Summary
Specific and non-specific protein-DNA interactions play a pivotal role in many of the fundamental processes inside cells including packaging, replication, recombination, restriction and transcription that ultimately regulate gene expression. Type II restriction enzymes are excellent models for studying the molecular basis of "sequence-specific" DNA-protein interactions because of their exquisite specificity of recognition and catalysis and comparatively uncomplicated reaction requirements.

Two kinds of complementary studies have been done to delineate the source of this exquisite specificity: i) to mutate the protein and study the effect of mutation on its interaction with cognate DNA substrate. ii) More profitably, to create a series of mutant or altered substrates and study the interaction of the native enzyme with these substrates. We have used the latter approach to map the contact sites for direct read-out within the recognition sequence of \textit{Ban} I, a type II restriction enzyme isolated from Bacillus aneurolyticus, IAM1077. Interestingly, the \textit{R.Bam}HI and the \textit{R.Bani} recognition sequences differ only by a central basepair inversion and both cleave between the two G's. Previous work on \textit{R.Bam}HI in our laboratory using mismatch oligonucleotide substrates revealed hydrogen bond donor acceptor groups essential for its substrate recognition and catalysis (Roy \textit{et al.}, 1994, 1995). Through a similar approach we wanted to examine the direct readout of the base sequence by the \textit{Bani} protein and how it differs from \textit{Bam}HI.
Although BanI has been cloned and sequenced (Maekawa et al., 1990), yet not much is mentioned about its biochemical and catalytic characteristics in the literature. Isolation and purification of BanI from Bacillus aneuropylicus IAM1077 was done to ascertain authenticity of the strain and the biochemical character of the enzyme. Some biochemical properties of BanI were characterised using commercially available enzyme. These included determination of kinetic parameters ($K_m$ and $K_{cat}$) for linear substrates of 141-bp and 181-bp lengths. The 181-bp substrate exhibited a lower $K_m$ value and higher $K_{cat}$ value than the 141-bp substrate. If BanI can locate its recognition site by a sliding or hopping mechanism then an enhancement of rate would be expected to occur when the recognition site is located in a more central position.

Furthermore, the effect of perturbation by different organic solvents such as glycerol and dimethylsulfoxide was studied to determine if BanI shows star activity. Surprisingly, inhibition of activity was observed at high concentrations of the organic solvents and no star activity could be detected.

BanI, like BamHI has three cysteines in its sequence. Since a role of cysteine has been implicated in BamHI catalysis (Nath et al., 1981) and one of our objectives was to compare BanI vis a vis BamHI, we studied the effect of sulfhydryl inhibitors on BanI also. The sulfhydryl inhibitors did not inhibit BanI activity in the concentration ranges they inhibited BamHI. Partial inhibition was observed only at very high concentrations of inhibitor. Hence our studies with thiol modifying agents suggested that cysteine does not play a role in catalysis by BanI.
To decipher the structural and molecular basis of the highly selective interaction of BanI with its cognate palindromic hexamer, different altered self-complementary oligodeoxynucleotide substrates of lengths varying from 12-18 bases were synthesised. Our substrates were created by placing G.T, G.A or C.A mismatch basepairs at the centre or next to the scissile phosphodiester bonds within the recognition sequence. A functional group reversal was also included in the study. These mismatches altered the functional group pattern and therefore hydrogen bond acceptor/donor sites in the major as well as the minor grooves of DNA. All the substrates were characterised by their UV absorption spectroscopy, UV melting curves and circular dichroism absorption spectroscopy.

The thermal transition data and the CD spectra of the mismatch substrates except the 18-mer substrates indicated that all the oligodeoxynucleotides were in the double-stranded B-DNA conformation under our experimental conditions thus satisfying the primary requirement for recognition and cleavage by a type II restriction enzyme like BanI. The 18-mers, however were shown to exist both as double-stranded B-form and hairpin structure, the latter predominating in case of TG-sequence.

Cleavage analysis was carried out on high percentage denaturing urea-PAGE gels. These experiments however showed that among the cognate and six mismatch substrates, only the cognate TA and the TG mismatch substrates were cleaved normally but at very different efficiencies, without any change in cleavage specificity.
Using the gel electrophoretic mobility shift assay we could also isolate the TG-13 BanI complex which formed only in the presence of Mg$^{2+}$ and persisted in the presence of poly dI-dC, thereby indicating that the complex formed was specific.

We also studied the interaction of the mismatch oligodeoxynucleotide substrates and the native sequence in terms of cleavage kinetics and inhibition kinetics for the R.BanI enzyme, to understand the effect of each alteration in the BanI recognition site. Thus comparing the interaction of these oligodeoxyribonucleotide substrates including the native sequence in terms of kinetic parameters, $K_{cat}$, $K_m$ and $K_i$'s with the enzyme, information about the mechanism of sequence specific recognition were obtained.

As the crystal structure of Ban I has not been elucidated, secondary structure prediction by various methods was done. We chose to compare our predicted secondary structure for BanI with those obtained from the crystal structures of BamHI and EcoRI and tried to identify common elements involved in BanI. We found considerable variations among the structures predicted by various programs for the same protein which in case of BamHI and EcoRI, differ considerably from actual X-ray crystal structures of these two enzymes. However, upon comparing in the most important region of common core motifs, the Chou-Fasman plot had a better predictability. We therefore chose to consider Chou-Fasman predictions for secondary structure of BanI and its isoschizomer, HgiCI.
We predicted a putative active site in BanI which looks similar to that seen in BamHI secondary structure, only change is the order in which the catalytic domain (central β-sheet) and recognition domain (adjacent α-helix) are arranged, is opposite to that in BamHI. Considering the symmetry of the dimeric protein bound to DNA, this reversal is perhaps necessary to compensate for the reversal of the two central A-T basepairs in the recognition sequence of BanI vis a vis the BamHI site. We predict that in BanI-DNA complex this crossover manner of contacts will be maintained as the positions of both thymines as well as the positions of the catalytic and the binding domains seem to be reversed in BanI when compared with the corresponding situations in BamHI.

In view of the above discussion, we conclude that BanI like EcoRI and BamHI interacts through the major groove possibly forming a similar symmetric complex of the DNA and the dimeric protein. From our BanI-mismatch substrate interactions approach, we put in evidence that the different mismatch substrates except CA-15 compete to bind to the same active site with varying affinities proportional to their \( K_i \). Among the various altered substrates, only the -TG sequence is weakly cleaved. However, the cleavage site remained unaltered in such cases. Also possibly, BanI, like EcoRV (Winkler et al., 1993) binds to specific and non-specific sequences with equal affinity and specific complex only forms in the presence of the cofactor Mg\(^{2+}\).