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Stereochemical course of site-specific DNA recombination revealed by methylphosphonate-substituted DNA substrates and active site variants of Flp and Cre recombinases

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Phosphoryl transfer reactions in RNA and DNA abound in living cells and are central to biological information processing. A common feature of self-catalyzed or protein-catalyzed phosphoryl transfer in nucleic acids is the role of divalent metal ions in stabilizing the penta-coordinate phosphate transition state. Most systems appear to follow the classical 'two-metal ion' paradigm or its variations, while recent evidence suggests the potential involvement of a third metal ion, at least in some systems. By contrast, members of the serine- and tyrosine-family site-specific recombinases exemplify metal-free mechanisms for mediating phosphoryl transfer associated with the DNA strand cleavage and strand joining steps that they perform. In the tyrosine family, the positively charged side-chains of two highly conserved arginine residues appear to functionally bypass metal ion requirement. By using Flp and Cre recombinases as representatives of the tyrosine family, we probed the individual roles of this arginine duo (Arg-I and Arg-II) in transition state stabilization. We find that Flp or Cre variants lacking either Arg-I or Arg-II can be rescued by replacing the scissile phosphate with methylphosphonate, thereby eliminating the negative charge on one of the non-bridging oxygen atoms in the transition state. Stereochemically pure RP and SP forms of the methylphosphonate substrates in conjunction with recombinase variants lacking either Arg-I or Arg-II have enabled us to dissect the stereochemical contributions of the individual arginines to the recombination reaction. The general strategies employed by us are of broad utility in the analyses of other recombination systems.

Biography

Makkuni Jayaram is a Professor of Molecular Biosciences at the University of Texas at Austin. His primary research interest is in the biochemical mechanisms of site-specific DNA recombination. Over the past three decades, his research group has used the Flp site-specific recombinase as a template for understanding the chemistry, conformational dynamics and topological features of strand breakage/exchange reactions in nucleic acids. His second interest is the laboratory concerns strategies devised by selfish DNA elements for moderating their selfishness to establish long-term peaceful coexistence with their host genomes.

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