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Review

# The spindle assembly checkpoint: More than just keeping track of the spindle

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## ABSTRACT

Genome stability is essential for cell proliferation and survival. Consequently, genome integrity is monitored by two major checkpoints, the DNA damage response (DDR) and the spindle assembly checkpoint (SAC). The DDR monitors DNA lesions in G1, S, and G2 stages of the cell cycle and the SAC ensures proper chromosome segregation in M phase. There have been extensive studies characterizing the roles of these checkpoints in response to the processes for which they are named; however, emerging evidence suggests significant crosstalk between the checkpoints. Here we review recent findings demonstrating overlapping roles for the SAC and DDR in metaphase, and in response to DNA damage throughout the cell cycle.

**KEYWORDS:** cell cycle, checkpoint, chromosome segregation, DNA damage, spindle assembly

## INTRODUCTION

For successful cell division, the genome must be accurately duplicated and equally segregated into daughter cells, which requires exquisite kinetics and regulation. Once the genome is duplicated, proper chromosome segregation is ensured by the spindle assembly checkpoint (SAC). The SAC is best characterized for its role in preventing premature separation of sister chromatids until proper chromosome alignment has been achieved at the metaphase-to-anaphase transition [1]. The SAC is composed of several members that are conserved from yeast to mammals; the core components include MAD1, MAD2, MAD3 (BUBR1 in mammals), BUB1 and BUB3. During metaphase, MAD1 and BUB1 monitor proper spindle microtubule-kinetochore attachment/tension. Until proper attachments are formed, the SAC inhibits the anaphase promoting complex (APC), an ubiquitin ligase required for downstream cleavage of sister chromatid cohesion. When problems are sensed and SAC is activated, MAD2 interacts with MAD1 at the kinetochore, facilitating conformational changes in MAD2 to promote the formation of the mitotic checkpoint complex (MCC) with MAD3 and BUB3. The MCC binds to CDC20 and prevents its interaction with, and activation of, the APC. Upon proper spindle-kinetochore binding/tension, inhibition of CDC20 is alleviated and CDC20-APC is free to ubiquitinate targets, leading to the cleavage of cohesion by the protease separase (Figure 1), and progression through metaphase [2].

Consistent with their role in monitoring microtubule-kinetochore attachment/tension, SAC components localize to the kinetochore during metaphase. However, SAC components MAD1 and MAD2 localize to the nuclear periphery during interphase in several organisms through interactions with the nuclear pore complex (NPC) [3-5], suggesting SAC has roles outside of metaphase. Indeed, there is emerging evidence

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that the SAC can be activated by additional chromosomal perturbations other than chromosome-microtubule connection defects and this can occur in both interphase and metaphase. This review focuses on recent data that indicates that the SAC is integrated with the DNA damage response and that these pathways function together throughout the cell cycle to ensure genome integrity.

**The DDR and SAC networks overlap during metaphase**

Newly replicated chromatin must be segregated equally into daughter cells, but it is imperative that DNA replication is complete before segregation occurs and that all DNA damage has been resolved. If cell division occurs with DNA damage or while replication is ongoing, it can result in fragmentation of the genome and loss of genetic material. Genomic stability is monitored by a large network of proteins termed the DNA damage response (DDR), which senses DNA damage and facilitates repair. Following DNA damage, DDR sensor proteins associate with the lesion to recruit transducers of the damage signal, which in turn help to recruit effectors for repair. ATM (ataxia telangiectasia mutated) and ATR (ataxia telangiectasia and Rad3 related) are highly conserved damage transducer kinases that phosphorylate both additional transducer kinases, such as CHK1 and CHK2, and effectors to repair the damage. Double stranded breaks (DSBs) can be repaired by two main mechanisms, non-homologous end joining (NHEJ) where two broken DNA ends are ligated back together without regard for homology, or homologous recombination (HR), where homologous sequences on the sister chromatid or homolog are used as a template for repair. ATM recognizes DSBs and helps recruit components to process the ends. If the break is to be repaired by HR, components such as the MRN exonuclease complex (Mre11-Rad50-Nbs1) are recruited to the break, leading to resection of the 5' strand of DNA leaving a 3' overhang. The single stranded DNA is then coated with the recombinase RAD51, which facilitates repair through strand exchange with homologous sequence. ATR recognizes single stranded DNA and contributes to break signaling [6]. If the break is to be repaired by NHEJ, DNA resection is blocked by binding of components such as 53BP1 and KU70/KU80 [7] (Figure 2). The DDR can respond to DNA damage by inducing arrest in the G1, S or G2 phases of the cell cycle [8]; however, until recently, the SAC was thought to be the only checkpoint to function in mitosis, and that the SAC and DDR checkpoints were largely independent. Contrary to this, recent findings have uncovered a requirement for several DDR components to efficiently activate SAC in response to metaphase defects.

The breast cancer associated protein BRCA1 may contribute to SAC activity by up-regulating transcription of SAC genes BUB1, BUBR1, ZW-10, and MAD2. Loss of BRCA1 leads to lagging chromosomes, chromosome bridges, and failure to arrest in metaphase after treatment with microtubule destabilizer nocodazole [9, 10]. These mitotic defects are presumably due to decreased expression of SAC components in BRCA1 mutants. Other DDR components may regulate SAC function more directly. The Fanconi anemia (FA) gene family encompasses >19 genes and together these gene products facilitate DNA repair; loss of FA genes leads to genomic instability and cancer syndromes (reviewed in [11]). A directed RNAi screen identified 14 FA genes required for metaphase arrest after treatment with microtubule stabilizer taxol. Consistent with a role for FA genes in SAC activation, 8 FA components (FANC-A, B, E, G, L, D1, D2 and N) localize to the spindle and/or centrosomes in unperturbed mitosis [12].

The DDR kinases ATM and CHK1 have also been implicated in SAC activation. ATM is phosphorylated by SAC component Aurora B in response to spindle poisons and, in turn, ATM phosphorylates additional SAC components. Aurora B kinase facilitates proper chromosome segregation by localizing to microtubules near kinetochores and helping to bi-orient chromatids [13, 14]. Aurora B phosphorylates ATM on serine 1403 and loss of S1403 phosphorylation results in a shortened M phase and defective M phase checkpoint. Following ATM S1403 phosphorylation, ATM phosphorylates BUB1 on serine 314 and this phosphorylation is also required for M phase checkpoint function [15]. Additionally, ATM-dependent MAD1 phosphorylation on serine 214 is required for MAD1 homodimerization and

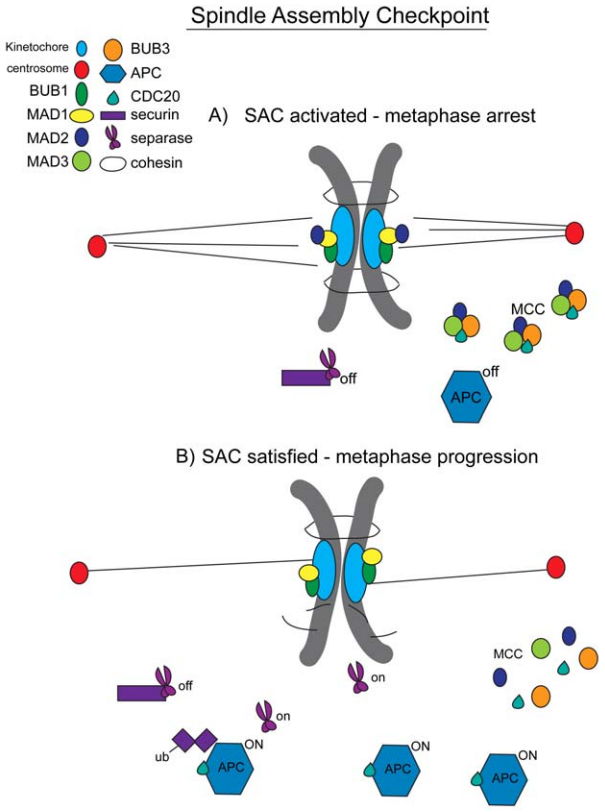


**Figure 1.** A) SAC is activated during metaphase by unattached kinetochores/spindle microtubules. The mitotic checkpoint complex (MCC) inhibits activation of the APC, which in turn prevents release of sister chromatid cohesion through securin/separase. B) Once all kinetochores are attached to the spindle, the MCC is disassembled and the APC is activated by CDC20, resulting in separase-dependent cleavage of cohesin and the onset of anaphase.

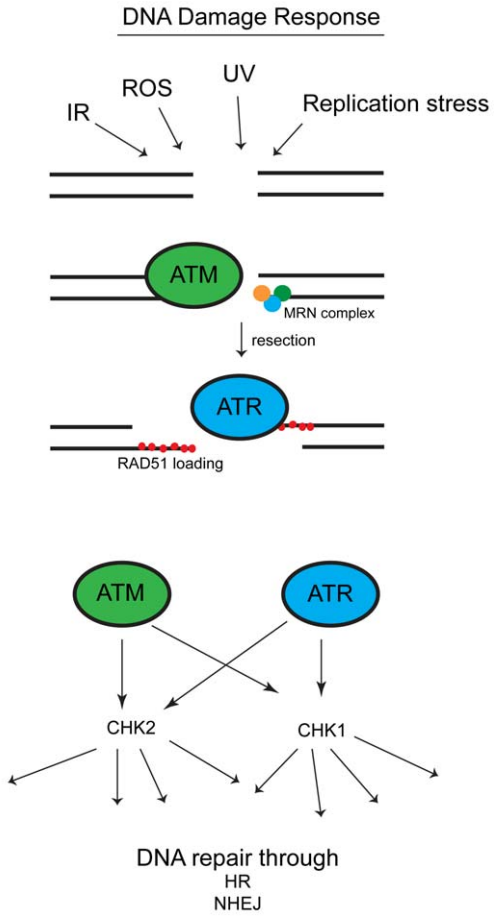


**Figure 2.** DDR activation after DNA damage where upstream kinases ATM and ATR recognize lesions and initiate signaling cascades for DNA repair.

heterodimerization with MAD2 after nocodazole treatment [16]. ATM is also responsible for the localization of DDR component MDC1 to kinetochores after spindle poisons. MDC1 is best known for its role in double strand break repair, where it binds to gamma-H2AX and recruits repair proteins to breaks [17, 18]. MDC1 may have a similar function in metaphase, as ATM-dependent gamma-H2AX at kinetochores is required for MDC1 localization, and together ATM and MDC1 aide in kinetochore localization of canonical SAC component MAD2 [19]. Additionally, high throughput screens in yeast and tissue culture cells have identified several other SAC or spindle-associated components as potential ATM/ATR target proteins, including CENPF, CLASP1 & 2, NUMA, and NUSAP1 [20, 21].

Where ATM is critical in executing efficient metaphase arrest after spindle poisons, another key DDR kinase CHK1 plays an integral role in unperturbed mitosis as well as contributes to checkpoint function. Evidence that CHK1 is required for proper chromosome segregation comes from the observations that CHK1 loss leads to chromosome misalignment, lagging chromosomes, and bi-nucleate cells in many cell types from avian DT40 cells to primary mouse fibroblasts and human cell lines [22-24]. CHK1's role is likely tied to interactions with Aurora B kinase at kinetochores, as CHK1 also localizes to kinetochores and loss of CHK1 leads to mis-localization of

Aurora B and decreased Aurora B kinase activity [22, 24]. Further, CHK1 can phosphorylate Aurora B and enhance its activity *in vitro* [24]. CHK1 can also phosphorylate SAC component MAD2 *in vitro*, and CHK1 depletion leads to MAD2 down regulation [25]. Similarly to loss of ATM, loss of CHK1 also affects the ability of cells to undergo metaphase arrest after spindle damage [24, 26]. We have shown in mitotic germ cells of *Caenorhabditis elegans* that loss of CHK1 renders cells incompetent to undergo metaphase arrest in the presence of monopolar spindles and compromises metaphase plate stability under persistent metaphase arrest. Consistent with a role in the checkpoint, CHK1 localizes to kinetochores under activating conditions [26]. Taken together, these studies provide clear evidence for integration of DDR components in canonical SAC activation in response to spindle defects.

Finally, a component important for meiotic recombination and DDR checkpoint signaling [27, 28] has recently been implicated in mitotic SAC regulation. TRIP12/PCH2 is a AAA+ ATPase that affects several processes in meiosis, likely through chromosome remodeling [29-32]. During mitosis, TRIP13/PCH2 deactivates the spindle checkpoint by binding to MAD2 and facilitating a conformational change to the inactive form [33-36]. Most recently, TRIP13/PCH2 was also found to be required for SAC activation and MAD2 accumulation on kinetochores in *C. elegans* embryos [37]. These recent studies exemplify we have more to learn about components that activate the spindle assembly checkpoint.

## SAC can be triggered by DNA damage

In addition to responding to spindle perturbation there is increasing evidence that DNA damage can induce signaling between the DDR and SAC resulting in metaphase arrest. *Saccharomyces cerevisiae* has been a key system for elucidating mechanisms of checkpoint signaling, and studies in yeast were the first to reveal that metaphase arrest in response to DNA damage is dependent on SAC. Both gamma- and UV-irradiation leads to an ATM-dependent phosphorylation of Pds1/Securin resulting in metaphase arrest [38] and arrest is compromised after loss of Pds1/Securin [39]. Metaphase arrest can also be induced by several types of replication stresses, either by introducing mutations or treating with drugs [40, 41]. Interestingly, arrest is dependent on SAC, ATM and ATR, but not kinetochore components [41-43].

The role of the SAC in response to DNA damage is not limited to yeast. Exposed telomeres are a unique type of DNA damage, and in Drosophila, uncapped telomeres can activate the DDR and result in a SAC-dependent metaphase arrest [44]. In human cells, DNA breaks induced in prophase of mitosis delay metaphase independent of p53 and ATM. Under these conditions, MAD2 localizes to kinetochores, suggesting the delay is SAC dependent. Additionally, the delay can be overridden by expression of a dominant negative MAD2 [45]. A SAC-dependent G2/M arrest can also be induced by treating cells with drugs that affect replication progression [46, 47]. Thus, SAC is responsive to both DNA and spindle perturbations and arrests cells in metaphase.

## SAC function outside of metaphase

SAC can clearly be activated by DNA damage to inhibit APC and metaphase progression, likely through CDC20 inhibition. However, in light of the increasing evidence for crosstalk between the DDR and the SAC, it raises the possibility that SAC can respond to DNA damage outside of its canonical role. Interestingly, a mouse line harboring a SAC-insensitive CDC20 allele survives longer during embryogenesis than SAC mutants [48], consistent with the idea that SAC components have additional functions independent of APC inhibition. We have shown that in *C. elegans* germ cells, SAC components MAD1 and MAD2 localize to the nuclear periphery after DNA damage is induced [26]. Not only are MAD1/2 important for localizing DNA damage to the nuclear periphery, they are also required for efficient DNA repair. This novel role of SAC components is active in S/G2 and is independent of CDC20 inhibition. Further, MAD2 also localizes to the nuclear periphery after damage in mammalian cells [26], suggesting this S/G2 role for SAC is conserved.

SAC component BUB1 is also required for efficient DNA repair in both budding yeast and mammalian cells. BUB1 localizes to DNA near

induced breaks in yeast and can interact with the NHEJ component 53BP1 to promote repair by NHEJ. Loss of BUB1 when there is no homologous template for repair leads to persistent DNA damage signaling and decreased cell survival [49]. Taken together, these studies suggest roles for SAC outside of metaphase and independent of the spindle/kinetochore.

**SAC, DDR, and damaged DNA localize to the nuclear periphery**

Interactions between chromatin and the nuclear periphery are emerging as important regulators of both gene expression and DNA repair. Chromatin domains localize to the nuclear periphery where gene expression is regulated through interactions with lamins [50-53] and NPCs [54, 55]. Interestingly, SAC component MAD1 also localizes to the nuclear periphery in interphase though associations with inner nuclear rim component NUP84/NUP107 [3, 5, 56]. We have shown in *C. elegans* that damaged DNA localizes to the periphery, and that this localization is dependent on MAD1 at NPCs and is important for repair [26]. There are also several examples of damaged DNA interacting with nuclear pore components or where depletion of nuclear pore components leads to DNA damage sensitivity [57-63]. In budding yeast, loss of NUP84/NUP107 specifically leads to DNA damage sensitivity and is required for localization of damage sites to nuclear pores [64-66]. NUP84/NUP107 also interacts with the SLX5-SLX8 (equivalent to mammalian RNF4), a STUbL (SUMO-Targeted Ubiquitin Ligase) complex whose loss leads to DNA damage sensitivity similar to that of loss of NUP84/NUP107 [66]. Damaged telomeres also associate with pores [67].

In Drosophila, DSBs induced in heterochromatin move to the nuclear periphery to complete repair in a STUbL and NPC-dependent process [68, 69]. Finally, translesion synthesis (TLS) polymerases eta and gamma have been shown to interact with nuclear pore components NPP2/NUP85, which complexes with NUP84/NUP107, and NPP22/NDC1, a core nuclear pore component, in *C. elegans*. Further, depletion of these Nups lead to damage sensitivity that is not exacerbated by co-depletion with TLS polymerases, suggesting that the NPC functions in DNA repair by tethering these repair polymerases [70]. Interactions among pores, damaged DNA and repair components are clearly important for efficient DNA repair. Whether these mechanisms function through MAD1/2 at the nuclear pores as they do in *C. elegans* has yet to be investigated.

**SAC responds to DNA damage and chromosome asynapsis in meiosis**

Meiosis is a specialized form of cell division and consequently the SAC plays an important role in regulating chromosome segregation. In meiosis, replicated homologous chromosomes must pair, synapse, form crossovers, and then undergo two cell divisions to produce haploid gametes. Mammalian female meiosis occurs in the fetus, and oocytes arrest prior to division until the organism reaches sexual maturity; arrest can last for decades. Additionally, female meiosis is associated with high levels of aneuploidy [71]. Long-term arrest coupled with weak SAC activity in oocytes has been postulated as the cause for increased aneuploidy in females. Although SAC is present in mammalian oocytes, it does not appear to play a robust checkpoint function as delays in bivalent alignment on the metaphase plate, or mono-oriented univalents, do not activate the checkpoint. The current model posits that SAC is satisfied in mid-prometaphase regardless of bivalent alignment, as MAD2 disassembles from all kinetochores at this time [72-77]. Although SAC does not respond to chromosome misalignment in meiosis as it does in mitosis, SAC can be activated by spindle poisons to arrest oocytes in metaphase [73-75]. Additionally, loss of SAC accelerates metaphase [78] suggesting a regulatory role in metaphase progression similar to what has been observed in mitosis [79]. Although the robustness of SAC in response to microtubule attachments in meiosis is debated, novel roles for the SAC in meiosis are emerging.

Two groups have recently discovered that DNA damage induced in mouse oocytes results in a metaphase arrest in meiosis I (MI). Under DNA damaging conditions, SAC components localize to kinetochores and depletion of SAC abrogates MI arrest [80, 81]. Interestingly, this MI arrest is independent of proper spindle formation and bi-orientation and congression of bivalents, even

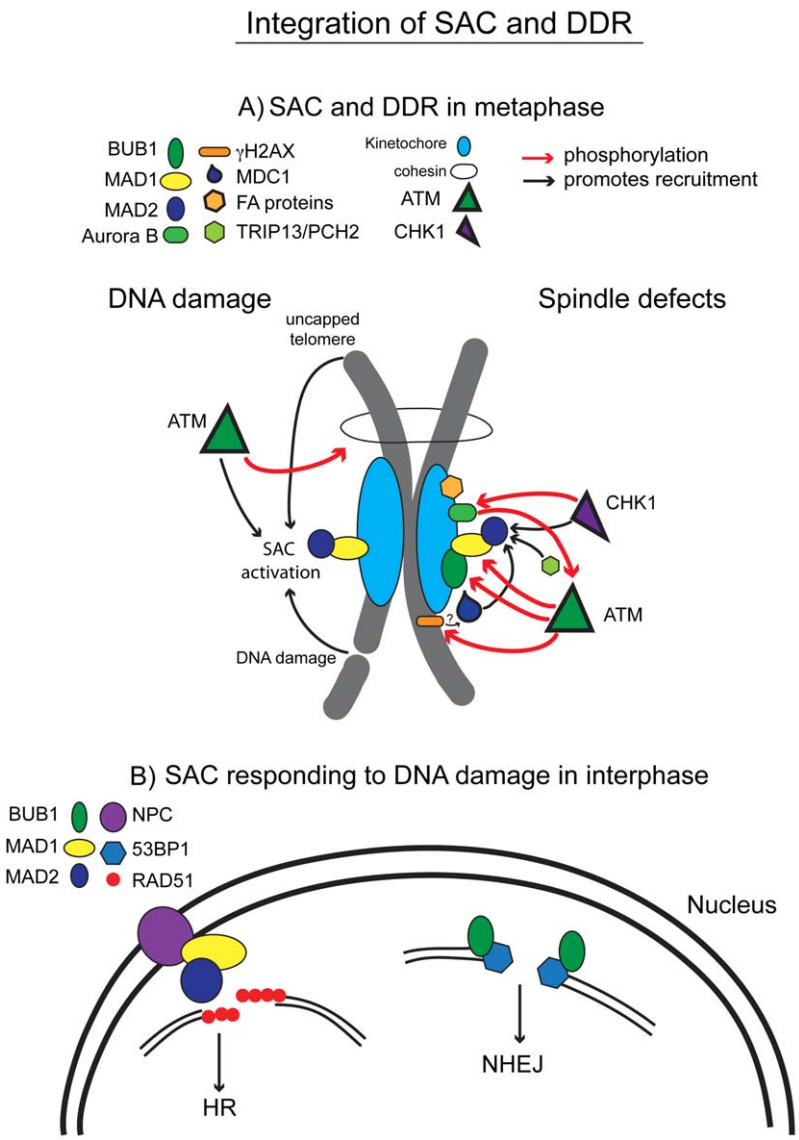


**Figure 3.** Model for SAC and DDR integration in A) metaphase in response to both damaged DNA and spindle defects. Damaged DNA and uncapped telomeres can lead to SAC activation. In some cases, this is through ATM. ATM can also phosphorylate securin to induce arrest. In response to spindle defects, ATM and CHK1 phosphorylate a number of SAC components (red arrows) and are required for metaphase arrest. B) SAC components respond to DNA damage in interphase and are required for localization of damaged DNA to the nuclear periphery and efficient DNA repair. Additionally, SAC component BUB1 localizes to break sites and interacts with 53BP1 to promote NHEJ.

in the presence of chromosome fragments [80] suggesting SAC activation is responding to DNA damage directly.

Bohr *et al.* discovered a unique role for SAC components at the nuclear periphery in prophase of meiosis in *C. elegans* [82]. They found that SAC components localize to the nuclear periphery throughout meiotic prophase and interact with inner nuclear membrane protein SUN1. During meiotic prophase, chromosome ends associate with SUN1 and are moved along the nuclear envelope to promote proper chromosome pairing [83], suggesting SAC could be monitoring chromosome pairing. Once pairing has occurred,

synaptonemal complex forms between the homologs to facilitate formation of a crossover, in a process termed synapsis [84]. SAC components negatively regulate synapsis, perhaps to destabilize incorrect pairing events and allow time for stabilization of proper homolog pairing. SAC is also required for the synapsis checkpoint, which induces apoptosis when synapsis is defective [82]. These results suggest that in meiotic prophase, SAC monitors tension between homologs similar to SAC's role in monitoring tension between sister chromatids in mitosis. While SAC may not be playing identical roles in mitosis and meiosis, SAC activation is clearly important for genomic stability and proper chromosome segregation in both mitosis and meiosis.

## CONCLUSION

The roles of the SAC and the DDR are not as defined as their names imply, as the SAC can respond to more than spindle assembly and the DDR can recognize lesions other than damaged DNA (Figure 3). Emerging evidence indicates that the SAC and DDR function together in both interphase and metaphase of mitosis and meiosis. Genomic stability is crucial for cell survival and fitness, and the two main cell cycle checkpoints collaborate throughout the cell cycle to coordinate damage signals, induce cell cycle arrest and facilitate repair. Given the expanded role of the SAC in monitoring genomic integrity, it is likely that additional roles await discovery.

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## CONFLICT OF INTEREST STATEMENT

No conflict of interests.

## ABBREVIATIONS

| | | |
|---|---|---|
| APC | : | Anaphase promoting complex |
| ATM | : | Ataxia telangiectasia mutated |
| ATR | : | Ataxia telangiectasia and Rad3 related |
| DDR | : | DNA damage response |
| DSBs | : | DNA double stranded breaks |
| FA | : | Fanconia anemia |
| HR | : | Homologous recombination |
| MI | : | Meiosis I |
| MCC | : | Mitotic checkpoint complex |
| MRN | : | Mre11-Rad50-Nbs1 |
| NHEJ | : | Non-homologous end joining |
| SAC | : | Spindle assembly checkpoint |
| STUbL | : | SUMO-Targeted Ubiquitin Ligase |

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