How to Remove Tangles in Your DNA

DNA damage is a fact of life. On any given day, an organism's DNA will suffer between 10,000 and 1,000,000 breaks or other damage. These problems are repaired by enzymes in our cells that fix the breaks, remove errors, and maintain the integrity of the genome. One of these DNA repair enzymes acts as a kind of molecular scissors to cut DNA at damage points and resolve tangles that can form when things go wrong. This must be done with great specificity in order to restore the DNA code to its previous state and not generate mutations. Researchers utilizing the APS and the Pohang Accelerator Laboratory in South Korea solved the structure of one of these molecular scissor proteins, called Mus81-Eme1, in a complex with DNA. Their analysis reveals the elegant way the enzyme recognizes, positions, and cuts the DNA, and then secures the two ends to avoid a new tangle and furthers our understanding of the mechanisms of DNA repair, with positive implications for fields such as reproduction, cancer, and aging.

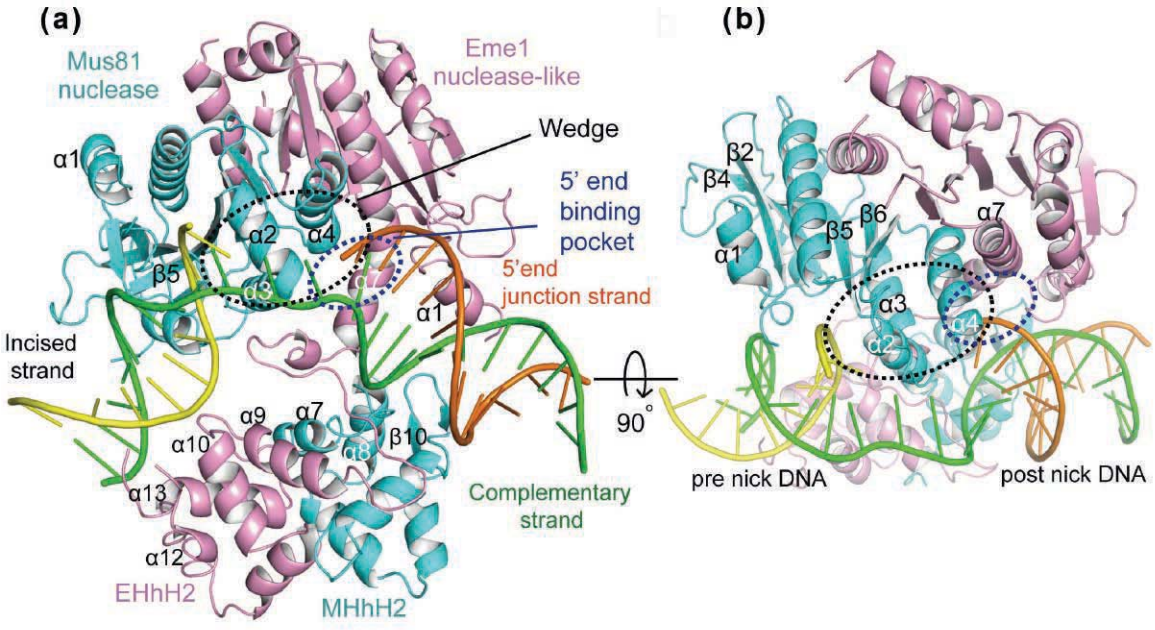


Fig. 1. a) A complex structure containing Mus81 (cyan) Eme1 (pink) bound to a 3′ flap DNA. The DNA strand to be cut is shown in yellow, the 5′ end (orange) is shown in the 5′ binding pocket (blue dotted circle) and the complementary DNA strand is shown in green. The wedge is shown as a black dotted circle. b) A 90° rotated view of 1a. On the right and left of the wedge, the 5′ junction of the 5′ end junction strand and the 3′ end of the strand to be cut are placed at the 5′ end binding pocket and the active site, respectively.

19-ID-D • SBC-CAT • Life sciences • Macromolecular crystallography, multi-wavelength anomalous dispersion, subatomic (<0.85 Å) resolution, microbeam, ultra-low-temperature (15K), large unit cell crystallography, single-wavelength anomalous dispersion • 6.5-19.5 keV • On-site, remote, mail-in • Accepting general users •

Most genes that have been found to affect lifespan are involved in DNA repair. If breaks and other DNA damage are not repaired, disease can result from adverse effects on normal gene expression and cell division functions that are critical to life.

One type of enzyme involved in DNA repair is a molecular scissors called an endonuclease. Mus81-Eme1 is one of a family of structure-selective endonucleases responsible for resolution of complex DNA overlaps that are the result of DNA damage. These overlaps, or flaps, come in two different flavors, 5′ and 3′ (pronounced "5-prime" and "3-prime" in DNA parlance) depending on the direction of the DNA break that occurred.

The researchers from the Pohang University of Science and Technology (South Korea) and Argonne were interested in why the Mus81-Eme1 endonuclease specifically recognizes only the 3′ flaps and not the 5′ flaps. To study this, they crystallized Mus81-Eme1 in complex with small pieces of DNA with either 3′ or 5′ flaps and collected diffraction data for the crystals at the SBC-CAT beamline 19-ID-D at the APS, and at the Pohang Accelerator Laboratory.

As one might expect for an enzyme that performs an important basic DNA repair function, Mus81-Eme1 works with elegance and precision. The team compared structures for Mus81-Eme1 in complex with one 5′ flap DNA and two 3′ flap DNAs. The 5′ flap structure showed that the DNA could bind to Mus81-Eme1 but the binding did not result in cutting of the DNA. Analysis of the 3′ flap structures revealed why.

When Mus81-Eme1 binds to the 3′ flap DNAs it undergoes a dramatic shape change and convertst from a compact structure to a more open structure (Fig. 1), unmasking a protein domain that acts as a wedge to keep the two cut ends separated. The 5′ end that is not being cut is held in place by a specialized binding pocket that keeps the end away from the action.

Next, Mus81-Eme1 bends the DNA to position the 3′ end right in the active site. Biochemically, the 5′ flaps don't work because the free 3′ ends don't fit properly into the binding pocket and so the DNA doesn't bend to place the 5′ end into the active site.

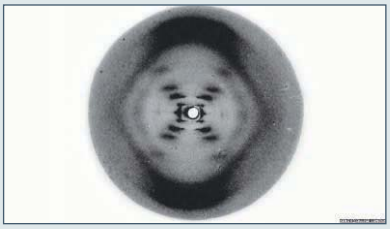
The team validated their model in two ways. First, they confirmed reduced enzyme activity in mutant versions of Mus81-Eme1 based on structure model predictions. Second, in experiments in which they labeled both ends of the DNA with fluorescent tags, the addition of Mus81-Eme1 resulted in the two ends of the DNA moving closer together, supporting the bending formation in the structure.

What's next? Because endonucleases like Mus81-Eme1 are responsible for repair of some of the most complex types of DNA damage, when these are not repaired due to a malfunction of an endonuclease, serious developmental and genetic disease can occur. The next step for the team will be to study the structures of other members of this endonuclease family, including one involved in a disease called Fanconi anemia, in order to provide a frame-work for curing these diseases.

— Sandy Field

See: Gwang Hyeon Gwon[1], Aera Jo[1], Kyuwon Baek[1], Kyeong Sik Jin[1], Yaoyao Fu[1], Jong-Bong Lee[1], YoungChang Kim[2], and Yunje Cho[1]*, "Crystal structures of the structure-selective nuclease Mus81-Eme1 bound to flap DNA substrates," EMBO J. **33** (9), 937 (2014).
DOI: 10.1002/embj.201487820
Author affiliations: [1]Pohang University of Science and Technology, [2]Argonne National Laboratory
Correspondence: * yunje@postech.ac.kr

This work was supported by grants from the National R&D Program for Cancer Control, the Ministry for Health and Welfare (1020280), a National Research Foundation of Korea grant funded by the Korea government (MEST, No. 2012004028, No. 2012-054226, and No. 20120008833), a rising star program (POSTECH), and the BK21 program (Ministry of Education). SBC-CAT is funded by the U.S. Department of Energy (DOE) Office of Biological and Environmental Research. This research used resources of the Advanced Photon Source, a U.S. DOE Office of Science User Facility operated for the DOE Office of Science by Argonne National Laboratory under Contract No. DE-AC02-06CH11357.



Rosalind Franklin was a brilliant x-ray crystallographer whose photograph of a fiber of DNA [above] was critical to James Watson and Francis Crick's discovery of the double helix. Her x-ray studies of DNA provided data that helped to bring into being the theoretical model constructed by Watson and Crick.

Her colleague at King's College, Maurice Wilkins, showed her photograph of the "B" form of DNA to Watson early in 1953, when she was preparing to leave King's to start a new project at Birkbeck College, London. Watson describes his reaction in *The Double Helix*: "The instant I saw the picture my mouth fell open and my pulse began to race... the black cross of reflections which dominated the picture could arise only from a helical structure... mere inspection of the x-ray picture gave several of the vital helical parameters." … The laboratory notebooks in which Franklin set out her findings and noted her own thoughts about the structure… contain annotations made ten years after her untimely death by her colleague Aaron Klug, who published a paper in *Nature* in 1968 showing that she had been ahead of Watson and Crick in establishing key parameters of the structure.

DNA was only an interlude in Franklin's career. She had made important contributions to the study of carbon at the British Coal Utilisation Research Association; subsequently she used her skills to reveal the hollow centre of the tobacco mosaic virus particle, and to trace the helical form of its genetic material within this cylinder.

Excerpted from: "Codebreakers: Makers of Modern Genetics, The Rosalind Franklin papers," the Wellcome Library, http://wellcomelibrary.org