

1049-92-150

Stephen D Levene* (sdlevene@utdallas.edu), Dept. of Molecular and Cell Biology, 800 West Campbell Road, Richardson, TX 75080. *New Mechanistic Insights into Flp and Cre Recombination from DNA Loop-closure Kinetics.*

The Flp and Cre recombination systems have become important tools for the genetic manipulation of higher organisms and paradigms for site-specific DNA-recombination mechanisms employed by the λ -int superfamily of recombinases. A hallmark of the int superfamily is that recombination takes place via a four-stranded, Holliday-junction DNA intermediate. High-resolution crystal structures of Flp and Cre synaptic complexes formed with duplex and junction DNAs suggest that the key mechanistic steps can be explained in terms of DNA strand exchanges taking place within an approximate square-planar arrangement of DNA duplexes. It is difficult, however, to reconcile the square-planar exchange mechanism observed in the co-crystal structures with evidence for a chiral recombination intermediate, which derives from the topological handedness of recombination products generated with circular DNA substrates. A rigorous kinetic analysis of intramolecular site-specific recombination as a function of target-site spacing, in concert with numerical analysis of loop-closure probabilities, shows that the rate-determining step in this mechanism involves a non-planar DNA intermediate. Implications of this finding regarding int-superfamily recombination pathways will be discussed. (Received March 02, 2009)