Unloxing the assembly and activation mechanism of Cre recombinase using cryo-EM

Kye Stachowski[1], Mark Foster[2]

[1]The Ohio State University [2]The Ohio State University

stachowski.7@osu.edu

Mechanistic understanding of the structural basis for DNA recombination in the Cre-loxP system has largely been guided by crystallographic structures of tetrameric synaptic complexes (intasomes). Those studies have suggested that conformational changes and DNA bending in presynaptic complexes underlie the assembly and activation mechanism of Cre recombinase. Here we used protein engineering to isolate Cre-loxP and Cre2-loxP complexes, and determined the structures of assembly monomer (54 kDa), dimer (110 kDa), and tetramer (220 kDa) intermediates using cryo-EM to resolutions of 3.9 Å, 4.5 Å and 3.2 Å, respectively. We found that as Cre assembles into an activated complex, the bend of the loxP DNA site becomes more pronounced as each intermediate is reached, and these deformations of loxP DNA are key selectivity determinants of Cre. The progressive DNA bending is accompanied by increased protein-protein interactions. Our work shows how tetramerization is required for Cre to become activated to recombine DNA. We also used 3D variability analysis to uncover conformational sampling that reveals how motion in the protein-protein interface of the tetramer is important for activation of Cre. These new insights could prove useful in design of new Cre variants with engineered site-specificity and improved recombination efficiency. These findings necessitate a reexamination of the mechanisms by which this widely utilized gene-editing tool selects target sites, avoids spurious DNA cleavage activity, and controls DNA recombination efficiency.