# Crystal Structure of Human Flap Endonuclease-1 (FEN1) Complexed to PCNA

DNA replication in eukaryotes is a highly coordinated process involving many proteins that work cooperatively to ensure the accurate and efficient replication of DNA. In this process, flap endonuclease-1 (FEN1) plays a crucial role in the removal of RNA primers during Okazaki fragment maturation in lagging strand DNA synthesis (Fig. 1). Flap DNA removal by FEN1 is also essential during long-patch base excision repair. FEN1 appears to be a key player in maintaining genomic stability by participating in the DNA replication and repair processes.

FEN1 endonuclease activity is markedly stimulated by proliferating cell nuclear antigen (PCNA), which is well known as the 'DNA sliding clamp'. This stimulation is induced by direct binding of FEN1 to PCNA, leading to a 10- to 50-fold increase in its nuclease activity. Mutations in FEN1 that disrupt the interaction with PCNA decrease the cleavage efficiency of flap DNA at the replication fork, thus leading to the generation of unfavourably long flap DNA strands.

Recently, we successfully crystallized full-length human FEN1 complexed to PCNA [1]. A set of diffraction data was collected at SPring-8 on beamline **BL38B1** using crystals cooled to 100 K with liquid-nitrogen vapour. The phases were determined by molecular replacement method using human PCNA and archaeal FEN1 monomers as a search model. The structure was finally refined at a resolution limit of 2.9 Å.

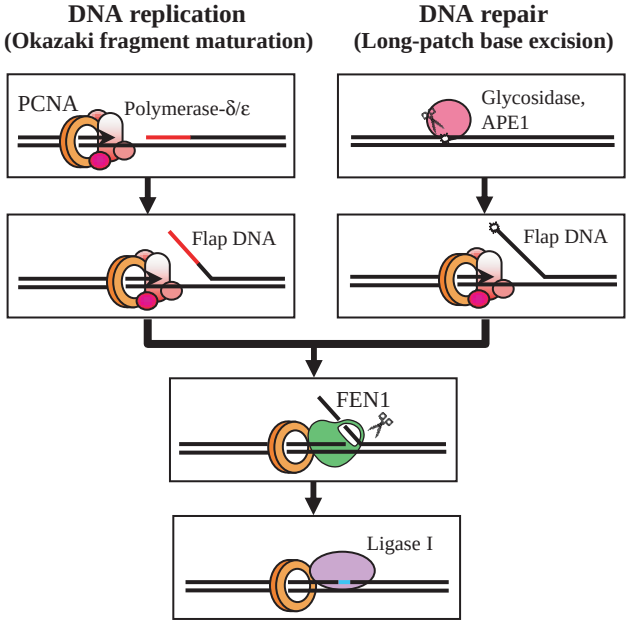


Fig. 1. FEN1 complexed to PCNA removes 5'-overhanging DNA flaps in the DNA replication (left) and repair (right) processes. RNA primer in Okazaki fragment is shown in red.

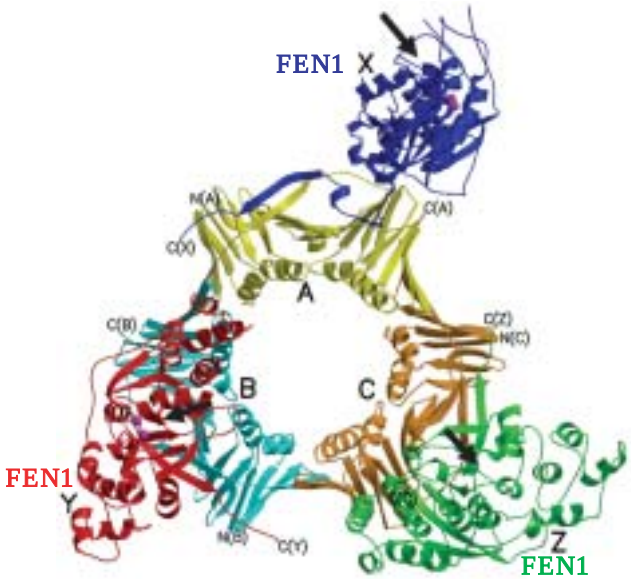


Fig. 2. Crystal structure of human FEN1-PCNA complex. Three FEN1 molecules are colored in blue (X), red (Y) and green (Z), and the three subunits of the PCNA trimer in yellow (A), cyan (B) and orange (C). The C-termini of FEN1 and PCNA are labeled. Metal ions bound to the active sites of FEN1 are shown in magenta. Proposed catalytic faces of FEN1 are indicated by arrows.

The structure revealed three FEN1 molecules (Fig. 2, molecules X, Y and Z) bound to one PCNA trimer [2]. Three PCNA subunits (subunits A, B and C) are tightly associated to form a closed ring. Each PCNA subunit binds one FEN1 molecule. The main interface involves the C-terminal tail of FEN1, which forms two β-strands connected by a short helix, the βA-αA-βB motif (Fig. 3). The FEN1 core domain is linked to this PCNA-binding tail by a short linker containing small residues. We found that this linker, $^{333}$QGST$^{336}$, functions as a hinge which endows FEN1 with a degree of freedom to swing the core domain.

In our complexed structure, the nuclease core domains of the Z and Y FEN1 molecules swing about 90º and 100º, respectively, from that of molecule X toward the center of the PCNA ring (Fig. 4). During this swing displacement, the active site tracks on a large spherical surface with a radius of 40-50 Å. FEN1 locates its core domain in front of the PCNA, while the active site of molecules Y and Z faces toward the conceivable position occupied by the DNA substrate. The swing-in motion might be utilized in threading the flap DNA through the clamp region. Suppose that dsDNA is passing through the center of PCNA ring and that the DNA occupies a linear orientation, approximately 50º more swing-in displacement of the FEN1 core domain would be needed to place its active site on the cleavage site (the junction of dsDNA and flap-ssDNA). The most likely trigger to allow FEN1 to swing into position for cleavage would be interactions between the single-stranded flap DNA and FEN1.

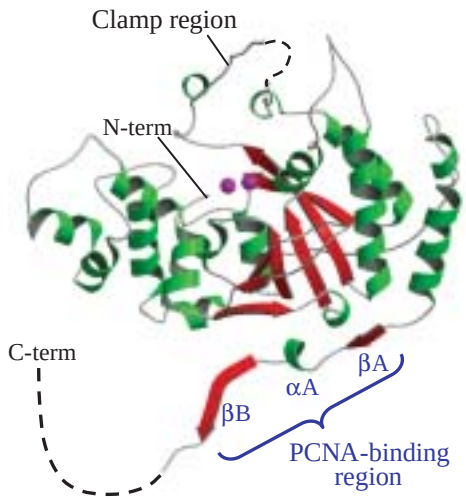


Fig. 3. Structure of human FEN1. Two metal ions in the active site are depicted in magenta. The clamp region is thought to thread along the single strand of the DNA substrate (flap DNA).

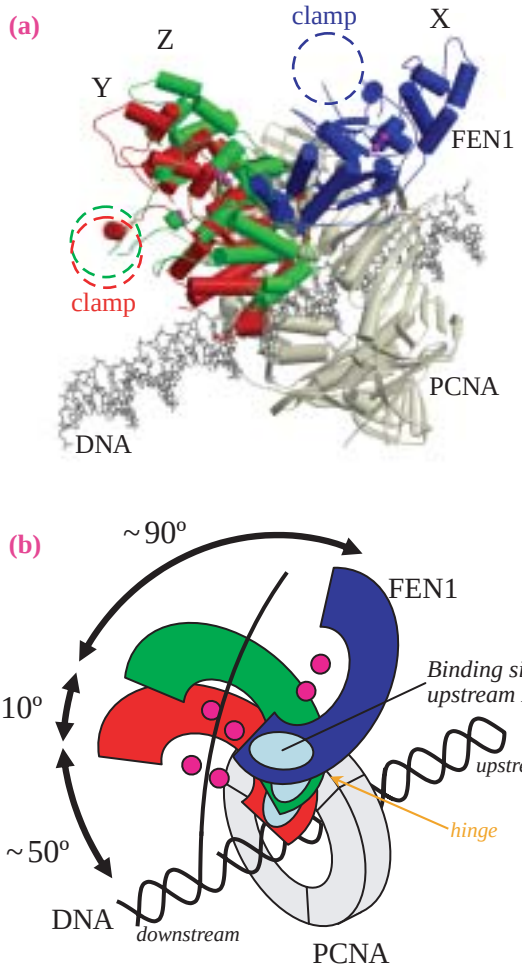


Fig. 4. The hinge flexibility of human FEN1. **(a)** Three FEN1 subunits in the crystal structure were superimposed on one site of PCNA (a gray model). The looping-out clamp regions are marked as dashed circles. The conceivable DNA model is superimposed at the center of PCNA. **(b)** Schematic model showing the orientation of the FEN1 core domains in **(a)**.

Ken Kitano[a], Shigeru Sakurai[a,b] and Toshio Hakoshima [a,c,*]

[a] Nara Institute of Science and Technology
[b] Japan Society for the Promotion of Science
[c] CREST, Japan Science and Technology Agency

*E-mail: hakosima@bs.naist.jp

## References

[1] S. Sakurai *et al.*: Acta Crystallogr. D **59** (2003) 933.
[2] *S. Sakurai, K. Kitano, H. Yamaguchi, K. Okada, K. Hamada, K. Fukuda, M. Uchida, E. Ohtsuka, H. Morioka, T. Hakoshima: EMBO J.* ***24*** *(2005) 683.*