The inner workings of replisome-dependent control of DNA damage tolerance

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Genomic DNA is continuously challenged by endogenous and exogenous sources of damage. The resulting lesions may act as physical blocks to DNA replication, necessitating repair mechanisms to be intrinsically coupled to the DNA replisome machinery. DNA damage tolerance (DDT) is comprised of translesion synthesis (TLS) and template switch (TS) repair processes that allow the replisome to bypass bulky DNA lesions and complete DNA replication. How the replisome orchestrates which DDT repair mechanism becomes active at replication blocks has remained enigmatic. In this issue of Genes & Development, Dolce and colleagues (pp. 167–179) report that parental histone deposition by replisome components Ctf4 and Dpb3/4 promotes TS while suppressing error-prone TLS. Deletion of Dpb3/4 restored resistance to DNA-damaging agents in ctf4Δ cells at the expense of synergistic increases in mutagenesis due to elevated TLS. These findings illustrate the importance of replisome-directed chromatin maintenance to genome integrity and the response to DNA-damaging anticancer therapeutics.

Faithful replication of DNA is essential for genome integrity. The fidelity of this process is further strained by endogenous and environmental genotoxic stresses that produce myriad DNA lesions that interfere with DNA replication. Bulky DNA adducts hamper replication fork progression and can result in fork stalling and collapse. A conserved DNA damage response, termed DNA damage tolerance (DDT), evolved to produce several strategies to effectively bypass replication-blocking lesions (Friedberg 2005). DDT encompasses two general repair mechanisms, termed translesion synthesis (TLS) and template switch (TS). The former, TLS, involves specialized DNA polymerases that gain access to the stalled replisome, allowing synthesis through bulky DNA adducts at the expense of increased mutation rates. On the other hand, TS exploits the undamaged DNA strand as a template to execute homology-directed repair synthesis in what is generally considered an error-free mechanism of repair (Friedberg 2005).

Nucleosomes are first disassembled to allow efficient replication fork movement, followed by rapid reassembly using naïve and parental histones [Fig. 1A]. This mechanism allows epigenetic information carried by parental histones to be transmitted to daughter cells following DNA replication, thus contributing to the maintenance of chromatin status across generations (Margueron and Reinberg 2010). Replisome components interact with histones and promote parental histone deposition, coordinating replication progression with the maintenance of chromatin status and genome integrity [Fig. 1A, Gan et al. 2018; Yu et al. 2018]. Chromatin is a well-established regulator of damage repair mechanisms at DNA double-strand breaks [Verma and Greenberg 2021], yet its contribution to the outcome of DDT is less clear. Moreover, how different components of the replisome orchestrate the DDT repair mechanism and whether this relates to assembly of nascent chromatin during DNA replication was unexplored.

The conserved replisome factor Ctf4 (chromosome transmission fidelity 4; AND-1 in humans) was defined as a central regulator of genome integrity during DNA replication. Indeed, Ctf4 links replicative helicases to DNA polymerase α, facilitates parental histone transfer, establishes sister chromatin cohesion, promotes template switching, and modulates rDNA damage repair [Branzei and Szakal 2016]. Elevated AND-1 (Ctf4) expression was associated with poor prognosis for patients with lung and esophageal cancers [Sato et al. 2010]. Additionally, AND-1 was identified as a promising cancer therapeutic target in genome-wide CRISPR screens in 324 human cancer cell lines encompassing 30 different cancer types [Behan et al. 2019].

Ctf4-deficient cells are hypersensitive to the DNA alkylating agent, methyl methanesulfonate (MMS) [Fumasconi et al. 2015]. MMS treatment primarily introduces 3-methyladenine (3MeA) lesions that inhibit DNA
replication and triggers DDT and other repair pathways. The Branzei laboratory (Fumasoni et al. 2015) reported that Ctf4 deficiency reduced TS but increased mutagenesis. This led Dolce et al. (2022) to exploit robot-assisted high-throughput suppressor screens of \(\textit{ctf4} \Delta\) sensitivity to MMS to understand the molecular basis underlying Ctf4-mediated DDT pathway choice and drug resistance. The investigators identified nonessential DNA polymerase \(\varepsilon\) subunits Dpb3 and Dpb4 as suppressors of MMS hypersensitivity in \(\textit{ctf4} \Delta\) strains (Fig. 1B). Drug resistance in \(\textit{ctf4} \Delta\ \textit{dpb3/4} \Delta\) double mutants was not associated with restored TS but instead occurred via hyperactivation of TLS with a commensurate increase in mutation rate (Fig. 1B). In accordance, Ctf4 and/or Dpb3 deficiency resulted in DNA polymerase \(\xi\) [Pol \(\xi\)]-dependent mutagenesis (Northam et al. 2006; Fumasoni et al. 2015). Nevertheless, when exposed to genotoxic agents, error-prone TLS protects the genome from life-threatening errors associated with fork stalling/collapse and deleterious large fragment deletions (Volkova et al. 2020). The investigators used rationally designed mutants of Dpb3/4 to show that its parental histone transfer activity promotes TS while suppressing TLS and the MMS hypersensitivity of \(\textit{ctf4} \Delta\) cells (Fig. 1B). An important distinction, however, is that Ctf4 and Dpb3/4 do not contribute equally to TS or to DDT, since \(\textit{ctf4} \Delta\) showed greater reductions in TS and more sensitivity to MMS (Fig. 1B). In contrast, \(\textit{dpb3/4} \Delta\) did not discernably affect MMS responses unless combined with Ctf4 mutation.

The work highlights the importance of chromatin assembly in modulating DDT pathway choice and drug resistance during replication-associated DNA damage responses. Parental histones and associated post-transcriptional modifications (PTMs) are preserved during DNA replication and transmitted to daughter cells for rapid and efficient responses to different stimuli, including DNA damage (Margueron and Reinberg 2010). It will be important to further investigate how parental histone transfer from Ctf4 and Dpb3/4 cooperatively works to regulates DDT and viability in the presence of bulky DNA
adducts. A second question of interest is whether histone H3 and H4 PTMs affect DDT. It is well established that PTMs on other replisome components profoundly influence DDT repair. For example, PCNA ubiquitylation by either monoubiquitin or K63-linked chains has been shown to dictate DDT by TLS or TS, respectively. Whether parental histone PTMs similarly influence DDT remains to be investigated. Ctf4 deficiency also impairs sister chromatid cohesion, fork reversal/regression, break-induced replication, and gene rearrangements. Moreover, ctf4Δ shows synthetic lethal relationships specifically with DNA recombination mutations Rad52Δ and Rad59Δ, but not Rad51Δ (Fumasoni et al. 2015). Whether these functions also require Ctf4-dependent parental histone transfer is unknown.

Intrinsic or acquired resistance to DNA-damaging agents limits the efficacy of widely used DNA damage-inducing anticancer therapies. The development of novel combination therapies that target compensatory repair mechanisms is a sought-after strategy for improving clinical outcomes. TLS inhibition may be a feasible approach to prevent resistance to potential therapy targeting Ctf4/AND-1. Induction of TLS has also been observed in other contexts, including in BRCA mutant cancer cells [Taglialetela et al. 2021; Tirman et al. 2021]. In these genetic contexts, it will be interesting to determine whether Dpb3/4 mutations in Pol ε or other alterations that affect parental histone deposition and contribute to cancer cell fitness arise. An affirmative result might also warrant the use of a TLS inhibitor. High-throughput genetic screening such as in the study from Dolce et al. (2022), including the more surgical use of emerging base-editing technologies to disrupt specific protein–protein interactions, may reveal a host of new vulnerabilities and resistance mechanisms within the DNA damage response that substantively benefit anticancer therapies.

Competing interest statement

R.A.G is a cofounder and scientific advisory board member for JAMM Therapeutics and RADD Pharmaceuticals.

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References

Behan FM, Iorio F, Picco G, Gonçalves E, Beaver CM, Migliardi G, Santos R, Rao Y, Sassi F, Pinnelli M, et al. 2019. Prioritization of cancer therapeutic targets using CRISPR–Cas9 screens. Nature 568: 511–516. doi:10.1038/s41586-019-1103-9

Branzi D, Szakal B. 2016. Priming for tolerance and cohesion at replication forks. Nucleus 7: 8–12. doi:10.1080/19491034.2016.1149663

Dolce V, Dusi S, Giannattasio M, Joseph CR, Fumasoni M, Branzei D. 2022. Parental histone deposition on the replicated strands promotes error-free DNA damage tolerance and regulates drug resistance. Genes Dev [this issue]. doi:10.1101/gad.349207.121

Friedberg EC. 2005. Suffering in silence: the tolerance of DNA damage. Nat Rev Mol Cell Biol 6: 943–953. doi:10.1038/nrm1781

Fumasoni M, Zwicky K, Vanoli F, Lopes M, Branzei D. 2015. Error-free DNA damage tolerance and sister chromatid proximity during DNA replication rely on the polα/primase/Ctf4 complex. Mol Cell 57: 812–823. doi:10.1016/j.molcel.2014.12.038

Gan H, Serra-Cardona A, Hua X, Zhou H, Lahib K, Yu C, Zhang Z. 2018. The Mcm2–Ctf4–Pola axis facilitates parental histone H3–H4 transfer to lagging strands. Mol Cell 72: 140–151.e3. doi:10.1016/j.molcel.2018.09.001

Margueron R, Reinesberg D. 2010. Chromatin structure and the inheritance of epigenetic information. Nat Rev Genet 11: 285–296. doi:10.1038/nrg2752

Northam MR, Garg P, Baitin DM, Burgers PM, Shcherbakova PV. 2006. A novel function of DNA polymerase ξ regulated by PCNA. EMBO J 25: 4316–4325. doi:10.1038/sj.emboj.7601320

Sato N, Koitaka J, Fujita M, Hosokawa M, Ito T, Tsuchiya E, Kondo S, Nakamura Y, Daigo Y. 2010. Activation of WD repeat and high-mobility group box DNA binding protein 1 in pulmonary and esophageal carcinogenesis. Clin Cancer Res 16: 226–239. doi:10.1158/1078-0432.CCR-09-1405

Taglialetela A, Leuzzi G, Sannino V, Cuella-Martín R, Huang JW, Wu-Baer F, Baer R, Costanzo V, Ciccia A. 2021. REV1–Polδ maintains the viability of homologous recombination-deficient cancer cells through mutagenic repair of PRIMPOL-dependent ssDNA gaps. Mol Cell 81: 4008–4025.e7. doi:10.1016/j.molcel.2021.08.016

Tirman S, Quinet A, Wood M, Meroni A, Cybulia E, Jackson J, Pegoararo S, Simoneau A, Zou L, Vindigni A. 2021. Temporally dependent ssDNA gaps. Mol Cell 81: 4026–4040.e8. doi:10.1016/j.molcel.2021.09.013

Verma P, Greenberg RA. 2021. Communication between chromatin and homologous recombination. Curr Opin Genet Dev 71: 1–9. doi:10.1016/j.gde.2021.05.006

Volkova NV, Meier B, González-Huici V, Bertolini S, Gonzalez S, Vühringer H, Abascal F, Martincorena I, Campbell PJ, Gartner A, et al. 2020. Mutational signatures are jointly shaped by DNA damage and repair. Nat Commun 11: 2169. doi:10.1038/s41467-020-15912-7

Yu C, Gan H, Serra-Cardona A, Zhang L, Gan S, Sharma S, Johanson E, Chabes A, Xu RM, Zhang Z. 2018. A mechanism for preventing asymmetric histone segregation onto replicating DNA strands. Science 361: 1386–1389. doi:10.1126/science.aat8849