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The Filament Forming Mechanism of SgrAI Endonuclease-Structural and Kinetic Analysis

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Abstract

Filament formation by enzymes has recently emerged as a new paradigm of enzyme regulation 1.

Wide-spread reversible self-assembly of enzymes in cells has also been shown to occur in all branches of life and diverse metabolic and biological pathways. We use the model system, SgrAI, to investigate the filament forming enzyme mechanism.

Filaments formed by SgrAI have been structurally characterized using cryo-electron microscopy 2 and the full reaction pathway including all rate constants has been determined using single turnover enzyme reactions, FRET, and global kinetic modeling 3– 4.

The structural studies uncover the mechanism of activation of enzyme activity in SgrAI by filament formation, as well as the origin of the unusual expansion of substrate specificity in SgrAI within the filament. A conformational change in the SgrAI dimer, stabilized by protein-protein and protein-DNA contacts in the filament, leads directly to a shift in the active site, hypothesized to lead to stronger binding of a second catalytic Mg2+ ion. DNA structure and energetics is hypothesized to control substrate specificity and filament formation by SgrAI such that the secondary class of DNA sequences are cleaved by SgrAI only upon joining a filament formed by SgrAI bound to a primary class of DNA sequences. The full kinetic model showed a slow second order step in filament formation which is shown to control enzyme activity such that only invading phage DNA is cleaved, leaving the host genome untouched. This model also shows that the filament forming mechanism is superior in fast activation of enzyme activity upon initial recognition of primary DNA sequences.

Using the SgrAI endonuclease as a model system to study filament forming enzymes, we have discovered that the enzyme is activated by stabilization of an activated conformation

by contacts made within the filament. Control of the equilibrium between a low activity, non-filamentous state and the high activity, filamentous state by DNA structural energetics gives rise to the unusual apparent expansion of DNA sequence specificity of SgrAI by filament formation. Simulations using the full kinetic pathway reveal an advantage in the filament mechanism over other non-filamentous mechanisms in the speed of activation in response to invading phage DNA, giving strong evolutionary advantage to this filament forming mechanism.

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