which functionally important C-domain salt bridge and triad residues influence proton conduction: their physical proximity to the apparent selectivity filter residue hints toward a possible direct coupling mechanism, but the details remain to be elucidated.

As for the question of channel desensitization, Chen et al. have already pinpointed a residue important for that process: the conserved Asp residue in TM9. When that Asp is mutated to Asn, Ala or Glu, XtOtop3 loses its desensitization phenotype. Mutation to Asn also results in a similar loss of function in mOtop1[6]. Interestingly, this Asp residue faces the solvated cavity at the interface between the N domain and C domain, and it can apparently engage in an interaction with the functionally important conserved His residue in TM12. We further note that since the charge-preserving mutation Asp-to-Glu is disruptive, something other than the ability to be protonated appears to be important in the desensitization mechanism.

Finally, these structures open the way to understand the functional variability among Otop proteins. Among the protonatable residues lining the permeation pathways, we note a His located at the top of TM11 in Otop3; in contrast, Otop1 features a charged or polar residue in that position. Intriguingly, the orientation of this residue in the chOtop3 structure is different from its orientation in the XtOtop3 structure (Fig. 2), which could indicate either a necessary rearrangement during conformational change or an ortholog-specific feature. Thus, the work by Chen et al.[6] and Saotome et al.[7] will pave the way to many other exciting mechanistic studies of Otop channels. ❐

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References

1. Tu, Y.-H. et al. *Science* **359**, 1047–1050 (2018).
2. DeCoursey, T. E. *Physiol. Rev.* **93**, 599–652 (2013).
3. Pielak, R. M. & Chou, J. J. *Biochim. Biophys. Acta BBABiomembr.* **1808**, 522–529 (2011).
4. Knight, C. & Voth, G. A. *Acc. Chem. Res.* **45**, 101–109 (2012).
5. Peng, Y., Swanson, J. M. J., Kang, S. G., Zhou, R. & Voth, G. A. *J. Phys. Chem. B* **119**, 9212–9218 (2015).
6. Chen, Q., Zeng, W., She, J., Bai, X. C. & Jiang, Y. *eLife* **8**, e46710 (2019).
7. Saotome, K. et al. *Nat. Struct. Mol. Biol.* https://www.nature.com/articles/s41594-019-0235-9 (2019).
8. van Keulen, S. C. et al. *J. Phys. Chem. B* **121**, 3340–3351 (2017).
9. Morgan, D. et al. *J. Gen. Physiol.* **142**, 625–640 (2013).
10. Chen, Q., Zeng, W., She, J., Bai, X. & Jiang, Y. *eLife* https://elifesciences.org/articles/46710#SA (2019).

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Competing interests

The author declares no competing interests.

DNA REPAIR

Crosslink and shield: protecting abasic sites from error-prone repair

Abasic sites are among the most frequent DNA lesions, and when they occur within single-stranded DNA, their repair can give rise to genomic instability and mutations. One mechanism for the protection of abasic sites involves covalent attachment of 5-hydroxymethylcytosine-binding, embryonic stem cell–specific (HMCES) protein to DNA. Now, two research groups have elucidated the structural basis of the action of HMCES and its bacterial equivalent, YedK, revealing a unique and intriguing chemistry of DNA–protein crosslink formation.

Marcin Nowotny

DNA is constantly subject to adverse chemical modifications that either occur spontaneously or are induced by external and internal damage agents, and such damage can distort genetic information. Among the most common modifications of DNA is loss of the base, which leads to the formation of abasic sites, also called 'apurinic/apyrimidinic' (AP) sites. Cells have efficient ways of removing AP sites from double-stranded DNA (dsDNA), mainly via the base excision–repair pathway[1]. Single-stranded DNA (ssDNA) arises transiently in various nucleic acid–processing pathways, including DNA replication. Some types of base loss may occur more rapidly in ssDNA[2], but no mechanism for the removal of AP sites from single-stranded nucleic acid has been described. When pathways that are normally used to repair AP sites in dsDNA act on such lesions in ssDNA, this can lead to dangerous double-strand breaks or can create mutations that may cause cancer in higher organisms, including humans[3,4]. Two research groups now report crystal structures of a protein that protects AP sites from such mutagenic pathways, and they reveal a unique chemistry for the formation of covalent links between protein and nucleic acid[5,6].

Mohni et al. recently reported an unconventional and intriguing mechanism that protects abasic sites in ssDNA and allows them to escape error-prone repair pathways[7]. (Fig. 1a). These authors observed enrichment of HMCES protein[8] at replication forks, sites of active ssDNA generation, and showed that inactivation of HMCES led to higher levels of DNA damage. One particularly intriguing finding was that the HMCES–DNA complexes were extremely stable and were not disrupted even by boiling, a hallmark of the formation of a covalent link between the protein and nucleic acid, known as a 'DNA–protein crosslink' (DPC). Crosslink formation was site specific, as DPCs occurred only within ssDNA or at a junction between ssDNA and dsDNA. Nuclease activity of the enzyme APE1 at abasic sites can lead to double-stranded breaks, and DPC formation blocked not only this activity but also the mutagenic action of translesion

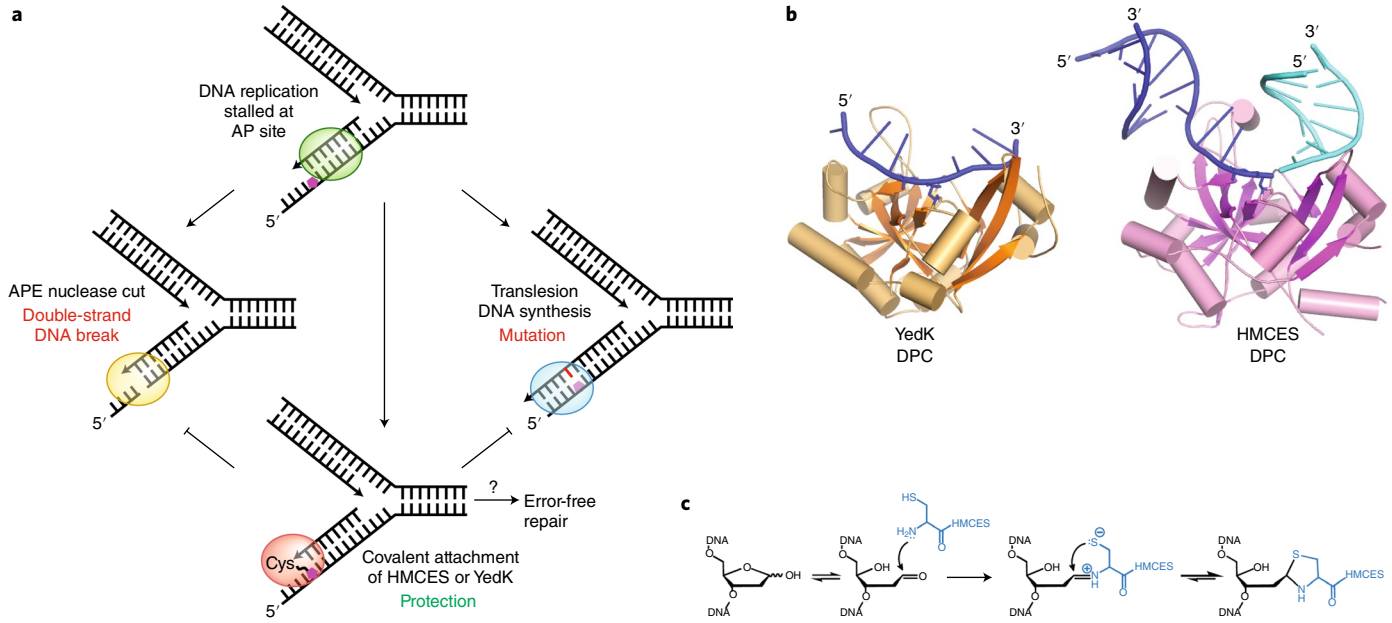

Fig. 1 | Mechanism of protection of abasic sites in single-stranded DNA. a, HMCES pathway, showing the DNA-replication machinery (green) stalled (top) at an abasic site (purple). The abasic site can be cleaved by the nuclease APE (yellow; left) to generate a double-strand DNA break. Alternatively, DNA can be synthesized through the AP site by a translesion polymerase (cyan; right), which can lead to mutations (red). AP sites can be protected from both of these mutagenic pathways via the covalent attachment of HMCES or YedK protein (orange, bottom). DPC formation needs to be followed by error-free repair through an as-yet-unknown pathway. **b**, Crystal structures of YedK protein (PDB 6NUA; left) and HMCES protein (PDB 6OE7; right) covalently crosslinked to DNA. The crosslink is shown as sticks; the DNA is shown in blue. DNA shown in cyan originates from a symmetry-related complex molecule (only the double-stranded portion is shown). **c**, Catalytic mechanism of DPC formation. The N-terminal cysteine that forms the crosslink is shown in blue, and the abasic-site DNA is shown in black (from ref. [6]).

polymerases. Thus, HMCES shielded abasic sites from two pathways that can potentially alter genetic information and promote genome instability. Further experiments suggested that subsequent removal of the DPC might be mediated by ubiquitin-mediated proteolysis.

While Mohni et al. elucidated a previously unknown pathway for the protection of AP sites within ssDNA, the molecular mechanism of function for HMCES and the precise chemistry of its covalent attachment to DNA remained unknown. This important gap in knowledge has now been resolved by two complementary reports that describe crystal structures of human HMCES protein and its *Escherichia coli* equivalent, YedK, in complex with DNA.

Thompson et al. have now determined the structures of YedK crosslinked to a seven-nucleotide ssDNA containing an AP site[6]. Halabelian et al. have determined structures of the catalytic domain of human HMCES protein in complex with dsDNA that comprises a 3′ overhang, including the structure of a DPC[5] (Fig. 1b). Within both structures, the ssDNA that is bound by the protein is sharply bent at the AP site. This DNA conformation cannot be accommodated by dsDNA, which explains the basis of the specificity of HMCES or YedK for AP sites within single-stranded nucleic acid. Analysis of the bacterial structure shows that the DNA immediately adjacent to the AP site and on the 3′ side can adopt a double-stranded conformation. A similar configuration is observed in the human structure, in which dsDNA from a symmetry-related molecule also occupies the position on the 3′ side of the AP site. This arrangement would lead to specific binding and crosslinking to an AP site located at ssDNA–dsDNA junctions that arise when the replication machinery stalls at an abasic site.

One notable strength of the work on YedK is that the structure has been solved at high resolution (1.6 Å), which is key for precise visualization of the chemistry of the DPC in a continuous stretch of DNA. Halabelian et al. solve a structure of the HMCES DPC at a resolution of 2.2 Å, which reveals the same chemical structure of the crosslink. On the protein side, the crosslink is formed by an N-terminal cysteine residue, previously shown by Mohni et al. to be required for the function of HMCES protein[7]. The ribose ring of the abasic site is open, and its C1′ carbon forms covalent links with the cysteine, closing a thiazolidine ring (Fig. 1b). On the basis of these findings, a catalytic mechanism of DPC formation is proposed: an intermediate that links the amide group of the cysteine with the C1′ of the ribose permits subsequent attack of the cysteine sulfhydryl group on C1′, which results in the formation a five-membered ring (Fig. 1c). This is a novel and interesting catalytic mechanism of DPC formation. After the reaction, the DPC is secluded from the solvent, which protects the AP site from the actions of other enzymes. The strength of the study by Halabelian et al. is that it not only visualizes the human DPC structure but also reveals two dsDNA-binding interfaces flanking three nucleotides of ssDNA (Fig. 1b). This implies that the protein can interact with gapped DNAs and 3′- and 5′-overhang substrates and may thus be directed to various DNA structures that comprise single-stranded regions.

Orthologs of HMCES protein are present in all forms of life, and their catalytic domains (the SRAP (SOS response–associated peptidase) domains) are conserved[9]. The structures of human HMCES protein and *E. coli* YedK protein, their mode of DNA binding and the trajectory of the substrate are remarkably similar. This strong

evolutionary conservation reinforces the critical functional roles of SRAP domain–containing proteins.

Considered together, the studies by Thompson et al.[6] and Halabelian et al.[5] provide comprehensive understanding of the molecular mechanism of action of the HMCES and YedK proteins. Future studies should concentrate on the biology of this system. Little is known about the roles of YedK protein in bacteria. Another issue is whether HMCES or YedK protein is involved in the protection of ssDNA-comprising intermediates other than replication forks. DNA protection by the covalent attachment of a protein is a rather counterintuitive strategy because large adducts, such as DPCs, are normally very toxic to cells. Thus, a key outstanding question is the mechanism of DPC removal and how error-free repair of the blocked abasic site is achieved in both eukaryotes and prokaryotes. Lesion resolution may involve the degradation of HMCES protein by the proteasome or by DPC-specific proteases[10]. Nucleotide-excision repair may also be involved, but the repair process would require the faithful reconstitution of dsDNA. Clearly, more exciting biology awaits discovery in this area. ❐

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References
1. Krokan, H. E. & Bjørås, M. *Cold Spring Harb. Perspect. Biol.* **5**, a012583 (2013).
2. Kavli, B., Otterlei, M., Slupphaug, G. & Krokan, H. E. *DNA Repair (Amst.)* **6**, 505–516 (2007).
3. Nik-Zainal, S. et al. *Cell* **149**, 979–993 (2012).
4. Sale, J. E. *Cold Spring Harb. Perspect. Biol.* **5**, a012708 (2013).
5. Halabelian, L. et al. *Nat. Struct. Mol. Biol.* https://doi.org/10.1038/s41594-019-0246-6 (2019).
6. Thompson, P.S., Amidon, K.M., Mohni, K.H., Cortez, D. & Eichman, B.F. *Nat. Struct. Mol. Biol.* https://doi.org/10.1038/s41594-019-0255-5 (2019).
7. Mohni, K. N. et al. *Cell* **176**, 144–153.e13 (2019).
8. Spruijt, C. G. et al. *Cell* **152**, 1146–1159 (2013).
9. Aravind, L., Anand, S. & Iyer, L. M. *Biol. Direct* **8**, 20 (2013).
10. Stingele, J., Bellelli, R. & Boulton, S. J. *Nat. Rev. Mol. Cell Biol.* **18**, 563–573 (2017).

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MICROTUBULES

Cytoskeletal cryptography: structure and mechanism of an eraser

The 'tubulin code', a set of post-translational modifications to the microtubule cytoskeleton that include removal of the C-terminal Tyr of α-tubulin, regulates the biological function of the polymer. Three studies now report structures of VASH1–SVBP and VASH2–SVBP heterodimers and provide insights into how these proteases recognize and cleave the terminal Tyr of α-tubulin.

Kevin C. Slep

The terminal, genetically encoded Tyr (or Phe, depending on the paralog) of α-tubulin is removed by vasohibin 1 (VASH1) and VASH2, a pair of enigmatic proteases that lack a canonical catalytic triad and require a second protein, called the 'small vasohibin-binding protein' (SVBP)[1,2]. VASH1–SVBP and VASH2–SVBP serve critical roles as tubulin code erasers, but how these proteases recognize and cleave the terminal Tyr of α-tubulin and what role SVBP serves in the function of VASH have remained open questions. Three articles in *Nature Structural & Molecular Biology* now present structural and functional analyses of the VASH1–SVBP and VASH2–SVBP heterodimers[3–5], providing structures of the proteases in their 'apo' (unbound) state, bound to three different inhibitors, and in complex with an α-tubulin C-terminal peptide. These studies demonstrate the similarities between the binding of an inhibitor and that of a substrate and delineate a unique Cys-His-(carbonyl)Leu catalytic triad responsible for the catalytic mechanism and thus provide the first atomic-level view of the Tyr eraser of the tubulin code.

Post-translational modifications greatly expand the functional repertoire of the proteome. For many biological systems, post-translational modifications represent a code that is written and erased by modifying enzymes and is decrypted by readers that translate the code into biological output. Post-translational modifications occur with spatial and temporal specificity. Although the functions of complex post-translational modifications have been delineated for some proteins, the tubulin code remains poorly understood.

Heterodimer isoforms of αβ-tubulin are post-translationally modified by a host of enzymes, including those that acetylate and deacetylate α-tubulin, and those that polyglutamylate and polyglycylate α- and β-tubulin C-terminal tails. In addition, there are proteases that remove C-terminal residues, and a tubulin Tyr ligase that can re-ligate the genetically encoded C-terminal Tyr to α-tubulin[6,7]. While the proteolytic removal of the terminal Tyr of α-tubulin by a 'tyrosine carboxypeptidase' was first reported in 1977 (ref. [8]), it was not until 2017 when two studies identified VASH1–SVBP and VASH2–SVBP as the proteases responsible[1,2] (Fig. 1a). Through the use of bioinformatics, a previously published report had proposed a non-canonical Cys-His-Ser catalytic triad for these Cys proteases[9]. While structures of related transglutaminases had been determined, on the basis of sequence homology, vasohibins appeared to be outliers, and their active-site mechanism remained an open question. In addition, little was known about the function of SVBP, which had been ascribed a chaperone role.

The three studies discussed here collectively present multiple crystal structures of VASH1–SVBP and VASH2–SVBP heterodimers in apo states, bound to a mutant α-tubulin C-terminal tail peptide, and in inhibitor-bound states involving epoY, parthenolide, and TPCK (Fig. 1b), along with small-angle X-ray-scattering analysis of