regulator of the DNA damage checkpoint response in mammalian cells [3,14]. Importantly, Chk1 also regulates mitotic entry during the unperturbed cell cycle, that is, in the absence of DNA damage [44,45]. It was initially reported that a relatively small fraction of human Chk1 localizes to centrosomes in interphase to prevent premature activation of Cdk1-cyclin B [44]. However, a more recent study shows that the anti-Chk1 antibody DCS-310 used cross-reacts with a different centrosomal protein as evidenced by persistent centrosomal staining in conditional Chk1-knockout mouse embryonic fibroblasts by this antibody [46]. Chk1 is phosphorylated at serines 286 (S286) and 301 (S301) by Cdk1 during mitosis [47]. Myc-tagged Chk1 harboring nonphosphorylatable mutations of S286 and S310 to alanine (S286A/S301A) localizes mainly in the nucleus, whereas wild-type Myc-Chk1 is detected in both nucleus and the cytoplasm in prophase by immunofluorescence [45]. Furthermore, expression of S286A/S301 Myc-Chk1 or Myc-Chk1-3xNLS in which Chk1 is fused to three nuclear localization sequences, delays entry to mitosis as judged by microscopic examination of cells after release from a double-thymidine block [45,46]. It is proposed that Chk1-S286/S310 phosphorylation by Cdk1 is required for cytoplasmic sequestration of Chk1 in prophase, to release nuclear Cdc25 inhibition, and promote robust Cdk1 activation in prophase (Fig. 1). Perhaps significantly, constitutive targeting of wild-type GFP:Chk1 to centrosomes by fusion with the pericentrin-AKAP450 centrosomal targeting (PACT) domain of AKAP450, increases the frequency of cells exhibiting phosphorylated Cdk1-Y15 (inactive Cdk1) at centrosomes by immunofluorescence compared with controls expressing kinase-dead GFP:Chk1:PACT, suggesting persistent Chk1 activity at centrosomes can delay Cdk1 activation [44]. However, the potential role of Chk1 in regulating centrosomal Cdk1 activity under physiological conditions requires further investigation [44,46].

Other DNA damage response proteins also have roles in mitotic progression: Loss of ATM or BRCA2 function reduces the time from nuclear envelop breakdown to anaphase onset in agreement with a role for these

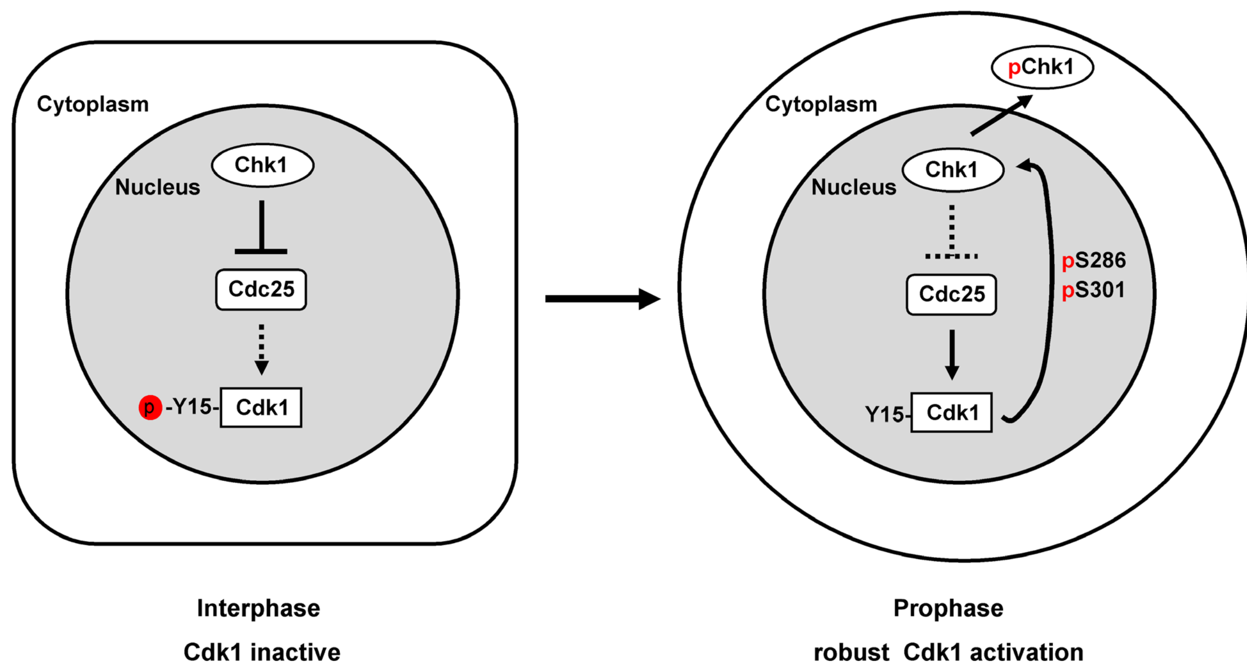

Fig. 1. A role for Chk1 kinase in mitotic entry. Cdk1 phosphorylates Chk1 to promote cytoplasmic sequestration of Chk1 in prophase. This releases inhibition of nuclear Cdc25 phosphatase to promote robust Cdk1 activation and mitotic entry. Dotted lines indicate suppressed molecular interactions. p, phosphorylation.

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proteins in spindle checkpoint signaling [48,49]. Also, 53BP1-depleted cells spent less time in mitosis compared with controls [50]. In contrast, Chk2-deficient cells exhibit a prometaphase delay compared with controls, which is consistent with a role for Chk2 in mitotic spindle formation [51]. The molecular mechanisms involved will be described in later paragraphs.

DNA damage response proteins in mitotic spindle formation

The mitotic spindle is responsible for faithful chromosome segregation during cell division, and errors in spindle formation can lead to aneuploidy or cytokinesis failure, which are associated with tumorigenesis [23–25,27]. For construction of the mitotic spindle, microtubules are nucleated from centrosomes, chromosome proximal regions, and preexisting spindle microtubules; however, the relative contribution of each pathway differs among species [52]. In the centrosomal spindle assembly pathway, microtubules nucleated from centrosomes are captured by kinetochores to form kinetochore–microtubule fibers ('search and capture' model, [53]). In the chromosomal pathway, microtubules are nucleated around chromosomes and organized into antiparallel arrays to generate the bipolar spindle ('self-assembly' model) [54,55]. The chromosomal pathway relies on the establishment of a RanGTP gradient around mitotic chromosomes: RanGTP binds to importins and dissociates nuclear import complexes that contain nuclear localization signal-bearing cargos. As a result, RanGTP directs the activity and/or localization of spindle assembly factors and mitotic regulators around chromosomes to promote spindle assembly [56]. In most animal somatic cells that contain centrosomes, the centrosomal cooperates with the chromosomal pathway with the centrosomal pathway being predominant.

Chk2 and BRCA1 in spindle formation

The early-onset breast cancer susceptibility gene *BRCA1* encodes a pleiotropic DNA damage response protein that functions in checkpoint activation and DNA repair [57]. Furthermore, Chk2 phosphorylates BRCA1 on serine 988 (S988) to promote repair of DNA double-strand breaks [58]. Using HCT116 Chk2-knockout cells and human cells depleted of Chk2 or BRCA1 by stable expression of shRNAs, Stolz *et al* showed that Chk2- or BRCA1-deficient cells exhibit delayed anaphase onset, increased frequency of disorganized spindles and misaligned chromosomes in metaphase, relatively high levels of missegregated chromosomes in anaphase and chromosomal instability compared with controls [51]. Chk2 localizes to mitotic centrosomes [59] and is phosphorylated at the activation sites threonine 68 and threonine 387 in mitotic cell extracts [51]. BRCA1 localizes to mitotic centrosomes according to one study [60] or forms foci that surround chromatin or the mitotic spindle according to a different study [61]. Expression of the phosphomimetic S988 to glutamic acid (S988E), but not the nonphosphorylatable S988 to alanine (S988A), BRCA1 suppresses abnormal metaphase spindles and rescues proper chromosome alignment in BRCA1-deficient or Chk2-deficient cells, suggesting Chk2 phosphorylates BRCA1-S988 to promote proper spindle formation in human cells [51]. Mechanistically, inhibition of Chk2 or expression of nonphosphorylatable mutant S988A BRCA1 increases activation of the mitotic kinase Aurora A at centrosomes in prometaphase (judged by Aurora A–threonine 288 phosphorylation on its activation loop) and enhances the microtubule polymerization rate compared with control cells [62]. Aurora A promotes microtubule assembly by inducing localization of microtubule-associated proteins at centrosomes and relatively high microtubule assembly rates in cells overexpressing Aurora A or deficient for Chk2 correlates with transient spindle abnormalities and generation of lagging chromosomes compared with controls [62]. Also, Chk2-knockout cells or cells expressing mutant BRCA1-S988A exhibit reduced association of the PP6C-SAPS3 phosphatase (a phosphatase that dephosphorylates the Aurora A activation loop to deactivate Aurora A) with BRCA1 by co-immunoprecipitation experiments and increased levels of active Aurora A at centrosomes compared with controls [62]. It is proposed that Chk2 phosphorylates BRCA1-S988 to induce localization of PP6C-SAPS3 phosphatase and restrain Aurora A catalytic activity at centrosomes to promote proper spindle assembly (Fig. 2A).

Xenopus egg extracts have the advantage of allowing independent examination of both centrosomal and chromosomal spindle assembly pathways: On one hand, adding sperm chromosomes to cytostatic factor (CSF)-arrested egg extracts can induce functional centrosome formation and spindle assembly from both centrosomal and chromosomal pathways [63]. On the other hand, adding plasmid DNA-coated beads or RanGTP to CSF-arrested egg extracts is sufficient to cause the formation of spindle-related structures in the absence of centrosomes, resembling chromosome-driven spindle formation [54,64,65]. The BRCA1-BARD1 (BRCA1-associated RING domain protein 1) heterodimer is also implicated in Ran-dependent mitotic

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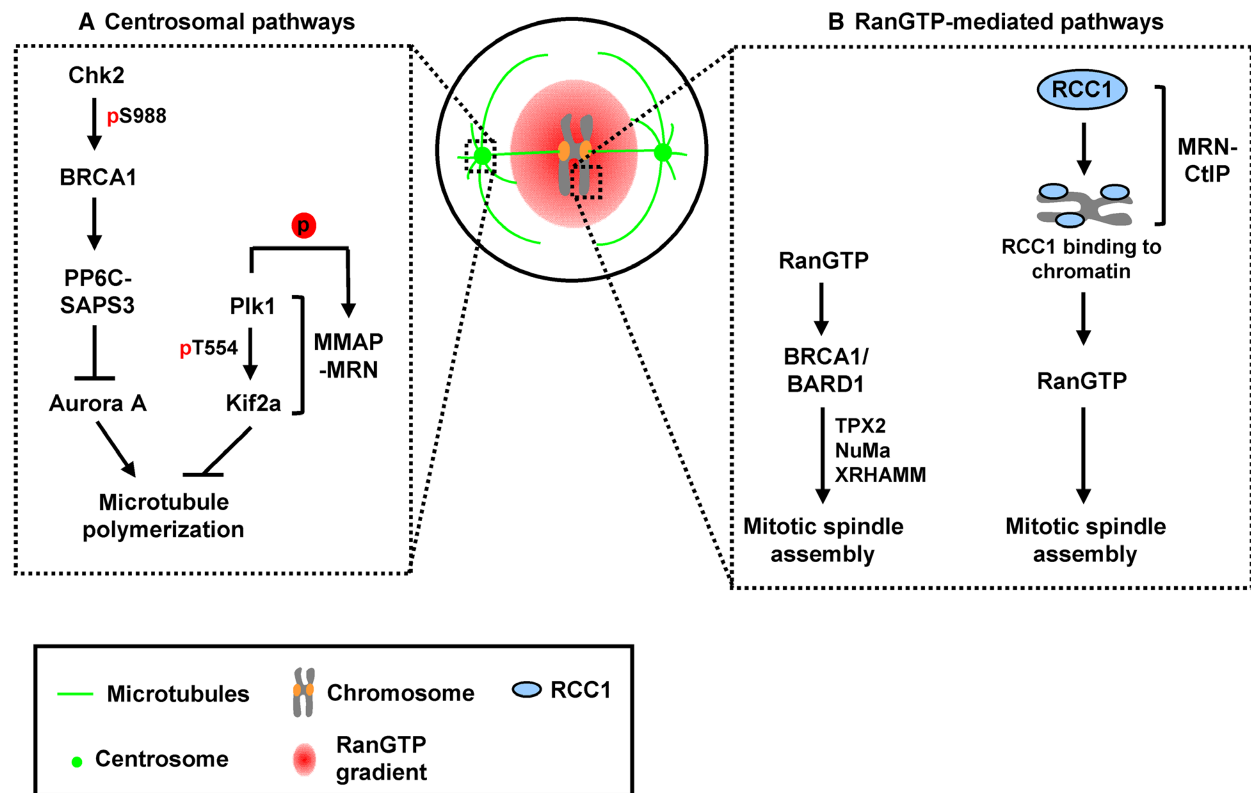

Fig. 2. DNA damage response proteins regulating mitotic spindle formation in higher eukaryotic cells. (A) Centrosomal spindle assembly pathways. Chk2 promotes proper spindle formation by phosphorylating BRCA1 to induce localization of PP6C-SAPS3 phosphatase and restrain Aurora A catalytic activity at centrosomes. The MMAP-MRN complex also acts as platform to facilitate Kif2a phosphorylation by Plk1 kinase to promote proper turnover of spindle microtubules. (B) RanGTP-mediated pathways. The MRN-CtIP complex promotes RCC1 chromatin association with establish a RanGTP gradient around the mitotic chromosomes. Also, the BRCA1-BARD1 complex participates in Ran-dependent mitotic spindle assembly by promoting proper localization of spindle pole-organizing proteins. Square brackets indicate a scaffolding role for the respective proteins. p, phosphorylation.

spindle assembly [61]. Immunodepletion of BRCA1/BARD1 from CSF-arrested Xenopus egg extracts supplemented with replicated chromatin or depletion of BRCA1/BARD1 from HeLa cells by siRNAs increases the frequency of disorganized spindles and unaligned chromosomes in metaphase compared with mock-treated egg extracts or control cells [61]. Furthermore, BRCA1/BARD1-depleted Xenopus egg extracts supplemented with a constitutively active Ran mutant to imitate chromosome-driven spindle assembly exhibit unfocused spindle poles compared with mock-treated controls, suggesting BRCA1/BARD1 is required for non-centrosomal spindle organization [61]. Both chromatin-induced and RanGTP-induced asters exhibit disorganized poles and diffused binding of the spindle pole protein TPX2 to spindle microtubules in BRCA1/BARD1-depleted egg extracts compared with mock-treated controls. Similarly, TPX2 mislocalizes on spindle microtubules in HeLa BRCA1/BARD1-deficient cells compared with controls. In addition, BRCA1/BARD1 interacts with the spindle pole-organizing proteins TPX2, NuMA, and XRHAMM by co-immunoprecipitation experiments in CSF-arrested Xenopus egg extracts [61]. It is proposed that the BRCA1-BARD1 complex promotes Ran-dependent mitotic spindle assembly by promoting proper localization of spindle pole-organizing proteins in Xenopus and perhaps in human cells (Fig. 2B).

The MRN complex in spindle formation

The evolutionary conserved MRN protein complex plays multiple roles in the DNA damage response, such as activation of the central kinase ATM and regulation of double-strand DNA repair [66]. Recent studies have also implicated MRN in proper spindle formation through at least two separate mechanisms [67,68].

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Firstly, MRN forms a mitosis-specific complex with the long isoform of C2orf44 protein (renamed mitosis-specific MRN-associated protein, MMAP) to regulate spindle dynamics [67]. Co-immunoprecipitation analysis showed that MMAP interacts with the C-terminal region of MRE11 in mitotic cell extracts; however, CtIP protein that interacts with MRN to initiate DNA resection is not detected in the MMAP-associated complexes [67]. Human HCT116 cells in which MMAP is disrupted by CRISPR-mediated gene targeting exhibit reduced levels of MRN proteins in mitotic cell extracts compared with controls, suggesting MMAP is required for optimal stability of the MRN complex in mitosis [67]. MMAP colocalizes with MRN proteins at spindle poles and in the mitotic spindle by fluorescence microscopy. Furthermore, MMAP-deficient cells or HCT116 cells depleted of MRN proteins exhibit increased levels of spindle microtubules, relatively slow microtubule turnover on metaphase spindles by fluorescence loss in photobleaching experiments, delayed anaphase onset, and higher frequency of unaligned chromosomes in metaphase compared with controls [67]. Mechanistically, the mitotic kinase Plk1 and the microtubule–depolymerase Kif2a associate with the MMAP-MRN complex by co-immunoprecipitation experiments; furthermore, the level of Kif2a is reduced in Plk1 immunoprecipitates from MMAP or MRN-deficient cells compared with controls [67]. Plk1 phosphorylates Kif2a at threonine 554 (T554) to enhance its microtubule–depolymerase activity *in vitro* and induce primary cilia disassembly in vertebrate cells [69,70]. Plk1 also phosphorylates MMAP and Mre11 at identified residues by *in vitro* kinase assays followed by mass spectrometry [67]. Expression of nonphosphorylatable MMAP or Mre11 mutant proteins in which the Plk1 target residues are changed to alanine reduces MMAP association with Mre11 by co-immunoprecipitation [67]. It is proposed that Plk1 phosphorylates MMAP and Mre11 to promote the formation of the MMAP-MRN complex. In turn, MMAP-MRN may act as platform to facilitate Kif2a phosphorylation by Plk1 to promote proper spindle microtubule turnover and optimal spindle formation (Fig. 2A). Because MMAP is expressed only in vertebrates, it is unclear whether a similar mechanism also operates in lower eukaryotes. Also, because Aurora A interacts with Kif2a by co-immunoprecipitation experiments and inhibits Kif2a depolymerase activity *in vitro* [69], functional interactions between the Chk2 and the MMAP-MRN centrosomal pathways that control spindle formation are likely to occur and require further investigation.

Secondly, MRN and its binding partner CtIP protein are required for chromatin-dependent mitotic spindle assembly. Depletion of Mre11 or CtIP, antibody-mediated inhibition of Mre11, or inhibition of Mre11 endonuclease activity by the small-molecule inhibitor, mirin, results in defects in metaphase chromosome alignment in Xenopus egg extracts [68]. Furthermore, MRN inhibition by the above treatments reduces the fidelity of assembled mitotic spindle structures around DNA beads in Xenopus egg extracts in the absence of functional centrosomes, suggesting MRN is required for the RanGTP-mediated chromosomal spindle assembly pathway. In HeLa cells, Mre11 depletion or treatment with mirin results in metaphase delay compared with control cells. Furthermore, treatment with mirin disrupts the RanGTP gradient in metaphase by using a Ran-regulated fluorescence resonance energy transfer (FRET) biosensor containing the importin-β-binding domain [68]. Binding of the RCC1 guanine nucleotide exchange factor to chromatin is required to establish the RanGTP gradient around the chromosomes [64,71]. Inhibition of MRN reduces binding of RCC1 to chromatin by live-cell imaging in HeLa cells compared with controls, and also by western blot analysis of chromatin-associated RCC1 in Xenopus egg extracts [68]. It is therefore proposed that MRN-CtIP contributes to Ran-dependent mitotic spindle assembly by promoting RCC1 chromatin association (Fig. 2B). However, the precise mechanism by which MRN-CtIP regulates RCC1 binding to chromatin has not been identified.

DNA damage proteins in chromosome segregation and spindle checkpoint signaling

The mitotic spindle checkpoint delays chromatid separation until all sister kinetochores are stably attached to spindle microtubules emanating from opposing spindle poles. This mechanism provides more time for the cell to correct erroneous kinetochore–microtubule attachments and avoid possible chromosome errors which can lead to carcinogenesis [72]. To achieve this, the mitotic spindle checkpoint monitors kinetochore–microtubule interactions: In the presence of unattached or improperly (unstably) attached kinetochores [73–75], conserved components of the mitotic spindle checkpoint such as the Mad (Mad1, Mad2, and BubR1) and the Bub (Bub1 and Bub3) proteins localize to unattached/misattached kinetochores and this step is essential to prevent activation of the anaphase-promoting complex/cyclosome to delay mitotic exit [28,29].

Aurora B is the catalytic subunit of the Chromosomal Passenger Complex (CPC) also comprising the

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scaffolding protein INCENP and the targeting subunits Survivin and Borealin (reviewed in Refs [76–78]). Aurora B localizes to centromeres and kinetochores in prometaphase where it regulates chromosome alignment and segregation by promoting detachment of misattached kinetochore–microtubules [76–78]. Aurora B is also involved in spindle checkpoint signaling: In higher eukaryotic cells, catalytic activity of Aurora B is required for sustained mitotic arrest in the presence of misattached kinetochore–microtubules [79,80]. Furthermore, more recent studies have shown that Aurora B is required for proper mitotic arrest in the presence of many unattached kinetochores by promoting efficient localization of the central mitotic kinase Mps1 to unattached kinetochores [81–83].

Chk1 in the mitotic spindle checkpoint

Interplay between the DNA damage and mitotic spindle checkpoints was first reported approximately 13 years ago [84,85]. Using a Chk1-knockout avian β-lymphoma DT40 cell line and human carcinoma BE, HCT116 or HEK293 cell lines depleted of Chk1 by siRNA, Zachos *et al.* [84] showed that Chk1-deficient cells exhibit high frequency of misaligned chromosomes in metaphase and missegregated chromosomes in anaphase compared with controls. These results were later confirmed and expanded in other studies using Chk1 +/− primary mammary epithelial cells isolated from Chk1 +/− mice [86], or other mammalian cell lines [87,88]. Furthermore, Chk1-deficient cells fail to properly accumulate in mitosis in the presence of taxol, a drug that stabilizes microtubules and induces improper kinetochore–microtubule attachments [84]. In contrast, Chk1 is dispensable for mitotic arrest after complete microtubule depolymerization by a relatively high (3.32 μM) concentration of nocodazole. Chk1 localizes to kinetochores in prometaphase and is required for optimal BubR1 kinetochore localization in the absence of spindle poisons or in cells treated with taxol [84]. Mechanistically, Chk1 phosphorylates human Aurora B at the conserved residue serine 331 (S331) in prometaphase in the absence of spindle poisons or after taxol treatment [88]. S331 is at the foot of the Aurora B C-terminal tail, which interacts with the IN box of INCENP in the partially active complex [89]. Phosphorylated S331 is required for optimal phosphorylation of INCENP at TSS residues and complete Aurora B catalytic activity by immunoprecipitation–kinase assays using histone H3 as substrate, but not for Aurora B localization to centromeres or Aurora B-binding to INCENP [88]. Overexpression of Aurora B-S331A harboring a nonphosphorylatable mutation of S331 to alanine in Chinese Hamster Ovary cells results in spontaneous chromosome missegregation, reduced localization of BubR1 to kinetochores, and impaired mitotic delay in the presence of taxol compared with controls expressing wild-type Aurora B [88]. It is proposed that Chk1 phosphorylates Aurora B-S331 to fully induce Aurora B catalytic activity and promote optimal chromosome segregation. Furthermore, Chk1-mediated Aurora B activation is required for anaphase delay in the presence of misattached kinetochores by promoting BubR1 and Mps1 localization to kinetochores (Fig. 3).

Chk2 in the mitotic spindle checkpoint

In human colon carcinoma BE cells, Chk2 localizes to prometaphase kinetochores after complete microtubule depolymerization by a relatively high (3.32 μM) concentration of nocodazole, but not in cells treated with taxol [90]. Chk2-deficient cells exit mitosis prematurely when most (or all) kinetochores are unattached by high nocodazole and this coincides with diminished localization of phosphorylated Aurora B-S331, BubR1, and Mad2 to kinetochores, reduced total levels of Mps1 kinase, and increased Cdk1-tyrosine 15 inhibitory phosphorylation compared with controls [90]. Chk2 phosphorylates Mps1–threonine 288 to stabilize Mps1 [91]; furthermore, the expression of a phosphomimetic Mps1-T288E in which T288 is changed to glutamic acid rescues total levels of Mps1 protein and diminishes Cdk1-tyrosine 15 phosphorylation in Chk2-deficient cells compared with controls [90]. Importantly, expression of both phosphomimetic Aurora B-S331E in which S331 is changed to glutamic acid and Mps1-T288E is required to prevent mitotic exit in Chk2-deficient cells compared with controls in the presence of high nocodazole [90]. In the absence of spindle poisons, Chk2 is required for localization of phosphorylated Aurora B-S331, Mps1, and Mad2 to kinetochores in early prometaphase, before the condensed chromosomes take a ringlike formation, and also for proper chromosome alignment and segregation. It is proposed that Chk2 delays mitotic exit by two mechanisms (Fig. 3): Firstly, Chk2 phosphorylates Aurora B-S331 when most kinetochores are unattached to induce Aurora B catalytic activity; in turn, active Aurora B enhances spindle checkpoint signaling by promoting BubR1 and Mps1 localization to kinetochores [81–83]. Secondly, Chk2 phosphorylates Mps1-T288 to stabilize Mps1 and inhibit Cdk1-Y15 phosphorylation to prevent mitotic exit after prolonged checkpoint activation by complete lack of microtubule attachment. These studies [88,90] demonstrate a

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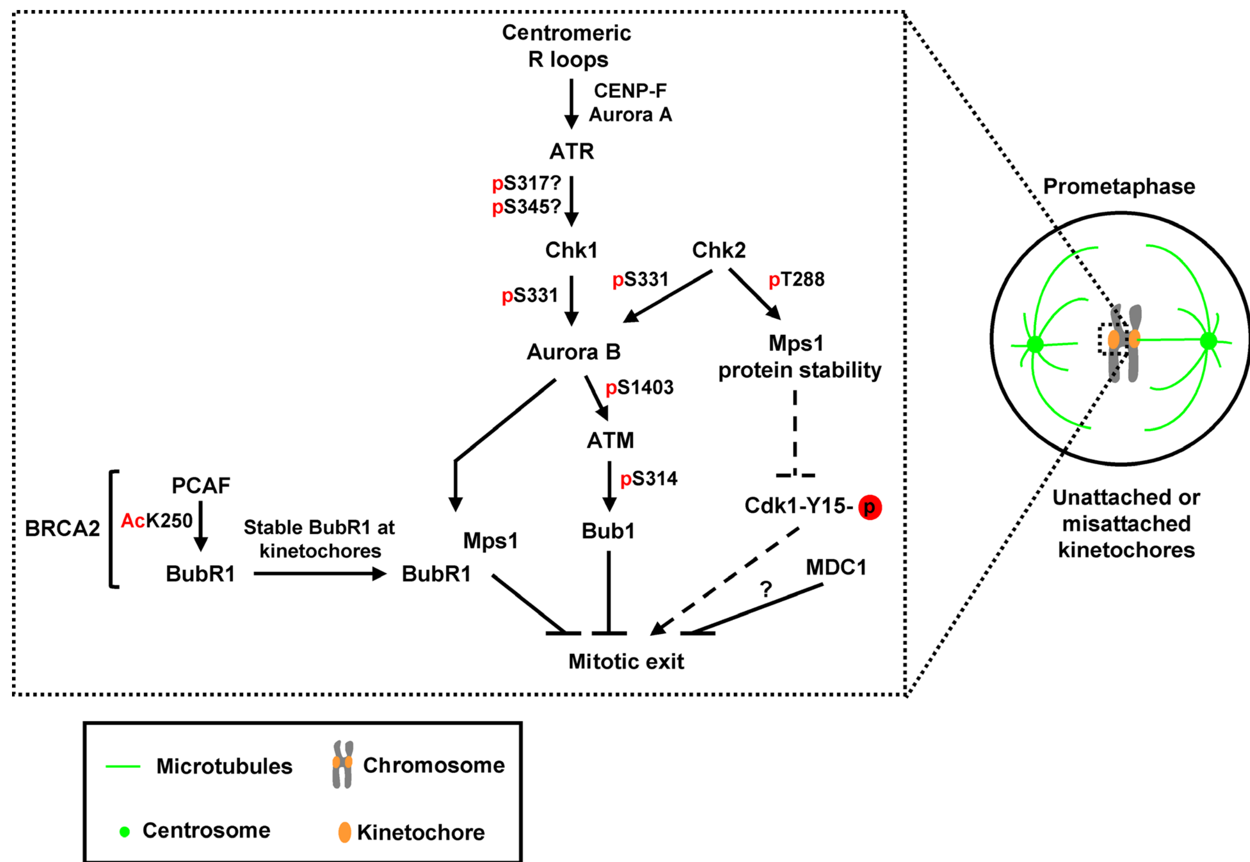

Fig. 3. Mechanisms by which DNA damage response proteins regulate the mitotic spindle checkpoint in human cells. Chk1 and Chk2 phosphorylate Aurora B-S331 in late or early, respectively, prometaphase to induce complete Aurora B kinase activity. In turn, active Aurora B enhances spindle checkpoint signaling by promoting BubR1 and Mps1 localization to kinetochores. Chk2 also phosphorylates Mps1 to stabilize Mps1 protein and inhibit Cdk1-Y15 phosphorylation to prevent mitotic exit after prolonged checkpoint activation by complete lack of microtubule attachment. Centromeric R loops activate ATR and this may result in Chk1 activation. Also, BRCA2 acts as scaffold to facilitate BubR1 acetylation by PCAF acetyltransferase to prevent untimely degradation of BubR1 by the APC/C in mitotic cells. Dashed lines indicate prolonged checkpoint activation. Unknown molecular events are indicated by question marks. Ac, acetylation; p, phosphorylation.

division of labor between Chk1 and Chk2 in activating Aurora B: Chk2 activates Aurora B in the presence of many unattached kinetochores/in relatively early prometaphase, whereas Chk1 activates Aurora B in the presence of misattached kinetochores/in late prometaphase. The biological significance of this phenomenon is unclear but may reflect different phases of mitosis in which Chk1 and Chk2 are activated. This division of labor is reminiscent of the G2 DNA damage checkpoint in which Chk1 prevents G2 cells with damaged DNA from entering mitosis, whereas Chk2 is solely responsible for mitotic delay in late G2 cells, which are ready to enter mitosis [92]. Also, the mitotic kinase Plk1 phosphorylates Chk2 to inhibit Chk2 kinase activity and inactivate the G2 DNA damage checkpoint in mammalian cells [93]. Whether a similar mechanism inactivates Chk2 in late prometaphase, in the absence of damaged DNA, to hand over control of Aurora B–S331 phosphorylation to Chk1 is unknown.

ATM in spindle checkpoint signaling

HeLa cells synchronized in mitosis after release from a thymidine block or treated with a relatively low (200 nM) dose of nocodazole that primarily generates misattached kinetochore–microtubules [90] exhibit phosphorylated ATM–serine 1981 (active ATM) in the absence of DNA damage, judged by lack of γ-H2AX foci formation [48]. Furthermore, ATM-S1981 phosphorylation is reduced after Aurora B inhibition compared with control cells, suggesting ATM is activated

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in mitosis in an Aurora B-dependent manner [48]. Aurora B phosphorylates ATM at serine 1403 (S1403) *in vitro* and in cell extracts. Phosphorylated ATM-S1403 exhibits diffused nuclear localization from prophase to anaphase and localizes to the midzone in anaphase and to the midbody in cytokinesis [48]. Expression of nonphosphorylatable S1403 to alanine (S1403A) mutant ATM reduces ATM-S1981 phosphorylation compared with the wild-type ATM by immunoprecipitation western blotting, suggesting ATM-S1403 phosphorylation is required for ATM activation. ATM-deficient cells or cells expressing mutant ATM-S1403A exhibit shortened times from nuclear envelop breakdown to anaphase onset and increased frequency of anaphases with lagging chromosomes compared with controls. Furthermore, ATM-deficient cells exhibit reduced levels of phosphorylated histone H3–serine 10 (S10, a marker of mitosis) compared with controls, suggesting ATM-deficient cells exit mitosis in the presence of low nocodazole [48]. It is proposed that ATM is required for proper mitotic progression and chromosome segregation, and for mitotic delay in the presence of improper kinetochore–microtubule attachments.

The mechanism by which ATM exerts its mitotic functions is a matter of active investigation. ATM phosphorylates Bub1 kinase on serine 314 (S314) after incubation of cells with low nocodazole or after DNA damage [48,94]. Depletion of ATM or expression of mutant Bub1-S314A that cannot be phosphorylated on this site impairs histone H2A–threonine 121 phosphorylation and reduces the percentage of phospho-H3-S10-positive cells in the presence of low nocodazole [48], suggesting S314 phosphorylation by ATM is required for Bub1 functions at kinetochores (Fig. 3). In human cells, Bub1 interacts with the kinetochore scaffold protein Knl1 and with Mad1-Mad2 to promote spindle checkpoint activation [95,96]. Furthermore, Bub1 mediates histone H2A–threonine 121 phosphorylation to promote CPC localization to centromeres [97,98]. It is therefore important to examine localization of downstream Bub1 proteins such as Mad1, Mad2, and Aurora B to centromeres/kinetochores in ATM-deficient cells or after expression of mutant Bub1-S314A protein to fully understand how ATM regulates spindle checkpoint signaling. It is also unclear whether phosphorylated S314 is required for optimal Bub1 catalytic activity and/or Bub1 localization to kinetochores in mitotic cells.

In addition, ATM phosphorylates Mad1–serine 214 *in vitro* and in nocodazole-treated cell extracts [99]. Overexpression of nonphosphorylatable mutant S214A Mad1 in HeLa cells reduces the mitotic index compared with controls in the presence of nocodazole [99]. However, because overexpression of even wild-type Mad1 can impair spindle checkpoint signaling by promoting Mad2 mislocalization [100], further experiments are required to assess the significance of Mad1-S214 phosphorylation for the mitotic spindle checkpoint.

ATR in chromosome segregation

Total ATR and autophosphorylated (active) ATR–threonine 1989 localize to centromeres on mitotic chromosomes in human diploid retinal pigment epithelium (RPE1) cells treated with a relatively low (330 nM) concentration of nocodazole [101]. Inhibition of ATR with two different small molecule inhibitors in RPE1 cells or rapid degradation of auxin-inducible degron (AID)-tagged ATR in mitotic AID:ATR avian DT40 cells increases the frequency of anaphases with lagging chromosomes compared with controls, suggesting ATR is required for proper chromosome segregation [101]. ATR and its partner protein ATRIP associate with the centromere protein CENP-F by co-immunoprecipitation experiments from mitotic cell extracts. Furthermore, inhibition of Aurora A kinase disrupts the interaction of ATR/ATRIP with CENP-F and diminishes localization of ATR, but not CENP-F, to centromeres compared with control cells, suggesting that ATR localizes to centromeres through Aurora A-regulated association with CENP-F [101]. R loops contain DNA-RNA hybrids and displaced ssDNA [102,103]. The single-strand DNA binding protein RPA (replication protein A) is a sensor of R loops and RPA activates ATR in response to DNA damage and DNA replication stress [104]. Experiments with antibodies that recognize RPA or DNA-RNA hybrids show that centromeres are positive for RPA and R loop staining by immunofluorescence; furthermore, ATR, RPA, and R loop-associated, but not CENP-F, centromere signals were reduced after expression of ribonuclease H1 that cleaves the RNA in DNA-RNA hybrids, suggesting that R loops activate ATR at centromeres to promote optimal chromosome segregation (Fig. 3) [101].

The downstream targets of ATR in regulating chromosome segregation are incompletely understood. ATR-deficient human cells treated with the Eg5 kinesin inhibitor S-Trityl L-cysteine (STLC) to inhibit bipolar spindle formation and exhibit reduced localization of phosphorylated (active) Aurora B-T232 at kinetochores and diminished phosphorylation of the Aurora B target histone H3 at serines 10 and 28 compared with controls [101]. Because the treatment of

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cells with STLC activates the spindle checkpoint in the presence of mono-attached kinetochores, ATR-deficient cells may exit mitosis prematurely compared with controls. In this case, it is unclear to what extent reduced phosphorylation of Aurora B targets in ATR-deficient cells is an actual marker of impaired Aurora B catalytic activity or a consequence of untimely mitotic exit. Whether/how Chk1 participates in ATR signaling in prometaphase cells is also unclear: Kabeche *et al* report that phosphorylated Chk1 at the DNA damage sites serine 317 and serine 345 localizes to prometaphase kinetochores in chromosome spreads and that this localization is reduced after ATR inhibition by fluorescence microscopy. However, other studies have failed to detect Chk1 phosphorylation at the above sites by western blotting analysis of cell extracts in the presence of spindle poisons or after treatment of nocodazole-arrested mitotic cells with ionizing radiation or UV light [47,84]. One possibility is that a relatively small population of phosphorylated-Chk1 at S317 and/or S345 that is hard to detect by western blotting localizes to kinetochores to mediate ATR signaling. Alternatively, the anti-phospho-Chk1 antibodies used may cross-react with kinetochore proteins by immunofluorescence. Restoring proper chromosome segregation in ATR-deficient cells by potentially expressing phosphomimetic Chk1 proteins at S317/S345 may help us understand the molecular mechanisms of ATR functions in mitosis.

BRCA2 in the mitotic spindle checkpoint

The DNA damage response protein BRCA2 is an established mediator of homologous recombination [57]. BRCA2 localizes to prometaphase kinetochores in nocodazole-arrested HeLa cells and interacts with BubR1 and PCAF acetyltransferase by co-immunoprecipitation analysis of mitotic cell extracts [49]. Depletion of BRCA2 in HeLa cells or disruption of the *Brca2* allele in mouse embryo fibroblasts derived from conditional *Brca2*-knockout mice reduces BubR1–lysine 250 (K250) acetylation and total BubR1 protein levels at kinetochores [49]. Acetylation of BubR1–lysine 250 by PCAF prevents untimely BubR1 degradation by the anaphase-promoting complex/cyclosome (APC/C) and is essential for proper mitotic progression and spindle checkpoint activity [49]. Consistently, mouse embryonic fibroblasts from *Brca2*-deficient or from transgenic mice that were engineered to have impaired BubR1-BRCA2 association, exit mitosis quicker by time-lapse microscopy in the absence or in the presence of nocodazole compared with control cells [49]. It is proposed that BRCA2 acts as scaffold to facilitate PCAF-BubR1 interaction and BubR1 acetylation in mitotic cells (Fig. 3). However, whether this function requires BRCA2–kinetochore localization is unclear.

MDC1 prevents mitotic exit and promotes chromosomal stability

MDC1 is an adaptor protein that directly binds to γ-H2AX to facilitate H2AX-phosphorylation by ATM and promote accumulation of DNA damage response proteins to DNA double-strand breaks [105]. In HeLa cells, approximately 35% of prometaphase kinetochores exhibit MDC1 staining and inhibition of ATM by a small-molecule inhibitor reduces MDC1 localization to kinetochores compared with controls [106]. It is unclear whether this relatively modest percentage reflects transient binding of MDC1 to all kinetochores or whether MDC1 binds to a subset of kinetochores, for example, depending on their level of microtubule occupancy. Depletion of MDC1 accelerates mitotic exit in the presence of taxol or 100 nM nocodazole that generate improperly attached kinetochores by time-lapse microscopy, but does not reduce Mad2 or BubR1 kinetochore staining compared with control cells [106], suggesting MDC1 prevents mitotic slippage in the presence of an active spindle checkpoint signaling. Depletion of MDC1 accelerates mitotic progression in the absence of spindle drugs in one study, but induces metaphase arrest in a different study perhaps reflecting different levels of MDC1 depletion [106,107]. Also, because MDC1 localization correlates with γ-H2AX phosphorylation at kinetochores [106], recruitment of MDC1 at kinetochores may reflect damage within centromeric DNA, which can lead to unstable kinetochore–microtubule interactions and spindle checkpoint activation. Further experiments are required to understand the potential role of MDC1 in unperturbed mitosis.

Also, it was recently shown that MDC1 interacts with the DNA damage response mediator protein TopBP1 [108]. Disruption of MDC1-TopBP1 interaction reduces TopBP1 recruitment to double-strand breaks in mitotic cells after irradiation and this correlates with increased mitotic radiosensitivity, micronuclei formation, and chromosomal aberrations compared with controls [108]. Using high-resolution confocal microscopy, Leimbacher *et al.* showed that TopBP1 forms filamentous intra- or interchromosomal structures capable of bridging MDC1 foci in mitosis after cell irradiation, suggesting MDC1-TopBP1 complexes tether double-strand breaks until repair is reactivated in the following G1 phase to preserve genome

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stability. It will be important to determine whether this mechanism also operates in response to spontaneous double-strand breaks in nonirradiated mitotic cells, for example, after DNA repair is prevented.

DNA damage response proteins in error correction

Merotelic attachments in which a single kinetochore simultaneously binds to microtubules emanating from both spindle poles occur spontaneously in early mitosis and do not activate the mitotic spindle checkpoint [109]. If uncorrected, merotelic attachments will lead to lagging chromosomes in anaphase and can result in chromosome missegregation and aneuploidy [110,111]. The Aurora B kinase plays a central role in correction of merotelic kinetochore–microtubule attachments by phosphorylating outer kinetochore proteins such as Hec1 to promote detachment of kinetochore–microtubules [112,113] and also by regulating proper localization of the microtubule depolymerizing kinesins MCAK and Kif2b to centromeres or kinetochores to depolymerize merotelic kinetochore–microtubules [114–116].

Chk1 in correction of merotelic attachments

Using Chk1-depleted human BE cells and avian DT40 Chk1−/− cells [117], Petsalaki and Zachos [118] showed that Chk1-deficient cells exhibit increased frequency of anaphases with merotelic attachments and lagging chromosomes compared with controls. Furthermore, Chk1 is required for correction of merotelic attachments in cells arrested in metaphase after treatment with the proteasome inhibitor MG132 [118]. Chk1-deficient cells exhibit diminished localization of MCAK and Kif2b to centromeres or kinetochores in prometaphase and reduced phosphorylation of Aurora B-target sites Hec1–serine 44 and serine 55, inside the Hec1 N-terminal tail that tunes the affinity of kinetochore–microtubule interactions, compared with controls [118]. Chk1 phosphorylates Aurora B-S331 in prometaphase and metaphase to promote Aurora B catalytic activity [88,118]. Furthermore, Chinese Hamster Ovary cells overexpressing a nonphosphorylatable mutant Aurora B protein in which S331 is changed to alanine (S331A) exhibit reduced localization of MCAK and Kif2b to centromeres or kinetochores and reduced phosphorylation of Hec1-S55 compared with cells expressing the wild-type Aurora B [118]. It is proposed that Chk1 phosphorylates Aurora B-S331 to promote binding of MCAK and Kif2b to centromeres or kinetochores, optimal Hec1 phosphorylation at serines 44 and 55, and correction of merotelic attachments before anaphase (Fig. 4).

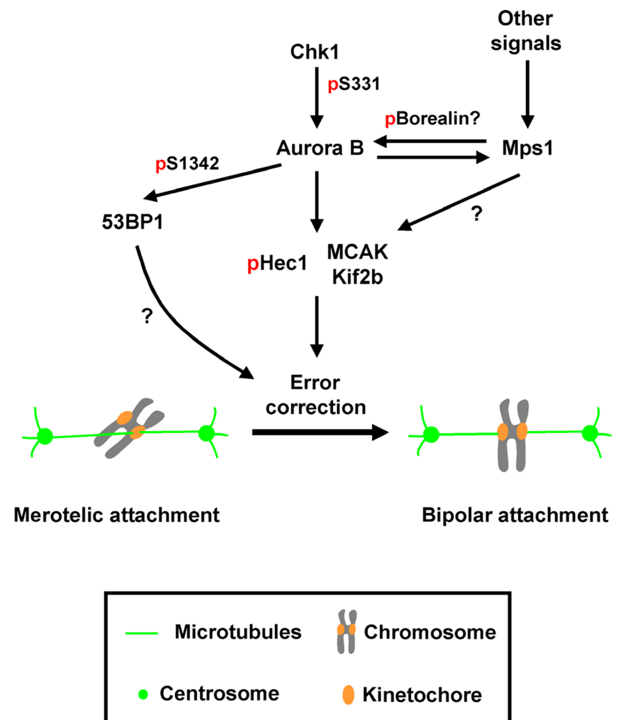

Fig. 4. Chk1 promotes correction of merotelic kinetochore–microtubule attachments before anaphase. Chk1 phosphorylates Aurora B-S331 to promote complete Aurora B catalytic activity. In turn, Aurora B phosphorylates the outer kinetochore protein Hec1 to promote detachment of misattached kinetochore–microtubules and promotes localization of the microtubule depolymerizing kinesins MCAK and Kif2b to centromeres or kinetochores to depolymerize merotelic kinetochore–microtubules. Aurora B also promotes kinetochore localization of Mps1 and 53BP1 that contribute to error correction by incompletely understood mechanisms. Unknown molecular events are indicated by question marks. p, phosphorylation.

Inhibition of Mps1 by siRNA or treatment with a small-molecule Mps1 inhibitor also increases the frequency of anaphases with merotelic attachments and lagging chromosomes compared with control cells, and this coincides with reduced Hec1-S55 phosphorylation and diminished localization of MCAK and Kif2b to centromeres or kinetochores compared with control cells [118]. Significantly, simultaneous inhibition of Chk1 and Mps1 exhibits an additive effect on anaphases with lagging chromosomes compared with cells defective for only one kinase; furthermore, cells expressing Aurora B-S331A exhibit reduced localization of Mps1 to kinetochores compared with those expressing wild-type Aurora B [118]. It is proposed that Chk1 promotes localization of Mps1 kinase to

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kinetochores through Aurora B-S331 phosphorylation; in turn, Mps1 promotes correction of merotelic attachments through incompletely understood mechanisms (Fig. 4). One possibility is that Mps1 phosphorylates the CPC protein Borealin to regulate Hec1, MCAK, and Kif2b [119]; however, an Aurora B-independent role for Mps1 in error correction cannot be excluded.

53BP1 in mitosis and error correction

53BP1 promotes ATM activity and DNA repair in response to double-strand DNA breaks [120]. 53BP1 also forms nuclear bodies around DNA lesions after replication stress, possibly to protect DNA against erosion [121]. 53BP1 was also initially proposed to participate in the mitotic spindle checkpoint based on its localization in the outer kinetochore from prophase until early anaphase and on its hyperphosphorylation in mitotic cell extracts [122]. However, 53BP1 is absent from prometaphase kinetochores after prolonged mitotic delay by centrosome loss or inhibition of Eg5 kinesin that activate the mitotic spindle checkpoint, suggesting 53BP1 is not a typical spindle checkpoint component [50,123]. Aurora B phosphorylates 53BP1 at serine 1342 (S1342) *in vitro* and in mitotic HeLa cells; furthermore, Aurora B inhibition by a small-molecule inhibitor or expression of nonphosphorylatable mutant 53BP1-S1342A protein reduces 53BP1 kinetochore staining compared with control cells, suggesting Aurora B phosphorylates S1342 to promote 53BP1 localization to kinetochores [124]. Depletion of 53BP1 or expression of mutant 53BP1-S1342A increases the frequency of lagging chromosomes compared with control cells, indicating that 53BP1 is required for optimal chromosome segregation [50,124]. 53BP1 colocalizes with the inner kinetochore marker ACA in merotelic kinetochores; furthermore, 53BP1 associates with MCAK by co-immunoprecipitation experiments in mitotic cell extracts, suggesting a potential role for 53BP1 in merotelic error correction (Fig. 4) [124]. However, a direct correlation between loss of 53BP1 kinetochore localization and increased frequency of merotelic attachments remains to be established. Also, 53BP1 inhibits APC/C activity in cell culture and *in vitro*; furthermore, 53BP1-deficient cells are often delayed entering mitosis and transit through mitosis faster than controls [50].

DNA damage response proteins in cytokinesis

In animal cells, cytokinesis begins immediately after chromosome segregation and is driven by an actomyosin ring that mediates cleavage furrow ingression and generation of the intercellular bridge that connects the two daughter cells [31,32]. However, contractile forces from the actomyosin ring cannot execute the final cut of the plasma membrane (abscission) to release the two daughter cells. Instead, this step is regulated by the Endosomal Sorting Complex Required for Transport (ESCRT) machinery that is assembled at the midbody in late cytokinesis [125,126]. For this purpose, the microtubule bundling protein Cep55 associates with the Mklp1-MgcRacGAP (centralspindlin) complex at the midbody; in turn, ESCRT-I/ ESCRT-II proteins such as Tsg101 and the ESCRT-associated protein Alix bind to Cep55 to recruit ESCRT-III proteins to the midbody to deliver the final cut ([127], reviewed in Refs [125,128]). After recruitment, ESCRT-III proteins mediate membrane deformation and scission by forming contractile helical filaments at the secondary ingression site (that will become the abscission site) at approximately 1 μm distance from the midbody [129–131]. Impaired abscission signaling can lead to furrow regression and binucleation in normally segregating cells [132,133].

The CPC translocates to the central spindle and the midbody during cytokinesis. Aurora B regulates abscission timing by inhibiting ESCRT-III function at the midbody, and a reduction in Aurora B catalytic activity is required for abscission in normally segregating cells [132–134]. Also, intermediate filaments, F-actin, and microtubules must be cleared of the abscission site to allow membrane scission by the ESCRT machinery [32]. In addition, chromatin bridges, nuclear pore defects, or DNA replication stress activate the Aurora B-mediated 'abscission checkpoint' to delay abscission in mammalian cells [135,136].

Chk1 regulates completion of cytokinesis

Chk1 localizes to the midbody in cytokinesis [84,86] and is phosphorylated at ATR-target residues in midbody-enriched cell extracts [137], suggesting that Chk1 is catalytically active at the midbody. Primary mammary epithelial cells isolated from heterozygous Chk1+/− mice exhibit increased binucleation compared with Chk1+/+ controls [86]. Furthermore, abrogation of Chk1 function by microinjection or transfection of an anti-Chk1 antibody in mouse NIH3T3 or human HeLa cells results in cleavage furrow regression and binucleation compared with IgG-transfected controls, suggesting Chk1 is required for completion of cytokinesis [86]. However, the molecular mechanism involved is under active investigation. Chk1 inhibition by the small-molecule inhibitor UCN-

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01 does not reduce Aurora B-S331 phosphorylation at the midbody compared with control cells, indicating that Chk1 does not regulate abscission through Aurora B-S331 phosphorylation; instead, the Cdc-like kinases 1, 2, and 4 are required for Aurora B-S331 phosphorylation and complete Aurora B activation in late cytokinesis [138]. Because Chk1-deficient HeLa cells exhibit increased frequency of intermediate filament (vimentin) bridges compared with controls in late cytokinesis [139], one possibility is that Chk1 prevents furrow regression by promoting vimentin severing through an incompletely understood mechanism (Fig. 5A).

Chk1 and ATR are also required for abscission delay in response to relatively mild DNA replication stress [137]. In addition, Chk1 is required for the generation of actin-rich structures (actin patches) at the base of chromatin bridges in cytokinesis to prevent them from breaking [140]. For a recent overview of Chk1 functions in the abscission checkpoint and in stabilization of chromatin bridges, please see [136].

BRCA2 in abscission

There is also evidence that BRCA2 regulates completion of cytokinesis. Using cells from BRCA2-heterozygous or knockout mice and various human cell lines depleted of BRCA2 by siRNAs, several studies have shown that inactivation or depletion of BRCA2 delays abscission, increases the frequency of furrow regression, and promotes binucleation compared with BRCA2-proficient control cells [141–143]. BRCA2 localizes to the central spindle and midbody; furthermore, the midbody localization of BRCA2 depends on BRCA2 interaction with the actin-binding protein Filamin A [143]. Disruption of the Filamin A-interacting domain in BRCA2 by introduction of an appropriate point mutation impairs completion of cytokinesis compared with the wild-type protein; however, it does not reduce the homologous directed repair activity of BRCA2, suggesting BRCA2 functions at the midbody and in DNA repair are separable [143]. In the absence

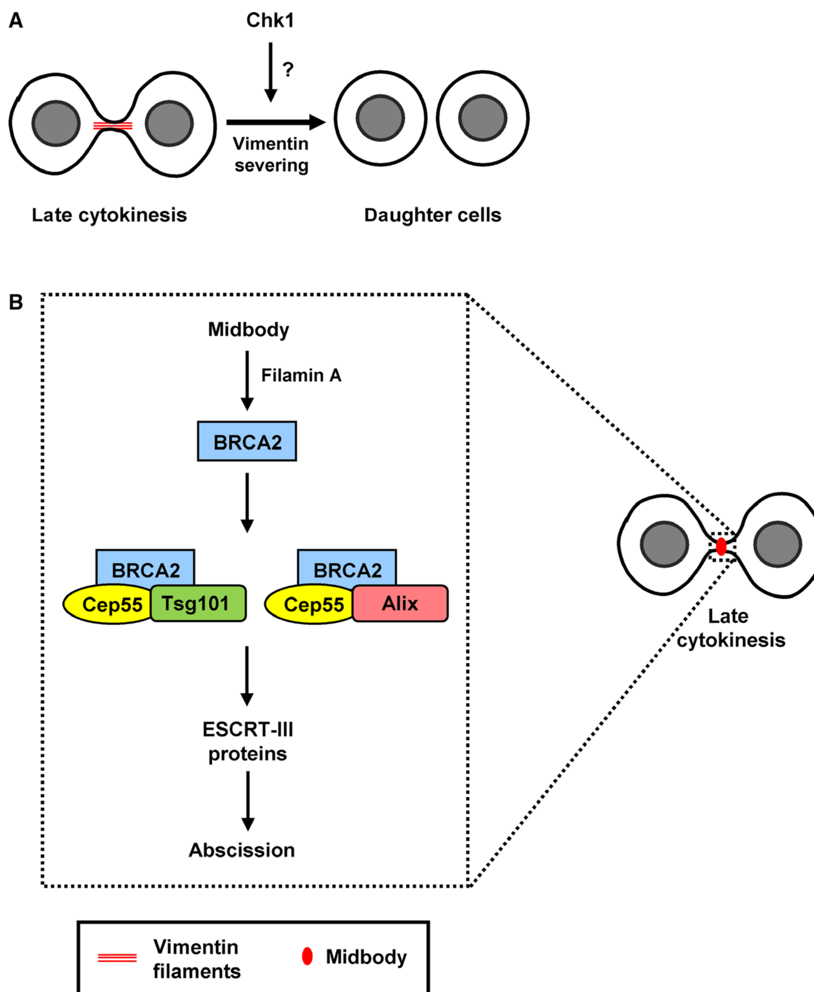

Fig. 5. Chk1 and BRCA2 regulate abscission. (A) Chk1 prevents furrow regression perhaps by promoting severing of vimentin filaments inside the intercellular canal. (B) BRCA2 localizes to the midbody by interaction with the actin-binding protein Filamin A. There, BRCA2 potentially acts as scaffold to facilitate the interaction of the midbody protein Cep55 with the ESCRT proteins Tsg101 and Alix and promote assembly of the abscission machinery. Unknown molecular events are indicated by question mark.

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of BRCA2, regulators of cytokinesis such as Mklp1, Mklp2, Alix, and Tsg101 mislocalize to the midbody. Biochemical analysis using ectopically expressed BRCA2 and Cep55 or purified recombinant proteins shows that BRCA2 promotes the interaction of Cep55 with Tsg101 and Alix. Also, expression of BRCA2 mutant proteins that disrupt interactions with Cep55, Tsg101, or Alix reduces localization of Tsg101 and Alix to the midbody and increases multinucleation compared with control human 293T cells expressing wild-type BRCA2 [143]. It is proposed that BRCA2 acts as scaffold to promote optimal assembly of the abscission machinery to the midbody (Fig. 5B).

However, a different study proposes that BRCA2 is not required for cytokinesis in human cells [144]. Using HeLa cells depleted of BRCA2 by three different siRNAs, Lekomtsev *et al*. did not detect increased cytokinesis failure compared with controls by live-cell imaging. Furthermore, BRCA2 depletion did not alter abscission timing in this study, judged by diffusion of a photoactivatable GFP protein from one daughter cell to the other or disassembly of midbody microtubules compared with controls by live-cell imaging [144]. Also, DLD1 colon cancer cell lines in which one or both *BRCA2* alleles are disrupted by gene targeting exhibit a modest increase in cytokinesis failure compared with controls [144]. Why these results are different from other studies is unclear. Perhaps of note, BRCA2-defective cells exhibit different levels of binucleation depending on the cell line and treatment even in the same study, ranging from a 4- to 5-fold increase in murine pancreatic tumor or human PEO1 ovarian cancer cells to approximately 1.6-fold increase in human 293T cells compared with controls [143]. One possibility is that BRCA2 has a relatively significant role in abscission in some cell lines but not in others, perhaps reflecting different genetic backgrounds. Another possibility is that binucleation of BRCA2-deficient cells is influenced by the level of DNA lesions that cells enter mitosis with, and this level can vary between cell lines [145,146]. Further analysis is required to fully understand the potential role of BRCA2 in abscission.

Conclusions and perspectives

For a long time, the DNA damage response and the mitotic cell division pathways were thought to be distinct and unrelated because the cell cycle response to damaged DNA was outside mitosis [33–35]. In the last few years, several studies have shown that DNA damage response proteins are also involved in mitotic cell division by regulating mitotic entry, spindle formation, correction of misattached kinetochore–microtubules, anaphase onset, or abscission in the absence of exogenous DNA damage (Fig. 6). It should be noted, however, that most of the applied model systems are tumor cell lines in which the DNA damage response is chronically activated [147]. It will be interesting to determine whether this chronic DNA damage response sustains the activity of at least some DNA damage proteins in mitosis and whether these proteins are required for optimal chromosome segregation also in nontransformed cells. Furthermore, expression of mutant DNA damage response factors can induce DNA lesions due to replication stress during S-phase [121]. These lesions can then be transmitted to mitosis leading to chromosome aberrations [148,149]. It is therefore important to investigate chromosome segregation/cytokinesis after small-molecule inhibition or rapid destruction of DNA damage response proteins upon mitotic entry [90,101], to exclude potential mitotic defects caused by unreplicated DNA in mutant cells. In any case, a growing number of papers in the literature suggest a cross-talk between the DNA damage and cell division machineries. However, the purpose of this cross-talk is incompletely understood. One possibility is that, by assigning mitotic duties in DNA damage response proteins, the cell can efficiently coordinate progression from interphase, a relatively long period of time during which the cell can stop and repair the damaged DNA, to the various stages of mitotic cell division where the main focus is on rapidly and accurately segregating its genetic material to the two daughter cells. Another possibility (that is not mutually exclusive with the first one) is that mitotic functions can alter the substrate specificity of DNA damage response proteins or help sequester them away from DNA damage sites to reinforce inhibition of the secondary DNA damage response in mitosis. Perhaps consistently, there is evidence that DNA damage proteins are wired differently in mitosis: For example, phosphorylation of Chk2-threonine 68 by ATM is induced both in response to nocodazole and ionizing radiation, whereas ATM-mediated phosphorylation of p53-serine 15 is not induced after nocodazole treatment [48]. In another example, active Chk1 is phosphorylated at mitotic but not DNA damage sites in the presence of spindle poisons according to certain studies [47,84]. It will be interesting to investigate whether expression of a phosphomimetic mutant Chk1 at the DNA damage sites can restore aspects of the secondary DNA damage response in mitotic cells.

Chromosomal instability is a hallmark of cancer; as a result, the identification of genes that safeguard chromosomal stability is important to understand the

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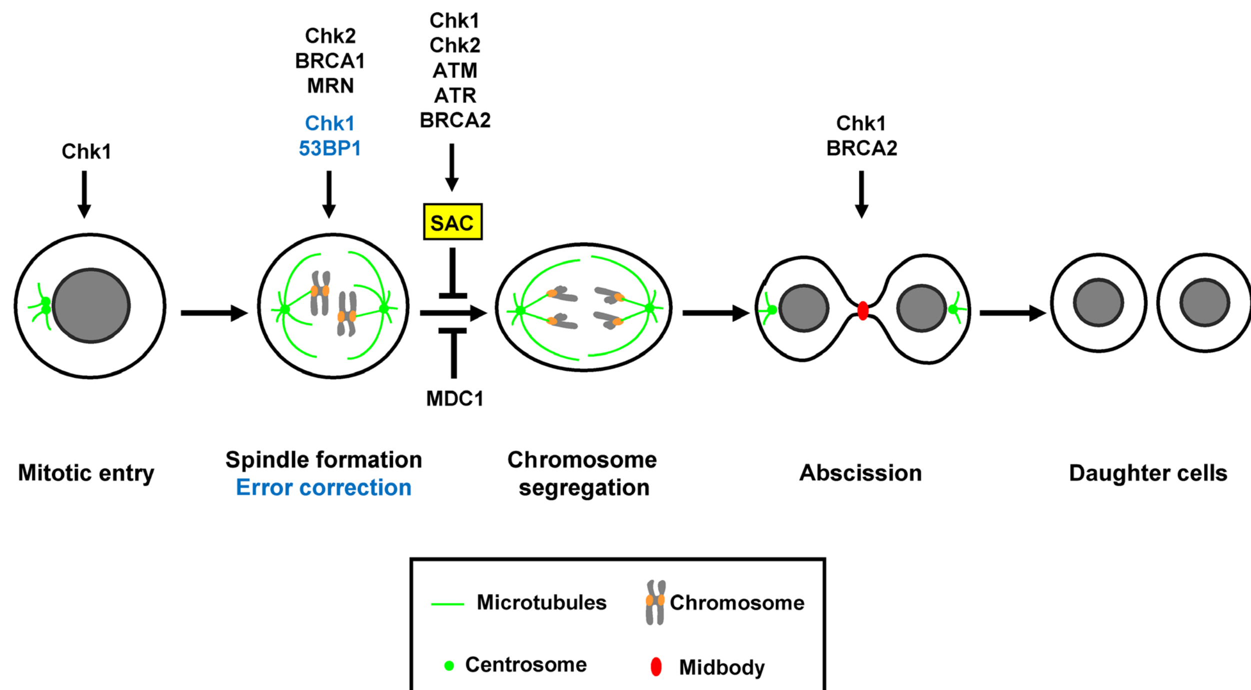

Fig. 6. Summary of DNA damage response proteins regulating mitotic cell division in higher eukaryotic cells. SAC, spindle assembly checkpoint.

etiology of cancer. In the last few years, several DNA damage response genes such as *CHK2, BRCA1, BRCA2*, *ATM*, and *ATR* were shown to preserve chromosomal stability through their roles in mitotic cell division [48,51,90,101,143]. The above genes frequently exhibit loss-of-function mutations in human cancers and were previously established as tumor suppressors on the basis of their role in the DNA damage response [3,150–152]. However, it is now recognized that maintaining chromosomal stability can be an important part of their tumor suppressor functions.

Almost the opposite is true for *CHK1*: Despite the important roles of Chk1 protein in cell cycle checkpoints, no homozygous loss-of-function mutation of *CHK1* has been detected in a wide range of human tumors [3,153]. Instead, Chk1 is overexpressed in a variety of human tumors including breast, colon, liver, gastric, and nasopharyngeal carcinoma; furthermore, loss of *CHK1* reduces tumor formation in mice after exposure to carcinogen, suggesting Chk1 promotes tumor growth [153,154]. The recent identification of Chk1 functions in mitosis may provide an explanation for this apparent paradox: Perhaps overexpression of Chk1 in human tumors can promote low-level chromosomal instability that is beneficial for tumor adaptation by deregulating Chk1 functions in the mitotic spindle checkpoint and error correction. Consistently, overexpression of other spindle checkpoint proteins such as Mad1 causes chromosomal instability and may promote tumor formation [100]. Pinpointing novel roles for DNA damage response proteins in unperturbed cell division may therefore help us understand mechanisms of tumorigenesis.

Finally, it is important to consider the implications of identifying connections between the DNA damage and mitotic machineries for cancer therapy. Chemotherapy and radiotherapy kill proliferating cancer cells through generating massive DNA lesions; however, off-target effects or cytotoxicity are frequent problems [153]. Microtubule poisons on the other hand, in particular taxanes, have been successfully used in the treatment of solid cancers; however, serious side effects such as peripheral neuropathy or drug resistance can limit their clinical utility [155]. Because relatively high rates of genomic instability can decrease tumor viability in some cancers perhaps by exceeding a threshold that is compatible with cancer cell fitness [156,157], the cross-talk between the DNA damage response and mitotic machineries can provide a framework for testing novel synthetic lethal interactions (i.e., when perturbation of either gene alone is viable but perturbation of both genes simultaneously results in loss of viability) to potentially kill cancer cells [158]. For example, BRCA1-deficient cancer cells exhibit

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spontaneous chromosome missegregation, at least in part due to relatively high microtubule polymerization rates preventing optimal correction of misattached kinetochore–microtubules [51]. Treatment of these cells with Plk1 inhibitors to further enhance microtubule polymerization (Fig. 2), or inhibition of error correction by Aurora B or Mps1 inhibitors may exacerbate chromosome segregation errors to a level incompatible with sustained tumor cell proliferation and inhibit tumor growth [156,157]. In another example, the treatment of ATM-deficient tumor cells exhibiting a weakened mitotic spindle checkpoint with Mps1 or Cdk1 inhibitors to induce mitotic slippage (Fig. 3) may lead to extensive chromosome missegregation followed by tumor cell death or senescence [155]. Investigating how the DNA damage response and mitotic machineries communicate with each other may help us devise novel strategies of cancer treatment without induction of exogenous DNA damage or addition of spindle poisons to minimize off-target effects.

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Conflict of interest

The authors declare no conflict of interest.

Author contributions

EP wrote the paper. GZ edited the paper and reviewed its final version.

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