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Mechanism of Activation of SgrAI via Enzyme Filamentation and Mechanism of DNA Sequence Specificity Expansion

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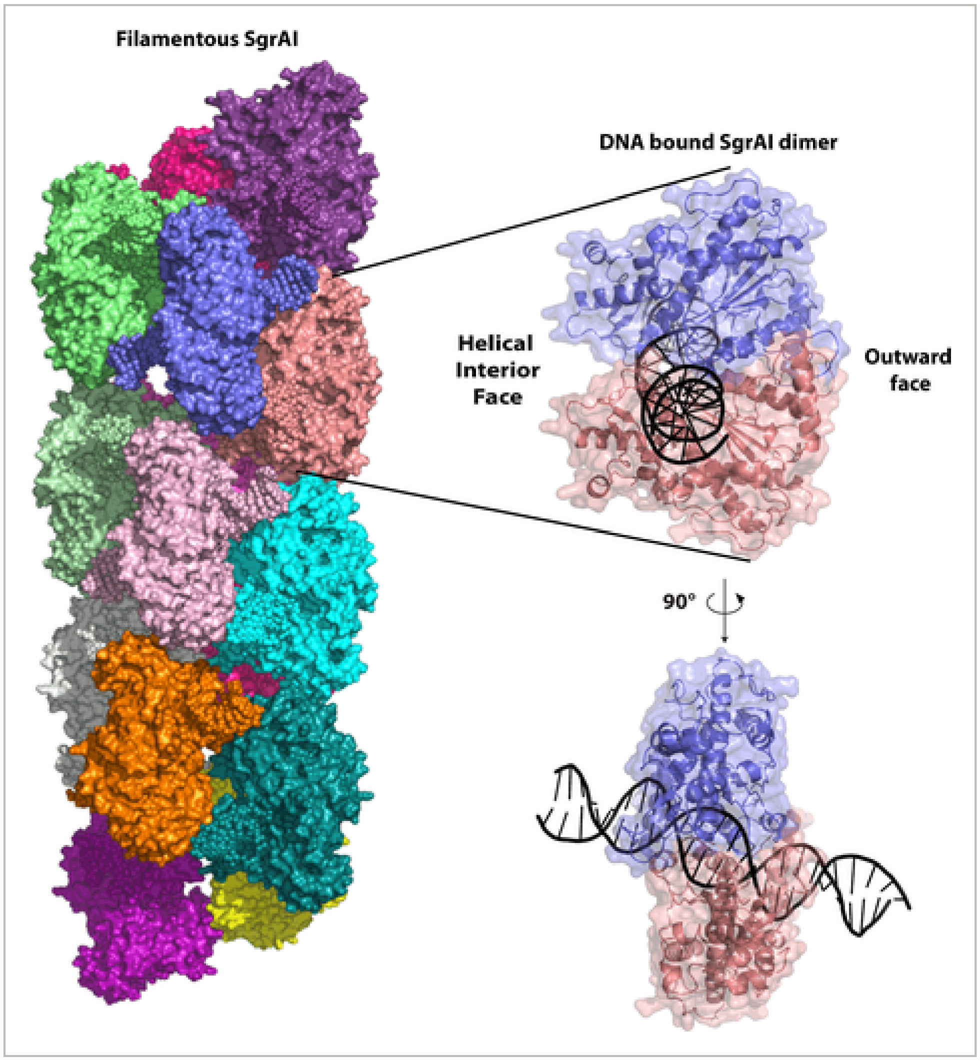
Abstract

We describe a new 2.7 Å CryoEM structure of a filament forming enzyme, SgrAI, in a pre-transition state captured just prior to nucleophilc attack. Filament forming enzymes are enzymes which form linear, helical, and even tubular structures that may act to regulate enzyme activity, play a role in signaling, nucleate phase separated membrane-less cellular compartments, or other possible functions. SgrAI is a sequence specific DNA endonuclease which plays a role in bacterial innate immunity. The phage-host arms race is arguably the oldest co-evolutionary system wherein bacteria evolve systems to defend against invading DNA, and phage evolve systems to outsmart bacterial defenses. SgrAI forms filaments with a left-handed helical symmetry of four copies per turn, which in principle could extend indefinitely. The kinetics of association and dissociation of the filament and concentrations of DNA bound SgrAI limit filament size under laboratory conditions to 2-20 enzyme copies, and the number of recognition sites on a phage genome limits filament size in vivo. Filamentation activates the SgrAI rate constant for DNA cleavage 200-1000 fold, and also expands the DNA sequence specificity of SgrAI to include fourteen additional recognition sequences. Our prior work has shown the details of the filamentous architecture, conformational changes in the bound DNA and protein, as well as the full enzymatic kinetic pathway including microscopic rate constants for each step. These studies suggest that the unusual regulatory mechanism involving filamentation evolved in SgrAI to maximize its anti-phage activity while protecting the unusually long genome of its host from damaging DNA breaks. Our new structure connects the conformational change seen in SgrAI in the filamentous form to the binding of a second divalent cation in the active site thereby resulting in the observed activated DNA cleavage rate. In combination with a newly reported x-ray crystal structure of apo SgrAI, we propose mechanisms for DNA sequence specificity

expansion upon filamentation involving a disorder-to-order transition, energetics of DNA structure, and protein-DNA interactions.



The SgrAI Homodimer Binds Duplex DNA and Forms a Filamentous Superstructure

This is the full abstract presented at the Experimental Biology meeting and is only available in HTML format. There are no additional versions or additional content available for this abstract.

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