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DNA repair

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POLQ to the rescue for double-strand break repair during mitosis

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DNA polymerase θ (POLQ) repairs mitotic DNA breaks; this requires RHINO and PLK1, averts genomic instability and may underlie effects of POLQ inhibitors in HDR-deficient cancer cells. We discuss recent work on mitotic DNA break processing and repair, the need for multiple DSB repair pathways and implications of therapeutic POLQ targeting in cancer.

DNA double-strand breaks (DSBs) can be highly disruptive for genome integrity in dividing cells. In the absence of efficient DSB repair, chromosome fragments can be mis-segregated during mitosis, leading to loss or gain of genome fragments and innate inflammatory responses.

To prevent the deleterious effects of DSBs, cells have evolved multiple repair pathways. Combined with cell cycle checkpoints that transiently arrest proliferation, these pathways are essential to maintaining genome integrity. DSBs are predominantly repaired by two mechanistically distinct pathways, non-homologous end-joining (NHEJ) and homologous recombination (HR). The choice between these is driven largely by the state of the cell cycle: whereas NHEJ is available through interphase, HR requires cyclin-dependent kinase (CDK) activity, essentially restricting templated repair to the S and G2 phases, when sister chromatids become available upon DNA replication (Fig. 1a).

Lack of DNA repair during mitosis?

As was already recognized in the 1950s[1], the DNA damage response (DDR) is rigorously reprogrammed during mitosis. Cells lose their ability to arrest cell cycle progression in response to mitotic DSBs, and both NHEJ and HR are inactivated[2–4]. Although sensing of DSBs occurs normally during mitosis, the combined activity of CDK1 and Polo-like kinase-1 (PLK1) blocks the activation of both NHEJ, by precluding the recruitment of repair proteins RNF8 and 53BP1 to DSBs[2], and HR, by preventing RAD51 accumulation at breaks[5]. Without canonical DSB repair, it was assumed that mitotic cells were extremely vulnerable to DSB induction, and they indeed show enhanced sensitivity to irradiation[4].

Rather than repairing DSBs, mitotic cells were thought to 'mark' DSBs, through H2AX phosphorylation (γH2AX) and subsequent recruitment of the upstream DDR component MDC1, to allow repair upon completion of mitosis[4]. However, 'marking' DNA breaks for repair in the next G1 phase is only sensible when the two ends of the broken chromosome are kept in close proximity. This appeared to be the case owing to the effects of MDC1-mediated recruitment of TOPBP1, which mediates tethering of broken chromosome fragments during mitosis[6]. More recently, TOPBP1 was shown to form a mitosis-specific complex with CIP2A, comprising filamentous structures that connect the two DNA ends of a mitotic DSB[7,8] (Fig. 1b). Whether the TOPBP1–CIP2A complex is solely involved in tethering DNA ends, or whether it also facilitates DSB processing, had remained unclear until now.

POLQ to the rescue: TMEJ repairs DSBs during mitosis

Two recent studies demonstrate that DNA beaks are actively repaired during mitosis, but through a third, stand-alone DSB repair pathway[9,10] (Fig. 1a). Over the past decade, numerous discoveries in a wide range of organisms have revealed that a previously ill-defined alternative end-joining activity is largely exerted by POLQ[11]. Alternative end-joining had initially been considered a backup pathway, observed only under experimental conditions in which NHEJ or HR are compromised. However, this perspective turned out to need revision when specific genetic contexts were identified in which polymerase θ-mediated end-joining (TMEJ) is the predominant method of DSB repair, in otherwise fully DSB-repair-proficient cells or organisms[12]. Mechanistically, POLQ extends the 3′ end of one DSB end, using as a template a partially resected strand from the end of the other DSB. This process relies on minimal base-pairing between both strands, serving as a minimal 'primer' to initiate DNA synthesis by POLQ; this explains the typical microhomology that characterizes sites of TMEJ repair and gave rise to the term microhomology-mediated end-joining.

Earlier work had already provided hints about when during the cell cycle TMEJ may act, as POLQ was implicated in repairing replication-associated DNA breaks[13], as well as HR repair intermediates in HR-compromised cells[14]. As TMEJ does not interfere with HR per se, these findings suggested that TMEJ operates temporally downstream of HR. The new studies now provide evidence for TMEJ as the key DNA DSB repair pathway during mitosis[9,10]. These findings greatly extend recent links between POLQ and mitotic processes, with POLQ being inhibited by RAD52 during interphase, yet becoming activated upon mitotic entry to repair replication-born DNA lesions[15]. POLQ – along with TOPBP1 and CIP2A – was also recently identified in a proteomics analysis of protein recruitment to mitotic DNA breaks[16].

The new work demonstrates actual DSB repair during mitosis: genomic scars at DSB sites induced by Cas9 in mitotic cells display the typical POLQ signature of microhomology, and this signature is lost in *POLQ*$^{-/-}$ cells[10]. Likewise, levels of the DSB biomarker γH2AX decrease progressively during mitosis in POLQ-proficient cells, reflective of active mitotic DNA repair, whereas γH2AX levels remain high in *POLQ*$^{-/-}$ cells[9,10]. Two distinct mechanisms were demonstrated for POLQ activation during mitosis in the two papers. Brambati et al.[9] identify RHINO, which, in conjunction with the 9-1-1 complex, specifically mediates POLQ recruitment to mitotic DNA breaks. The function of RHINO is restricted to mitosis, through PLK1-mediated activation upon mitotic entry and APC/C-mediated degradation when cells exit mitosis. Importantly, cells lacking RHINO or POLQ cannot repair mitotic DNA breaks. In parallel, Gelot et al.[10] identify PLK1 as a direct activator of POLQ, mediating its recruitment to mitotic DSBs. Combined with the recent finding that TOPBP1 functions as a phosphorylation-dependent

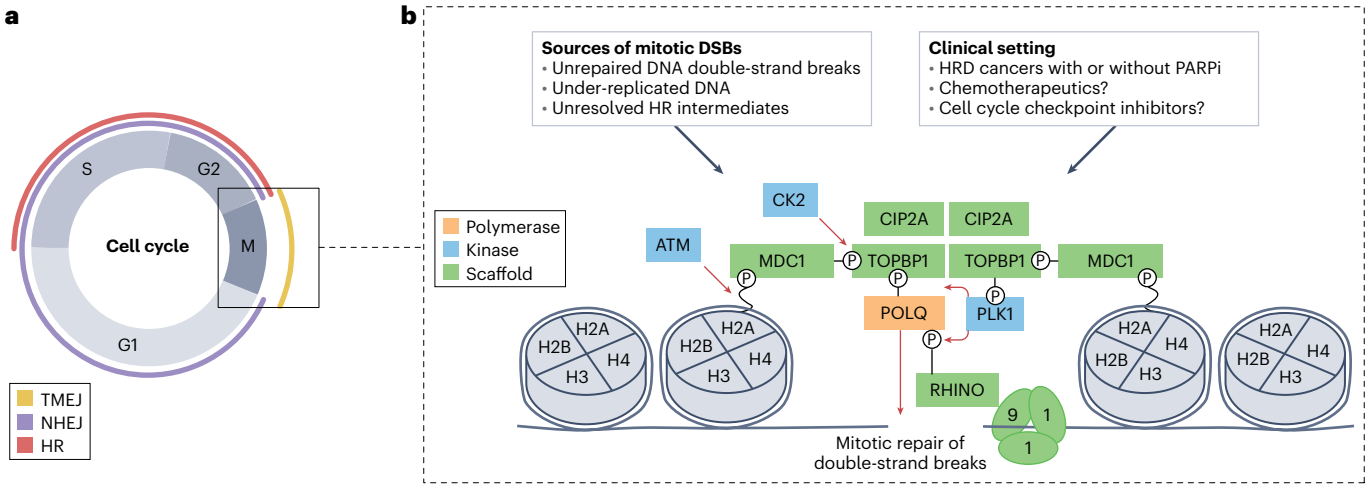

Fig. 1 | Regulation of DSB repair in mitosis by POLQ. a, Schematic representation of the cell cycle, with proposed activity of NHEJ, HR and TMEJ in the different cell cycle phases. **b**, Known complexes and modifications regulating DSB repair during mitosis.

recruitment platform for PLK1 (Fig. 1b)[17], these new studies position PLK1 as a key activator of TMEJ during mitosis. Interestingly, PLK1 is also responsible for the inactivation of NHEJ upon entry into mitosis, establishing this kinase as a master regulator of the DSB repair switch from HR and NHEJ during interphase to TMEJ during mitosis.

Clinical implications

The current studies are also relevant for the investigation of POLQ as a targetable vulnerability in HR-defective (HRD) cancer cells. Previous work revealed that POLQ inhibition enhances the cytotoxicity of PARP1 inhibitors (PARPi) and remains effective in PARPi-resistant HRD cancer cells[18]. Importantly, the DNA lesions induced by PARPi in HR-deficient cells are transmitted into mitosis[19], providing a rationale for why HRD cancers would rely on mitotic DSB repair. Indeed, Gelot et al.[10] now demonstrate that selective inactivation of POLQ during mitosis phenocopies the BRCA–PARP1 synthetic lethal interaction. With the recognition that TMEJ is the key pathway for mitotic repair of DSBs, these findings potentially expand the role of POLQ inhibitors to other cancer settings that involve mitotic DSB accumulation. These include several chemotherapeutic agents and recently developed cell cycle checkpoint inhibitors that cause cells to enter mitosis with under-replicated DNA or unrepaired DSBs. Of note, TMEJ signatures are also seen in human genomes and in healthy tissues, suggesting that mitotic POLQ activity is important in physiological contexts, which warrants long-term assessment of the effects of POLQ inhibitors on genome integrity in normal cells.

Outstanding questions

Building on these exciting new discoveries, numerous questions arise. With the description of two separate mechanisms of POLQ recruitment, through RHINO and through TOPBP1, an intriguing question concerns whether and how these mechanisms intersect. For instance, one could speculate that RHINO directly or indirectly interacts with the TOPBP1–CIP2A complex. Furthermore, our current understanding of the proteins involved in mitotic DSB repair remains limited. Therefore, there is a pressing need to uncover additional factors and explore the intricacies of post-translational regulation in this pathway. What also remains unclear is how TMEJ relates to other mitotic mechanisms that process DNA lesions, including mitotic DNA synthesis (or MiDAS) and the processing of joint DNA molecules at ultrafine bridges in anaphase[20].

On a more conceptual level, though intriguing, it is unclear why cells require a third DSB repair pathway (in addition to NHEJ and HR) and what the extent of the physiological significance of TMEJ may be. Although POLQ is evolutionarily conserved from plants to humans, not all eukaryotic species encode a POLQ ortholog (yeast, for example, does not)[21]. Additionally, *Polq* knockout mice develop normally, albeit with elevated levels of genome fragmentation, as evidenced by micronucleus formation in their blood cells[22]. With the knowledge that TMEJ operates in mitosis, it will be important to establish the nature and extent of DSBs for which neither NHEJ nor HR offers a repair solution; DSBs that remain unrepaired when cells enter mitosis may provide the raison d'être for TMEJ. Answering this question in the context of physiological conditions is a challenging endeavor, as the answer may vary across different tissues and developmental stages. Because of its intrinsically mutagenic mode of action, TMEJ leaves behind a specific genomic scar as a 'smoking gun' for its involvement[23]. The existence of a TMEJ signature within human genomes, which is also clearly apparent in disease-causing alleles[23], indicates that TMEJ has a substantial role in driving mutagenesis within healthy cells and populations. With this notion, another intriguing question emerges: does the repair of mitotic breaks by TMEJ function as a tumor suppressor by preventing chromosome fragmentation, or does it, due to its mutagenic mode of action and ability to generate translocations, have tumor-promoting capacity, as early work suggested[24]? These effects of TMEJ repair are not mutually exclusive; it is possible that TMEJ provides short-term benefits by safeguarding cells against gross genomic rearrangements, while its mutagenic activity may lead to adverse consequences in the long term.

Although this intriguing research significantly expands the functional implications of TMEJ and its mechanistic wiring, it also underscores that we are only in the early stages of understanding the potential functions and implications of TMEJ concerning genome stability, evolution and cancer. Exciting prospects lie ahead.

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Competing interests

M.A.T.M.v.V. has acted on the scientific advisory boards of Nodus Oncology and RepareTx.