

Structures and mechanism of a type IIS restriction endonuclease: PaqCI

Madison Kennedy¹, Barry Stoddard²

¹N/A ²Basic Sciences, Fred Hutchinson Cancer Center / Seattle, WA

arzakennedy@gmail.com

Restriction endonucleases are an essential component of innate, 'preprogrammed' phage restriction systems that protect bacteria from foreign DNA. Type II restriction endonucleases are invaluable tools in research because of their ability to identify and cleave specific DNA sequences with extremely high fidelity, as well as their unique mechanisms of cleavage. Type IIS restriction endonucleases contain separate DNA recognition and cleavage domains and recognize asymmetric targets to cleave at exact distances outside of their target sequences. Type IIS restriction endonucleases are employed for a variety of biotech purposes, including the creation of gene-targeting zinc finger and TAL effector nucleases and DNA processing for genome mapping and deep sequencing applications. The most well-studied type IIS enzyme, FokI, has been shown to require multimerization and engagement with multiple DNA targets for optimal cleavage activity; however, details of how it or related enzymes forms a DNA-bound reaction complex have not been described at atomic resolution. Here we describe a series of crystallographic and CryoEM structures in the presence and absence of bound DNA targets that reveal aspects of DNA recognition and cleavage by the type IIS PaqCI restriction endonuclease. The structures illustrate the enzyme's tetrameric domain organization in the absence of bound substrate and the subsequent formation of a tetrameric reaction complex (involving significant domain rearrangements and reformation of the dimer complex) poised to deliver the first of a series of double strand cleavage events. Understanding the diversity of form and function within type II restriction endonucleases by investigating members such as PaqCI can reveal the differences between (1) catalytic architectures and cleavage mechanisms, (2) DNA binding domains and recognition mechanisms, and (3) the transient formation of higher order oligomeric assemblages to help ensure discrimination between self- and non-self. Additionally, PaqCI could be used to create novel artificial enzymes with new specificities that could be used in biotech applications.