SYNOPSIS

Restriction modification (RM) systems are important components of bacterial immune system. Their primary function is to protect the bacteria from invading bacteriophages. Based on the subunit composition, enzyme properties and cofactor requirements, they are classified into four different types (Type I to Type IV). The Type II group comprises of two enzymes with contrasting activities- a restriction endonuclease (REase) and a methyltransferase (MTase). A Type II REase recognizes and cleaves specific DNA sequences on incoming foreign DNA while the MTase recognizes and methylates the base within the DNA sequence and protects the bacterial genome. These REases bind DNA in a sequence specific manner and cleave in the presence of metal ions. The work presented in this thesis deals with R.KpnI, which is isolated from *Klebsiella pneumoniae* OK8. The enzyme recognizes a palindromic double stranded DNA sequence, GGTAC ↓ C, and cleaves as indicated. It belongs to the HNH superfamily of nucleases and is characterized by the presence of a ββα- Me finger motif. R.KpnI has inherent promiscuous activity in the presence of Mg^{2+}. It also shows activity in the presence of both the alkaline earth and transition metal ions. However, the enzyme catalyses very high fidelity DNA cleavage in the presence of Ca^{2+} unlike other REases. The basis for this property is yet to be investigated. The *in vivo* promiscuous activity of the enzyme could be advantageous for the bacteria to target the incoming foreign DNA. However, the importance of the promiscuous activity of REases in bacterial physiology is not completely understood. The work presented in this thesis describes the mechanism of Ca^{2+} mediated cleavage specificity and understanding the biological significance of the promiscuous activity of REases.

Chapter 1 provides a general introduction and overview of the literature on Type II REases. It deals with general features of Type II REases, DNA binding, mechanism of phosphodiester

bond hydrolysis and also the role of the metal ions in modulating specificity of these enzymes. New developments in engineering of new specificities in the REases are dealt with in this chapter. The wide prevalence and diversity of RM systems indicate that they might have additional biological roles and such functions have been discussed at the end of this chapter.

One of the unusual features of R.KpnI is Ca^{2+} mediated specific DNA cleavage. **Chapter 2** describes the mechanism of unusual Ca^{2+}-mediated activity of R.KpnI. Substitution of residues in a putative Ca^{2+} binding motif, E132xD134xD136, showed decreased levels of DNA cleavage and Ca^{2+} coordination. However, the invariant His of the catalytic HNH motif acts as a general base for nucleophile activation, and the other two active site residues, D148 and Q175, also participate in Ca^{2+}-mediated cleavage. Insertion of a 10- amino acid linker to disrupt the spatial organization of the ExDxD and HNH motifs impairs Ca^{2+} binding and affects DNA cleavage by the enzyme. This study showed the role of distinct Ca^{2+} binding motif in conferring site specific cleavage upon Ca^{2+} binding; the motif is needed for the promiscuous activity of the Mg^{2+}- bound enzyme. From this study, it is evident that metal ion-mediated modulation of DNA cleavage is one of the key features of R.KpnI.

The effect of altering the metal ion binding residues on the cleavage specificity of the enzyme is presented in **Chapter 3**. A conservative mutation of the metal-coordinating residue D148 to E results in the elimination of the Ca^{2+}-mediated cleavage but imparts high cleavage fidelity with Mg^{2+}. High cleavage fidelity of the mutant D148E is achieved through better discrimination of the target site at the binding and cleavage steps. Biochemical experiments and molecular dynamics simulations suggest that the mutation inhibits Ca^{2+}-mediated cleavage activity by altering the geometry of the Ca^{2+}-bound HNH active site. The present study showed that plasticity in active site to accommodate different metal ions is related to

promiscuous activity, and altering of the metal ion coordination is a plausible way to reduce the promiscuous activity of metalloenzymes.

The *in vivo* promiscuous activity of R.KpnI may have additional cellular functions. In **Chapter 4** a new intracellular function of the promiscuous REase and elucidation of its biological significance is presented. Induction of programmed cell death in bacteria by the REase is described. Bacterial survival studies and microscopic analysis were carried out to examine the role of enzyme promiscuity in bacterial cell death. It is observed that REase triggered DNA damage leads to cell death, releasing nutrients during stationary phase which supports the growth of rest of the population. These observations may open up new avenues to understand the additional roles of REases in bacterial physiology.