1 nuclease, 2 domains, 3 stages of catalysis

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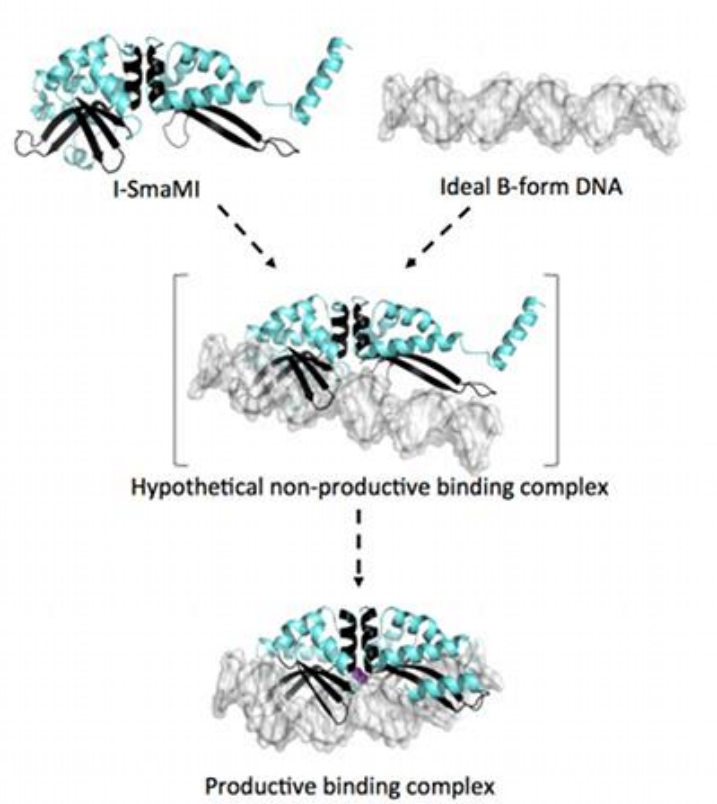

Structural changes during DNA binding by the I-SmaMI meganuclease. An alpha helix in the extreme C-terminus (Helix 11) of the enzyme is positioned away from the rest of the structure in the unbound and hypothetical non-productive binding form. The N-terminal domain of the enzyme contacts the DNA before the C-terminal domain makes close contacts (seen in the middle hypothetical non-productive binding complex). Finally, helix 11 rotates to contact the DNA in the final productive binding form of the enzyme. Changes in the structure of the DNA are also apparent upon binding.

Image provided by Betty Shen.

As research on gene editing technology is rapidly expanding, the need for investigation into how specificity and delivery of gene-editing agents can be achieved is becoming more acute. The LAGLIDADG meganucleases are highly specific DNA cleaving enzymes that are relatively small and create 3' overhangs in the DNA, which is desirable for homology-driven repair of double-strand breaks (i.e. gene editing). In nature, LAGLIDADG meganucleases are found as either homodimers or pseudosymmetric single-polypeptide monomers, forming either two identical or pseudosymmetric domains, which together contact 20-22 base-pairs of DNA. While the homodimeric nucleases recognize palindromic DNA sequence motifs, the monomeric enzymes target and cleave fully asymmetric sequence motifs, allowing for a wider range of target specificity. The two pseudosymmetric domains make distinct contributions to DNA binding and cleavage activity and this has made re-engineering of these enzymes challenging. A detailed view of the structure of an enzyme in different stages of catalysis may reveal crucial information that would aid efforts toward its re-design for gene editing purposes.

High resolution structural and biochemical information on one of these monomeric pseudosymmetric meganucleases, called I-SmaMI, was gathered and recently published in the *Journal of Molecular Biology*, following a fruitful collaboration between Fred Hutch investigators in Barry Stoddard's Lab (Basic Sciences Division) and scientists in Brett Kaiser's Lab at Seattle University. Staff scientist Betty Shen (Stoddard Lab) determined the structure of I-SmaMI by X-ray crystallography in three different states: unbound (no DNA), bound to DNA, and bound to cleaved DNA (see Figure, high-resolution here). She observed that the enzyme undergoes significant structural changes between the free and DNA-bound state; a C-terminal helix goes through a rigid-body rotation toward the DNA and subtle but significant conformational changes occur in the left and right domains of the enzyme. Given the obvious change in the position of the C-terminal helix between unbound and bound states, the scientists wanted to test the contribution of this feature to catalysis. They purified a mutant of I-SmaMI lacking the helix (Δhelix11) and found that this mutant had significantly reduced ability to bind as well as cleave DNA. These results suggest that this helix is required for full enzymatic activity. Next, the authors wanted to further interrogate the contribution of each domain to DNA-binding. Under the direction of Brett Kaiser, formerly a postdoc and staff scientist at Fred Hutch (Strong Lab, Stoddard Lab), a student at Seattle University uncovered important biochemical properties of the enzyme.

They created strands of DNA containing the I-SmaMI target sequence but mutated either the "left" or "right" DNA target sequence (called "half sites"), which bind the N- or C- terminal domain of the enzyme, respectively. They found that mutating the right half-site did not severely compromise binding but mutating the left DNA half-site completely abolished binding. These results suggest that the N-terminal domain contributes the majority of the DNA-binding affinity of the enzyme just prior to the formation of the transition state that precedes full binding and cleavage. Turning to mutagenesis of the protein itself, the authors investigated the contribution of the catalytic residues in each domain to cleavage activity. They found that disrupting the N-terminal domain catalytic residues abrogated cleavage activity more than perturbing the catalytic site of the C-terminal domain. They hypothesize that the binding of a "number of arginine and lysine residues at the surface of the N-terminal domain may be responsible for the recognition and anchoring of I-SmaMI to a specific patch of DNA bases preceding the 'central four' bases which in turn, triggers the conversion between the "non-productive" and "productive" complexes," said Betty Shen. Investigations are currently underway to investigate this implication.

This research has illuminated the asymmetric contribution of the N-terminal domain of monomeric LAGLIDADG meganucleases to DNA binding and cleavage and also the importance of the final C-terminal helix for function. This is valuable information not only for those interested in engineering

enzymes for gene editing but also for advancing our understanding of protein-DNA interactions in general.

Shen BW, Lambert A, Walker BC, Stoddard BL, Kaiser BK. 2015. "The structural basis of asymmetry in DNA binding and cleavage as exhibited by the I-SmaMI LAGLIDADG meganuclease." *Journal of Molecular Biology*. 428(1):206-20. doi: 10.1016/j.jmb.2015.12.005.

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