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Importance of homo-dimerization of Fanconi-associated nuclease 1 in DNA flap cleavage

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ABSTRACT

Fanconi-associated nuclease 1 (FAN1) removes interstrand DNA crosslinks (ICLs) through its DNA flap endonuclease and exonuclease activities. Crystal structures of human and bacterial FAN1 bound to a DNA flap have been solved. The *Pseudomonas aeruginosa* bacterial FAN1 and human FAN1 (hFAN1) missing a flexible loop are monomeric, while intact hFAN1 is homo-dimeric in structure. Importantly, the monomeric and dimeric forms of FAN1 exhibit very different DNA binding modes. Here, we interrogate the functional differences between monomeric and dimeric forms of FAN1 and provide an explanation for the discrepancy in oligomeric state between the two hFAN1 structures. Specifically, we show that the flexible loop in question is needed for hFAN1 dimerization. While monomeric and dimeric bacterial or human FAN1 proteins cleave a short 5′ flap strand with similar efficiency, optimal cleavage of a long 5′ flap strand is contingent upon protein dimerization. Our study therefore furnishes biochemical evidence for a role of hFAN1 homodimerization in biological processes that involve 5′ DNA Flap cleavage.

1. Introduction

Interstrand DNA crosslinks (ICLs) interfere with DNA replication and transcription. Failure to remove ICLs can induce cell cycle arrest, cell death, and genome instability. The Fanconi-associated nuclease 1 (FAN1) is a DNA structure-specific nuclease involved in ICL repair via interaction with FANCI-FANCD2 complex [1–4]. FAN1 may also have a repair function that is independent of proteins in the Fanconi anemia (FA) pathway of DNA damage response [5]. Importantly, FAN1 mutations are thought to lead to the renal disease Karyomegalic Interstitial Nephritis (KIN) [6–8]. FAN1 possesses both 5′ flap endonuclease and 5′ to 3′ exonuclease activities [2,3]. In cells, FAN1 functions in concert with or in parallel to other nucleases, including the endonucleases XPF-ERCC1, MUS81-EME1, SLX1 and the exonucleases SNM1A and SNM1B, to unhook ICLs [9]. SLX4 interacts with XPF-ERCC1, MUS81-EME1 and SLX1 and recruits these nucleases to DNA lesions [9]. It is important to note that FAN1 is structurally distinct from FEN1 (Flap endonuclease 1), which removes 5′ RNA and DNA flaps during lagging strand DNA replication and long-patch base excision repair [10].

The crystal structures of human FAN1 (hFAN1) and a bacterial FAN1 ortholog have been solved by several groups [11–13]. These structures provide insights into how FAN1 binds 5′ flap DNA with a 1-nt overhang (Fig. 1A and Fig. S1). Surprisingly, the two available structures of the hFAN1 differ both in the oligomerization state (dimer vs monomer) and DNA-binding mode. The dimeric structure has an interface with DNA that spans both hFAN1 protomers [11], while the monomeric hFAN1 structure largely resembles the crystal structure of the *Pseudomonas aeruginosa* FAN1 (PaFAN1) bound to DNA [12,13] (Fig. 1A, right panel). It is unclear whether the two hFAN1 crystal structures capture physiologically relevant oligomeric states of this enzyme in different substrate engagement/cleavage modes.

In this study, we resolve the activity differences between the dimeric and monomeric forms of hFAN1 in the cleavage of 5′ DNA flaps. Interestingly, we find that even though both dimeric and monomeric hFAN1 forms can cleave a short DNA flap strand efficiently, monomeric hFAN1 is less capable than the dimeric enzyme in cleaving a longer flap. We independently verify this activity profile using bacterial orthologs of FAN1 that are intrinsically dimeric or monomeric.

Abbreviations: FAN1, FANCD2/FANCI-associated nuclease 1; FANCI, Fanconi anemia complementation group I; FANCD2, Fanconi anemia complementation group D2; ICL, interstrand DNA crosslink; HR, homologous recombination; TLS, translesion DNA synthesis

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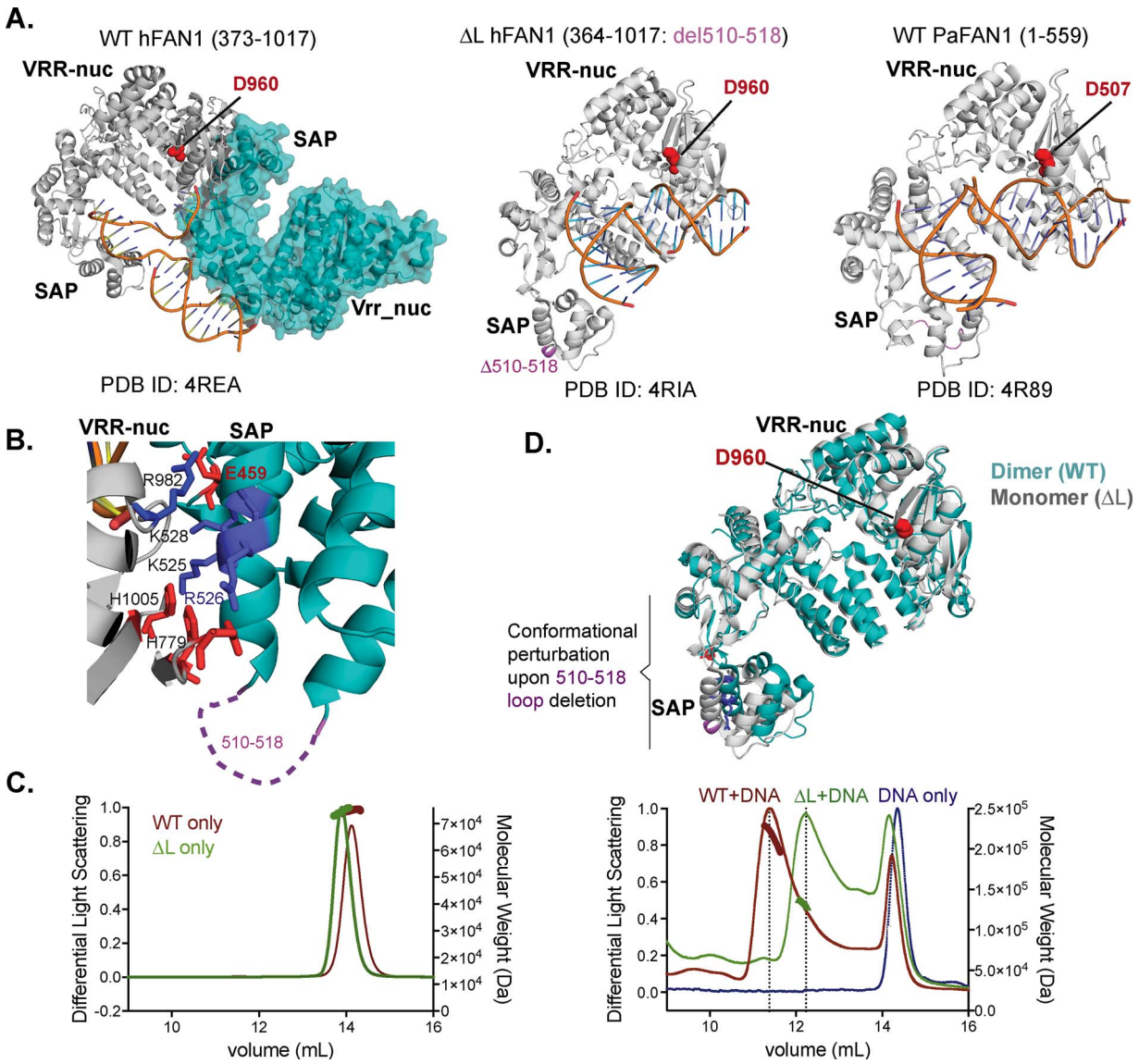

Fig. 1. Dimerization of FAN1 upon DNA binding. (A) Previously solved crystal structures of WT hFAN1 [11], hFAN1 with residues 510–518 deleted (ΔL hFAN1) [12], and *Pseudomonas aeruginosa* FAN1 [13] are compared, with the catalytic site aspartate highlighted in red. The catalytic VRR-nuc domain and the DNA binding SAP domain are labeled in bold for all FAN1 molecules. (B) Zoomed-in view of the dimerization interface of WT hFAN1, formed by the VRR-nuc domain of one monomer (grey) and the SAP domain of the 2nd monomer (teal). The location of the 510–518 loop is marked with a dash line. (C) Multi-angle laser light scattering (MALLS) chromatographic profiles for WT and ΔL hFAN1 alone (left panel) and with a 5′ flap substrate added (right panel). (D) Superposition of the monomeric FAN1 structure (PDB ID: 4RIA, grey) over one molecule of the dimeric FAN1 structure (PDB ID: 4REA, teal) highlighting the conformational perturbation induced by deletion of the flexible loop between residues 510–518.

2. Results and discussion

2.1. Requirement for an interdomain loop in DNA-induced hFAN1 dimerization

The crystal structure of hFAN1 previously solved by us shows a homodimeric configuration of the protein [11], where the two hFAN1 molecules make contacts with the duplex regions of the 5′ flap DNA so as to position one of the subunits to cleave the single-stranded DNA flap. This DNA-bound hFAN1 structure was derived from a truncated form of hFAN1 (residues 373–1017) that lacks the UBZ domain but otherwise covers all the essential domains for DNA processing and possesses nuclease activity comparable to that of full length hFAN1. We refer to this construct as WT hFAN1 or simply WT (Fig. 1A, left panel and Fig. 1B). Intriguingly, another published hFAN1 structure shows a monomeric conformation and a different mode of DNA binding [12]. This structure was similarly derived from hFAN1 (residues 364–1017) that lacks the UBZ domain but in addition harbors the deletion of a flexible loop spanning residues 510–518 in the SAP domain, hereafter referred to as ΔL hFAN1 or simply ΔL (Fig. 1A, middle panel).

To confirm that dimeric and monomeric hFAN1 forms exist in solution, we used size exclusion chromatography followed by multi-angle laser light scattering (SEC-MALLS) to characterize the WT and ΔL proteins. The data were collected either for the proteins alone or in the presence of a 5′ flap DNA substrate (Fig. 1C). We found that both WT and ΔL hFAN1 are monomeric in solution with a measured molecular weight of ~75 kDa. Upon pre-incubation with a 5′ flap DNA substrate, however, the SEC elution peak shifted to the dimeric position for WT, but the ΔL form remained monomeric. This provides direct evidence that deletion of the flexible loop prevents hFAN1 dimerization upon DNA binding.

We note that the results from the SEC-MALLS analysis are consistent with the proximal location of the flexible loop to the dimer interface in our hFAN1 structure (Fig. 1B). As a result, deletion of this loop (510–518) induces changes in the relative placement of the two helices abutting the loop (Supplemental Table 1) and a significant perturbation