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COMMENTARY

Turning end-joining upside down in mitosis

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ABSTRACT

How cells deal with DNA breaks during mitosis is not well understood. While canonical non-homologous end-joining predominates in interphase, it is inhibited in mitosis to avoid telomere fusions. DNA polymerase θ mediated end-joining appears to be repressed in interphase, but promotes break repair in mitosis. The nature and induction time of breaks might determine their fate during mitosis.

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DNA-double strand breaks (DSBs) are pernicious lesions whose incorrect repair can lead to genome instability and cell death. To deal with such injuries, cells developed a complex network of pathways termed the DNA damage response (DDR). An essential component of the DDR is the activation of checkpoints, which halt cell cycle progression and ensure enough time for repair through different mechanisms. The main DSB repair pathways are canonical non-homologous end-joining (c-NHEJ) and homologous recombination (HR); while other pathways, such as DNA polymerase θ (POLθ) mediated end-joining (TMEJ), are used in specific genomic contexts. c-NHEJ is active throughout the cell cycle and involves direct break re-ligation, with no or limited processing. Conversely, resection of the break ends channels repair into HR which is active during S/G2 and uses the sister chromatid as a template for high-fidelity repair. Alternatively, TMEJ can also act on resected breaks to mediate the re-ligation of break ends with exposed micro-homologies but harbors the risk of introducing mutations. Therefore, these two pathways can compete for the same type of breaks; in fact, it has been described that cells with deficiencies in HR are dependent on POLθ for DSB repair and survival.[1,2] Although these responses are well delineated in interphase, less is understood about how cells cope with DNA damage in mitosis. It is generally assumed that repair is blocked during mitosis to prioritize cell division, as there are no DNA damage checkpoints in place after late prophase until the next G1 phase. However, recent work from our group and others shows proof of DNA repair occurring in mitosis.[3–5] Below we discuss how these new findings fit to previous models supporting the inactivation of end-joining in mitosis and consider the factors that may contribute to pathway choice in this cell cycle stage.

In our latest publication, we illustrate how cells deficient in the breast cancer type 2 susceptibility protein (BRCA2), which are defective in HR, delay repair of endogenous breaks arising in S phase until the onset of the following mitosis.[3] We show that the radiation sensitive 52 (RAD52) protein localizes to resected breaks in G2, actively preventing their processing by TMEJ until cells enter mitosis (Figure 1a). Premature activation of TMEJ in the absence of RAD52 in BRCA2-deficient G2 cells leads to the formation of chromatid fusions, therefore compromising genomic stability. Regulation of TMEJ is likely also relevant for BRCA2-proficient cells, as BRCA2 itself inhibits POLθ possibly at different DSB intermediates than RAD52.[6] We hypothesize that the chromatin compaction of mitotic chromosomes helps to align the correct break ends, preventing fusion formation by TMEJ during mitosis and explaining how a seemingly error-prone pathway is required for survival. Our findings illuminate the mechanisms underlying the synthetic lethality between BRCA2 and POLθ and support the notion of active DSB repair in mitosis.

A parallel study in *Drosophila melanogaster* has also pinpointed the use of Polθ-dependent repair to mitosis, along with the fly protein Fanconi anemia complementation group D2 (Fancd2).[4] Clay and colleagues use cells of the *Drosophila* hindgut, which lack DNA damage checkpoints and enter mitosis with unrepaired breaks, similar to our BRCA2-deficient human cells with a leaky G2/M checkpoint.[7] The authors show how Fancd2 and Polθ are required for the processing and repair of DSBs to promote faithful segregation of broken acentric DNA, thus, preventing the formation of micronuclei. Using human cells, the Stucki laboratory has also shown how breaks induced during mitosis by ionizing radiation are tethered by mediator of DNA damage checkpoint 1 (MDC1) and DNA topoisomerase II binding protein 1 (TOPBP1) (Figure 1b). These two proteins form bridging structures that may stabilize broken chromosomes until they get repaired in the next G1 phase.[5] One can speculate whether the time when a break is generated plays a role in its mitotic fate: if the break is induced prior to mitosis, the cell may have time to process it accordingly, generating a suitable substrate for TMEJ. If the break is induced strictly within mitosis, it may be better to use different strategies (such as tethering) until the full repair mechanisms are again available.

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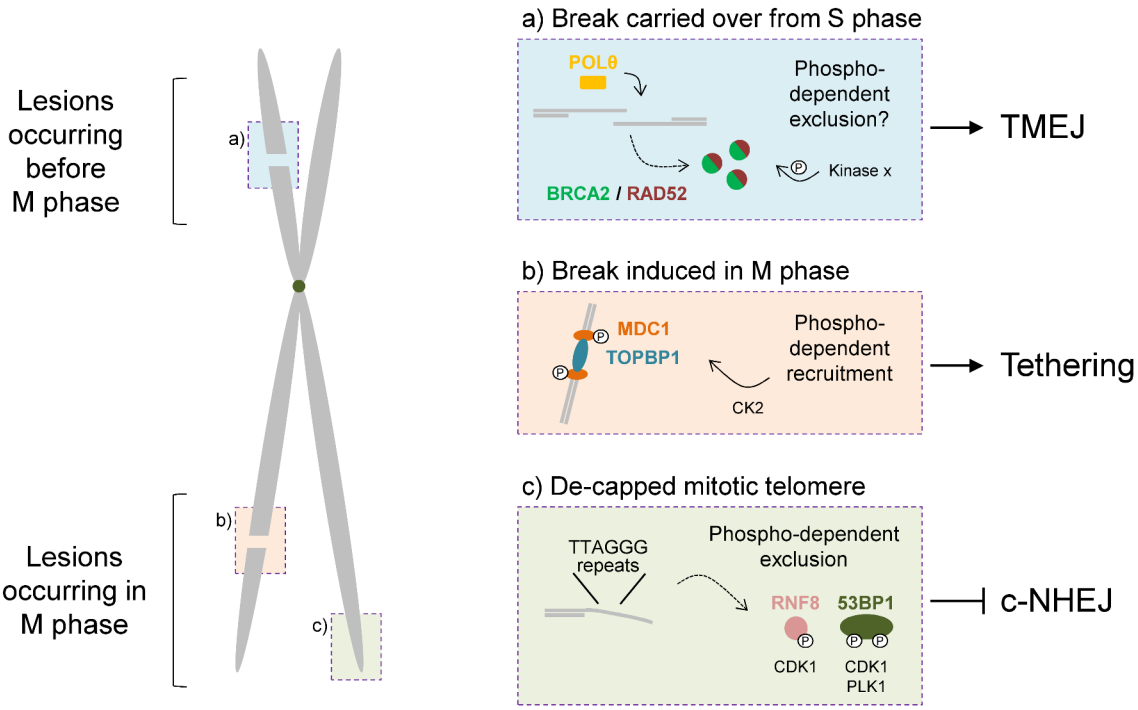

Figure 1. DSB repair in M phase. The time (before or during M phase) and the type of lesion (telomere de-capping or internal break) affect pathway choice in M phase. While DNA polymerase θ (POLθ) mediated end-joining (TMEJ) is delayed until mitosis to repair breaks carried over from S phase (a), internal breaks directly occurring in M phase are tethered to avoid loss of genetic material during cell division (b). In addition, canonical non-homologous end-joining (c-NHEJ) is blocked in M phase to protect naturally de-capped mitotic telomeres from being fused (c). Phosphorylation plays an important role in the regulation of these responses. Abbreviations: breast cancer type 2 susceptibility protein (BRCA2), radiation sensitive 52 (RAD52), mediator of DNA damage checkpoint 1 (MDC1), DNA topoisomerase II binding protein 1 (TOPBP1), ring finger protein 8 (RNF8), tumor protein P53 binding protein 1 (53BP1), casein kinase 2 (CK2), cyclin-dependent kinase 1 (CDK1), Polo-like kinase 1 (PLK1).

These reports present surprising new evidence on the usage of TMEJ, expanding previous models supporting the idea that classic end-joining is blocked during mitosis.[8] While apical DDR components, such as phosphorylated histone variant H2AX (γH2AX) and MDC1, are detected at mitotic breaks, the downstream factors ring finger proteins 8 (RNF8) and 168 (RNF168), as well as tumor protein P53 binding protein 1 (53BP1) are excluded.[8,9] This is essential to prevent unprotected mitotic telomeres from being fused by c-NHEJ, a process that is regulated by post-translational modifications: phosphorylation of RNF8 by cyclin-dependent kinase 1 (CDK1) and 53BP1 by CDK1 and polo-like kinase 1 (PLK1) are needed to block the recruitment of these factors to mitotic breaks[8] (Figure 1c); whilst, de-phosphorylation of 53BP1 by cyclin-dependent kinase 5 (CDK5) has also been shown to be necessary to re-activate repair once the cells progress into G1.[10] This highlights the role of mitotic kinases and phosphatases in regulating the DDR during the cell cycle. In fact, phosphorylation of MDC1 is also crucial for TOPBP1 recruitment to breaks in mitosis and therefore their role in the tethering of acentric chromosomes.[5] In addition, it remains to be elucidated whether the removal of RAD52 and BRCA2 from mitotic breaks to allow the activation of TMEJ is also controlled *via* phosphorylation.

Given that the end-joining pathways are differentially used during mitosis, several questions remain unanswered. Why is c-NHEJ suppressed, while TMEJ is preferentially delayed until mitosis? Why is TMEJ not a threat to telomeres? Do the nature and structural differences of breaks play a role? One can imagine that telomeres may not be a suitable substrate for TMEJ, supported by the fact that TMEJ activation at telomeres only occurs when the full shelterin complex as well as c-NHEJ are disrupted.[2] Consequently, it is possible that the cell can afford the activation of TMEJ to repair lesions at resected internal breaks with exposed homologies during mitosis, without it being potentially harmful for telomere stability. Answering these questions is important for the understanding of DSB repair through the cell cycle and could be of value for targeting tumor cells with defects in specific checkpoints or repair factors.

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