Published in final edited form as:

Nat Struct Mol Biol. 2010 August ; 17(8): 926–928. doi:10.1038/nsmb0810-926.

A new nuclease member of the FAN club

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Abstract

To cope with the life-threatening crisis of a DNA interstrand cross-link (ICL), human cells must invoke the Fanconi anemia (FA) DNA repair pathway. The FA pathway is a multistep repair process, requiring multiple nucleolytic incisions and translesion DNA synthesis. Recent work from four laboratories has identified a novel Fanconi anemia-associated nuclease, FAN1, that binds directly to monoubiquitinated FANCD2, resolving a decade-long puzzle regarding the function of this FANCD2 modification.

DNA interstrand cross-links (ICLs) create a serious threat to cell survival by posing a complete blockade to DNA replication and transcription. The molecular basis of ICL repair has therefore drawn increasing attention in the field of DNA repair. Fanconi anemia (FA) is a recessive genetic disorder, and the thirteen known FA genes encode proteins that cooperate in ICL repair^{1–4}. Eight of the FA proteins comprise a multisubunit ubiquitin E3 ligase complex (referred to as FA core complex). This ligase monoubiquitinates the heterodimeric substrate FANCD2–FANCI to generate FANCD2-Ub–FANCI, a surrogate marker of the activation of the FA pathway^{5,6}. Downstream FA proteins, such as the products of the *FANCD1*, *J* and *N* genes, are also required for the resolution of ICLs. A possible 14th FA gene, *RAD51C*, has recently been identified⁷.

The underlying molecular mechanism of ICL repair by the FA pathway has long remained elusive. Recently, a cell-free system derived from *Xenopus laevis* eggs has elucidated important general principles of this mechanism^{8,9}. ICL repair involves nucleolytic incisions on both sides of the cross-link when two replication forks converge on the ICL. The unhooked cross-link allows the bypass of the lesion by a DNA polymerase, followed by homologous recombination, which completes the repair. Importantly, these studies in the *Xenopus* system show that the FANCD2-Ub–FANCI complex is required to promote both the nucleolytic incision step and the translesion bypass step, but the sequence of the steps and the identity of the nucleases have remained largely a mystery. Recent work from four laboratories has at least partially resolved this mystery by identifying a novel FA-associated nuclease, FAN1, and demonstrating its essential role in the FA pathway^{10–13}.

FAN1 was discovered independently using three different approaches, including bioinformatics searching for nucleases containing ubiquitin-binding domain^{12,13}, a MS-based proteomic approach for deciphering protein-protein interaction¹¹ and a genome-wide small hairpin RNA screen for sensitivity to cross-linking agents¹⁰. Originally known as human KIAA1018, FAN1 contains a conserved nuclease domain at its C terminus, which endows the protein with endonuclease and 5'-to-3' exonuclease activity. Notably, FAN1 shows a

COMPETING FINANCIAL INTERESTS

The authors declare competing financial interests.

preference for a 5'-flap DNA substrate and shows the opposite polarity to the two endonucleases previously implicated in ICL processing, MUS81/EME1 and XPF/ERCC1, which both preferentially cleave 3'-flaps¹⁴. Depletion of FAN1 leads to increased cellular sensitivity to DNA cross-linking agents. This ICL hypersensitivity can be rescued by wild-type FAN1 but not by mutants harboring point mutations at the nuclease domain, suggesting that the nuclease activity of FAN1 is required for ICL repair. The link between FAN1 and the FA pathway is mediated by monoubiquitinated FANCD2-Ub-FANCI and the ubiquitin-binding zinc finger (UBZ) domain at the N terminus of FAN1. FAN1 interacts with FANCD2-Ub-FANCI, and this interaction is abolished by disrupting the UBZ domain of FAN1. Upon ICL damage, FAN1 is recruited to nuclear foci by FANCD2-Ub, whereas a FANCD2 mutant that cannot be ubiquitinated (K561R) fails to complete this event.

The discovery of FAN1 provides a better understanding of how the FA pathway regulates ICL repair (Fig. 1). Cross-link repair is initiated by the stalling of one replication fork on each side, 20–40 nucleotides from the ICL. This stalled structure can be recognized by the FANCM-FAAP24-MHF complex^{15,16}. The FANCM complex then recruits the FA core complex to the damage site and simultaneously activates ataxia telangiectasia and rad-3-related (ATR) kinase via the recruitment of replication protein A¹⁷. ATR phosphorylates FA pathway components and promotes FANCD2 monoubiquitination, and one of the forks continues to progress to the cross-link site. By the time the fork reaches position -1, FANCD2 has been monoubiquitinated by the FA core complex. In turn, FANCD2-Ub-FANCI recruits FAN1 to the damage site, where FAN1 performs nucleolytic incisions and promotes the unhooking of the cross-link, allowing lesion bypass by translesion synthesis (TLS) polymerases. Given its exonuclease activity, FAN1 may also be involved in later steps in this model, such as DNA excision and homologous recombination.

Regardless of the actual mechanism, it is clear that multiple nucleases act at multiple steps in ICL repair (Fig. 1). First, in the ICL unhooking step, MUS81/EME1 acts alone or in conjunction with FAN1 (or perhaps with some other, unidentified nuclease). Second, the unhooked oligonucleotide may be trimmed by 5'- and 3'-exonuclease activities to allow the TLS polymerase to proceed; the 5'-to-3' exonuclease activity of FAN1 may contribute to this step. Third, the blunt-ended double-strand breaks, generated by replication through the single-strand gap, must be resected to generate the necessary 3'-overhang and to allow strand invasion and homologous recombination to occur; despite its function in homologous recombination repair, FAN1 does not appear to be involved in this part of the process^{11,12}. Finally, at a later step of homologous recombination, the D-loop structure created by strand invasion may be incised by FAN1, using its 5'-exonuclease activity. The resulting 5' end may be ligated to the extended invading strand, which results in the regeneration of an intact replication fork, where DNA replication can restart.

Although the identity of the specific nuclease at each step is unclear, several new candidate nucleases are evident. For instance, the SNM1A nuclease appears to have a direct role in ICL repair. SNM1A-deficient cells are hypersensitive to ICL, causing agnethesis^{18,19}. Also, ICLs can lead to monoubiquitination of proliferating cell nuclear antigen (PCNA) by RAD18, generating PCNA-Ub, which will recruit the UBZ-containing SNM1A to the site of ICL for repair²⁰. The SNM1A exonuclease activity¹⁸ may contribute to ICL repair, perhaps by trimming the unhooked oligonucleotide and therefore allowing the TLS step to occur.

Hence, SNM1A recruitment may be analogous to FAN1 recruitment (Fig. 2). It is interesting that FANCD2-Ub and PCNA-Ub specifically recruit nucleases—FAN1 and SNM1A, respectively—to the ICL site. Whether FANCD2-Ub can recruit SNM1A, or whether PCNA-Ub can recruit FAN1, remains to be determined. The TLS polymerases Pol η and Rev1, which

also have UBZ or ubiquitin-binding motif (UBM) domains, are recruited to PCNA-Ub^{21,22} and perhaps to FANCD2-Ub.

Other nucleases are also possible candidates. Recent evidence suggests that FANCD2 itself has intrinsic nuclease activity²³. MLH1, found in complex with FAN1 (refs. 10,11), also has endonuclease activity²⁴. Finally, other nucleases, such as the SLX4 complex^{25–27} and the newly identified EXDL2 (ref. 10), may be required for efficient ICL repair. Importantly, the *in vitro* *Xenopus* system may prove useful in determining the precise function and timing of the nucleolytic events in ICL repair.

Several outstanding issues remain unresolved. First, previous studies indicated that FANCD2 monoubiquitination is required to recruit FANCD2 to chromatin⁵. However, FANCD2-Ub is chromatin bound in foci, even in the absence of its binding partner, FAN1. This suggests that the ubiquitin moiety of FANCD2 may bind other chromatin-bound partners that also contain ubiquitin interaction motifs. Alternatively, because FAN1 preferentially binds FANCD2-Ub¹³, the FANCI-Ub subunit of the ID complex may be critical for chromatin binding. Hence, the identification of other ubiquitin-binding partners for FANCD2 and FANCI will be important. Second, how the FAN1 and SNM1A nucleases are released from FANCD2-Ub and PCNA-Ub is unknown, but the release may be accomplished by the USP1–UAF1 deubiquitinating complex^{28,29}. Third, it remains unclear how FANCD2–FANCI regulates TLS machineries and whether nuclease events come before or after the TLS events⁹. Finally, it will be important to determine whether FAN1 is itself a FA gene (that is, a gene with biallelic germline mutations in FA patient) or it is somatically mutated or silenced in human cancers with ICL hypersensitivity.

Acknowledgments

We thank J.M. Kim and Y. Kee for helpful discussions and critical reading of the manuscript. A.D.D is supported by US National Institutes of Health grant NIH-RO1-DK43889.

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Figure 1.

FA-regulated ICL repair. ICL-stalled replication forks (one fork on each side of the ICL) are recognized by FANCM-FAAP24-MHF complex, which in turn recruits FA core complex to the damage site and activates ATRIP/ATR signaling via the recruitment of replication protein A. ATR phosphorylates FA pathway components and promotes FANCD2 (D2) monoubiquitination, and one of the forks continues to progress to the cross-link site. By the time the fork reaches position -1, FANCD2 has been monoubiquitinated by the FA core complex. FANCD2-Ub-FANCI recruits FAN1 to the damage site, where FAN1 performs nucleolytic incisions and promotes the unhooking of the cross-link, allowing lesion bypass by

TLS polymerases. FAN1 may also be involved in later steps in this model, such as DNA excision and homologous recombination (HR). P, phosphate; I, FANCI.

Figure 2.

Monoubiquitination of FANCD2 (D2) and PCNA in ICL repair. ICL damage activates monoubiquitination of FANCD2 and PCNA by the E3 ligases (FA core complex or RAD18, respectively). These modifications lead to the recruitment of nucleases FAN1 or SNM1A, respectively, via their UBZ domains, and these nucleases may be involved in subsequent incisions and/or excision steps during ICL repair. Similarly, the UBZ or ubiquitin-binding motif (UBM) containing TLS DNA polymerases, such as Rev1 and Pol η , may be recruited to FANCD2-Ub or PCNA-Ub and, in turn, may participate in the repair. The removal of the ubiquitin moiety, performed by the USP1–UAF1 deubiquitinating enzyme complex, is also required for ICL repair. I, FANCI.