Cell Research

www.nature.com/cr
www.cell-research.com

RESEARCH HIGHLIGHT

A new varietal of DNA interstrand crosslink repair

Ravindra Amunugama[1,2] and Johannes C. Walter[1,2]

Cell Research (2020) 30:459–460; https://doi.org/10.1038/s41422-020-0321-x

Knipscheer, Patel, and colleagues have identified a new mechanism that repairs alcohol-induced DNA interstrand crosslinks with a minimum of collateral DNA damage.

Our cells are under constant assault by endogenous and exogenous agents that damage DNA and thereby threaten genome integrity. One of the most pernicious forms of damage is a DNA interstrand crosslink (ICL), which covalently crosslinks the two strands of the double helix. ICLs block DNA replication and transcription, and in the absence of repair, a single ICL can kill a eukaryotic cell. Given their toxicity, ICL-inducing agents are widely used in cancer chemotherapy, as exemplified by cisplatin and nitrogen mustard. Importantly, the most essential cellular pathways of ICL repair are coupled to DNA replication, most likely because failure to resolve ICLs during this phase of the cell cycle causes lethality due to a failure to complete genome duplication. Until recently, only two mechanisms of S phase ICL repair were known, both of which involve DNA intermediates that could cause gross chromosomal rearrangements (GCRs). Now, a third pathway has been discovered that repairs alcohol-induced, endogenous ICLs without the possibility of major concomitant DNA damage.[1]

The first pathway of S phase ICL repair that was discovered involves a large group of 'FANC' genes that are mutated in Fanconi anemia (FA), a human genetic disease characterized by bone marrow failure (BMF) and cancer predisposition.[2] The 22 FANC proteins include recombinases, structure-specific endonucleases, and translesion synthesis (TLS) polymerases. Over a decade ago, repair of a plasmid bearing a site-specific cisplatin ICL was recapitulated in frog egg extracts.[3] In this system, cisplatin ICL repair requires the convergence of two replication forks on the lesion (Fig. 1a). Following replisome disassembly and fork remodeling, the ubiquitylated FANCI-FANCD2 heterodimer promotes dual incisions surrounding the ICL to unhook the lesion.[4,5] The double-stranded (ds) DNA break formed in one sister chromatid is repaired by homologous recombination, while the ICL remnant remaining in the other sister chromatid is bypassed by TLS polymerases. This FA pathway can likely repair any ICLs, since it incises the phosphodiester backbone adjacent to the lesion. However, its creation of a double-strand break intermediate has the potential to cause chromosomal translocations and other GCRs, major causes of genomic instability and cancer.

More recently, a second pathway of ICL repair was revealed when plasmids containing a psoralen ICL were exposed to egg extracts (Fig. 1b). These ICLs are unhooked by a DNA glycosylase called NEIL3, which cleaves one of the glycosyl bonds forming the ICL.[6,7] Unlike the FA pathway, the NEIL3 pathway avoids incision of the phosphodiester backbone. However, NEIL3 generates an abasic (AP) site in one sister chromatid, whose bypass is highly mutagenic. Moreover, inadvertent cleavage of the chemically labile AP site can potentially create a dsDNA break, followed by GCRs.

Although the above studies identified pathways that process chemotherapy-induced ICLs, a major unanswered question concerned the identity of the endogenous ICLs that cause FA and how they are repaired. In 2011, Patel and colleagues focused on acetaldehyde, a reactive byproduct of alcohol metabolism that is converted to acetate by aldehyde dehydrogenase (ALDH2). They showed that combined inactivation of FANCD2 and ALDH2 recapitulates key features of FA in mice.[8] Moreover, mutations in *ALDH2* exacerbate the FA phenotype in humans.[9] Together with the fact that acetaldehyde can form ICLs in the test tube,[10] these observations strongly suggest that endogenous acetaldehyde is an underlying cause of the genomic instability associated with FA. A two-tiered protection model was proposed. The first tier involves acetaldehyde detoxification by ALDH2; in the second tier, the FA pathway repairs any ICLs formed by acetaldehyde molecules that escape the first tier.

To explore how acetaldehyde ICLs (AA-ICLs) are repaired, the Patel and Knipscheer laboratories joined forces.[1] They generated a synthetic AA-ICL, which normally forms when two acetaldehyde molecules react with guanine to form a propanoguanine intermediate that subsequently reacts with a neighboring guanine in the complementary strand. Addition of this AA-ICL to frog egg extracts revealed that roughly half of the lesions are repaired by the FA pathway, consistent with deficiency in the FA pathway causing acetaldehyde sensitivity. However, the other half of the AA-ICLs are repaired by a new pathway (Fig. 1c). Like the FA and NEIL3 pathways, the new pathway requires fork convergence. However, it does not involve incision of the phosphodiester backbone or cleavage of a glycosyl bond. Instead, the data suggest that the AA-ICL undergoes partial reversal, regenerating guanine in one strand and probably propanoguanine in the other strand. Thus, repair avoids the dsDNA break and AP site intermediates that might lead to GCRs in the FA and NEIL3 pathways. The only genome instability generated by the new pathway involves a low level of mutagenesis during TLS of the propanoguanine.

It is now critical to determine whether unhooking of the AA-ICL by the new pathway is enzymatic or involves mechanical forces generated by the converging replisomes. Assuming the former scenario, it will be fascinating to see whether mutations in the relevant enzyme eventually turn up as a new complementation group in FA patients. Another question concerns why cells have three pathways of S phase ICL repair. Apparently, the NEIL3 and AA-ICL repair pathways cannot compensate for each other.[1,6] Thus, these pathways probably evolved to neutralize specific types of ICLs with minimal concomitant damage. In contrast, the

[1]Department of Biological Chemistry and Molecular Pharmacology, Blavatnik Institute, Harvard Medical School, Boston, MA 02115, USA and [2]Howard Hughes Medical Institute, Boston, MA 02115, USA
Correspondence: Johannes C. Walter (johannes_walter@hms.harvard.edu)

Published online: 28 April 2020

© CEMCS, CAS 2020

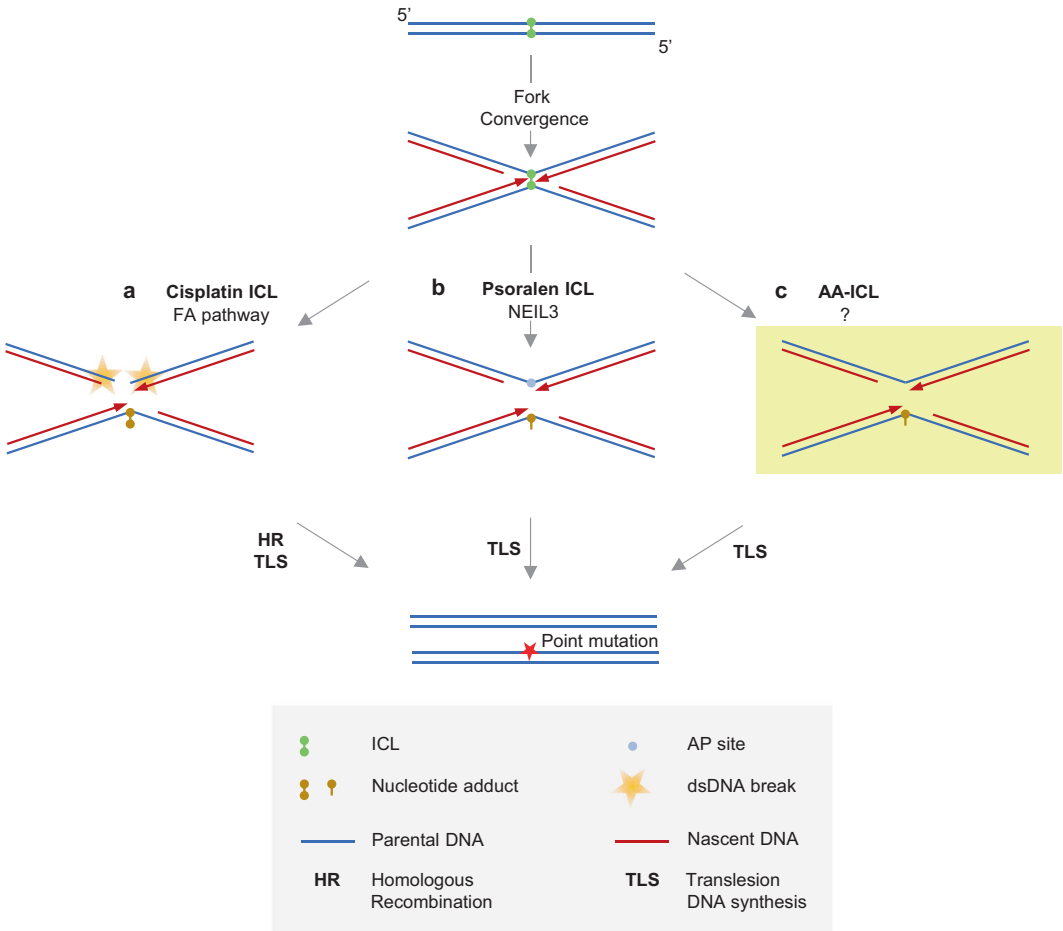

Fig. 1 Replication-coupled DNA ICL repair pathways. a The FA pathway repairs cisplatin ICLs via dual incisions in the phosphodiester backbone. The resulting double-strand break is fixed by inter-sister chromatid recombination. **b** The NEIL3 pathway cuts one of the two glycosyl bonds forming the ICL, generating an AP site in one strand and an ICL remnant in the other strand, both of which are bypassed by TLS. **c** Acetaldehyde ICLs are unhooked via direct reversal, generating a guanine in one strand and a propanoguanine in the other strand, which is bypassed by TLS.

incision-based FA pathway, although able to repair all ICLs tested so far, incurs a higher risk of genomic rearrangements and therefore should be used as a last resort. Indeed, the NEIL3 pathway is favored over the FA pathway[11] via TRAIP-dependent ubiquitin signaling at the converged replisomes.[7] Whether and how the putative AA-ICL unhooking enzyme is prioritized remains an important question for future investigation.

With this latest work, it is now abundantly clear that vertebrate cells have at their disposal a versatile and sophisticated arsenal to repair ICLs with a minimum of collateral DNA damage. Through further investigation, it may one day become possible to manipulate the flux through these pathways for therapeutic benefit.

REFERENCES

1. Hodskinson, M. R. et al. *Nature* **579**, 603–608 (2020).
2. Niraj, J., Farkkila, A. & D'Andrea, A. D. *Annu. Rev. Cancer Biol.* **3**, 457–478 (2019).
3. Räschle, M. et al. *Cell* **134**, 969–980 (2008).
4. Amunugama, R. et al. *Cell Rep.* **23**, 3419–3428 (2018).
5. Knipscheer, P. et al. *Science* **326**, 1698–1701 (2009).
6. Semlow, D. R., Zhang, J., Budzowska, M., Drohat, A. C. & Walter, J. C. *Cell* **167**, 498–511 (2016).
7. Wu, R. A. et al. *Nature* **567**, 267–272 (2019).
8. Langevin, F., Crossan, G. P., Rosado, I. V., Arends, M. J. & Patel, K. J. *Nature* **475**, 53–58 (2011).
9. Hira, A. et al. *Blood* **122**, 3206–3209 (2013).
10. Cho, Y. J. et al. *Chem. Res. Toxicol.* **19**, 195–208 (2006).
11. Li, N. et al. *Nucleic Acids Res.* **48**, 3014–3028 (2020).