Role of Dimeric Endonuclease G in DNA Degradation

The complex structures of CPS-6 with DNA provide a clue how endonuclease degrades DNA and exhibits endonuclease activity as a homodimer.

Endonuclease G (EndoG) is a highly conserved mitochondrial endonuclease in eukaryotes that plays a role in mitochondrial DNA biogenesis. EndoG is also a pro-apoptotic enzyme that promotes cell death in a caspase-independent path.[1] During apoptosis, a fraction of EndoG is translocated from mitochondria into a nucleus to degrade chromosomal DNA. In mammals, EndoG is highly expressed in cardiomyocytes. EndoG-deficient mice have elevated levels of reactive oxygen species in cardiomyocytes leading to cardiac hypertrophy independent of blood pressure.

CPS-6 is a homologue of EndoG identified in C. *elegans* displaying a nuclease activity similar to that of EndoG. CPS-6 was identified to be involved in an elimination of the paternal mitochondrial DNA following fertilization during early embryogenesis.[2] A decreased nuclease activity was observed for both CPS-6 and EndoG when the dimeric conformation was dissociated into monomer under oxidative conditions,[3] but how EndoG/CPS-6 binds and cleaves DNA as a homodimer and why it loses its activity when it is oxidized and dissociates into monomers remain unknown.

Hanna S. Yuan of Academia Sinica and her co-workers constructed a CPS-6 double mutant, H148A/F122A, which retained its ability to bind DNA but had no activity in DNA cleavage. CPS-6 H148A/F122A mutant was crystallized in space group $P2_1$ with one dimer per asymmetric unit. The crystal structure of CPS-6 was determined by molecular replacement with CPS-6 H148A (PDB entry: 3S5B) as a search model. X-ray diffraction data were collected at **TLS 15A1**. They made a further attempt to co-crystallize CPS-6 H148A/F122A and a single-stranded DNA and determine the complex crystal at 2.3 Å resolution in different space group $P2_12_12_1$.

The overall structure[4] of dimeric CPS-6 H148A/F122A bound to two single-stranded DNA in one asymmetric unit is illustrated in **Fig. 1**. Only five of eight nucleotides (5′-TTTTTGT-3′) were visible in the omitted density map; they were all assigned as thymidine (T1 to T5) based on the shape of each base (**Fig. 2**). These five nucleotides could represent a digested 5-nt DNA molecule or an intact 8-nt DNA that has disordered ends (**Fig. 2**).

The two DNA molecules are located remotely from each other and face opposite directions. Inspection of the CPS-6-DNA complex structure reveals that CPS-6 binds DNA mainly at the phosphate backbones via hydrogen-bonded interactions. The DNA bases point away from the protein surface and in no way interact with CPS-6, thereby avoiding sequence-specific interactions.

Based on the structural and functional information obtained, a DNA hydrolysis mechanism was proposed. The active site is located in the ββα-metal motif that is bound with a Mg^{2+} ion. His148 acts as a general base to activate a water molecule, which in turn makes an in-line attack

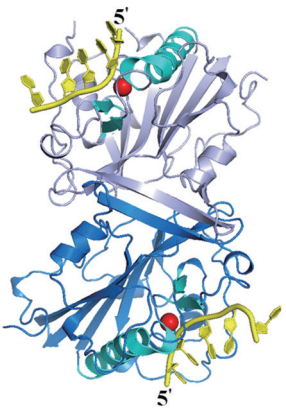

Fig. 1: Crystal structure of CPS-6 H148A/F122A mutant bound with single-stranded DNA at 2.3 Å resolution. [Reproduced from Ref. 4]

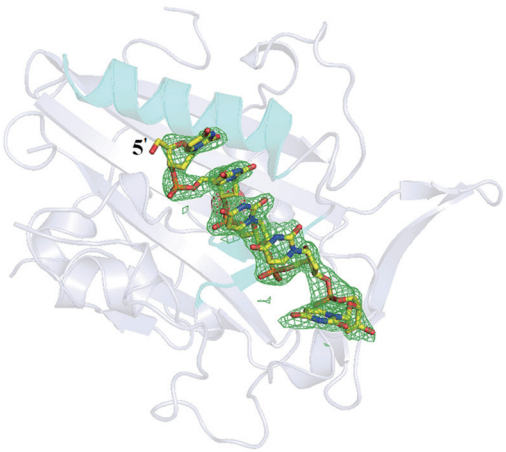

Fig. 2: Omit ($2F_o$-F_c) density map illustration for the DNA substrate that was bound to one CPS-6 protomer (contoured at 3.0 σ). [Reproduced from Ref. 4]

on the scissile phosphate. The Mg-bound water functions as a general acid to provide a proton to the cleaved DNA product. The bound DNA substrate is thereby hydrolyzed in a sequence-independent manner to produce cleaved products with a 5′-end phosphate and a 3′-end OH group.

Why EndoG has a diminished nuclease activity when it is dissociated into monomers is intriguing, as each protomer can bind and degrade DNA separately. To answer this question, they further constructed two obligatory monomeric CPS-6 mutants (P207E and K131D/F132N), which degrade DNA with a diminished activity because of a DNA-binding affinity poorer than that of wild-type CPS-6. Unexpectedly, the P207E mutant exhibited predominantly a 3′-to-5′ exonuclease activity, indicating a possible activity change from endonuclease to exonuclease. The dimer conformation of CPS-6 is thus essential to maintain its optimal DNA-binding and endonuclease activity. Compared to other non-specific endonucleases, which are typically monomeric enzymes, EndoG is a unique dimeric endonuclease, of which the activity can be modulated with oxidation to induce a dimer-to-monomer conformational change.

In summary, these results provide a molecular basis to explain how EndoG degrades DNA substrates without a sequence preference and why EndoG exhibits optimal endonuclease activity as a homodimer. The authors suggest that stabilizing the dimeric conformation of EndoG might provide a way to promote its endonuclease activity and to combat diseases induced by oxidative stress. (Reported by Chun-Jung Chen)

This report features the work of Hanna S. Yuan and her co-workers published in Nucleic Acid Res. ***44****, 10480 (2016).*

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- Protein Crystallography
- Biological Macromolecules, Protein Structures, Life Sciences

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Molecular Averaging Is Powerful for Crystal Structures

A new method, molecular averaging in real space, is developed to evaluate effectively the phasing power and to enhance the success of determining new protein structures.

X-ray crystallography of proteins remains a predominant method to determine three-dimensional structures of biological macromolecules. Despite great progress towards its automation and efficiency, phasing massive diffraction reflections remains a critical step in the determination of structures. *Ab initio* phasing, which requires only one native data set, is eagerly expected but is still a challenging method. One *ab initio* phasing is a method to utilize many equivalent molecules in a cell unit. Supposing electron densities of each equivalent molecule to be the same, the phases of amplitude data are greatly restricted; a correct molecular density is obtained. It is called molecular averaging. This technique is generally common for phase improvement after molecular replacement to eliminate the initial model biases. *Ab initio* phasing by molecular phasing is a challenging topic. Many trial calculations and much discussion of *ab initio* phasing with molecular averaging have been reported. In particular, a viral particle composed of many well ordered capsid proteins is an effective target for application with *ab initio* molecular averaging, but there has been no successful case of *ab initio* trials by molecular averaging to a novel protein structure.