Structural Characterization of σ^{54} Core-Binding Domain Truncation

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Transcription is the process by which a cell makes mRNA from template DNA genes. These mRNA strands are later translated by ribosomes to build the proteins necessary for all cellular functions. Transcription is regulated by cells to control the timing and amount of gene expression for each individual gene. This, in turn, determines how the cells grow and function. Bacterial transcription requires core RNA polymerase (RNAP) to synthesize new RNA strands, as well as a modular σ factor to open the double helix DNA and grant RNA polymerase access to the gene. Different σ factors target different genes providing one mechanism of transcription regulation. The σ^{54} class is unique in that its ability to initiate transcription is further regulated by a variety of AAA+ ATPase activators. σ^{54}-RNAP holoenzyme can bind near genes to be transcribed, but cannot open DNA to initiate transcription without the additional AAA+ ATPase activator protein. Little is known about the details of this activation mechanism, but structural similarities to other AAA+ ATPases suggest these proteins may provide a tugging force that results in a conformational change to σ^{54} that renders it capable of opening DNA. Molecular tweezer experiments show an unfolding intermediate under applied force that amounts to about ~20% of the σ^{54} core binding domain (CBD) losing structure. A possible interpretation is that the C-terminal helix of the CBD unfolds before the rest of the protein. To test this idea, we made a variant of the protein in which the C-terminal helix of CBD was removed, and compared this construct to wild-type CBD using NMR spectroscopy**.** We conclude that the truncation did not affect the secondary and tertiary structure of the rest of the domain. Future molecular tweezers experiments will investigate its unfolding profile under force to determine if CBD represents a conformational fracture point in σ^{54}. Studying the structural details of σ^{54} activation will broaden our understanding of the general mechanisms of AAA+ ATPase activation and DNA opening.