Summary
5. Summary

Section 4.1.1. Studies on the effect of ions on the DNA binding capacity, structure and stability of L1 repressor

1. Of the monovalent cations tested, Na⁺ was found to be the most favorable ion for preserving the biologically active conformation of L1 repressor.
2. Polyvalent anions but not the monovalent anions affected the interaction between L1 repressor and cognate operator DNA.
3. Mg²⁺ severely affected the secondary structure, function and thermal stability of L1 repressor.
4. Conformation and thermal stability of the C-terminal domain (CTD) of L1 repressor were affected by the Mg²⁺ as well.

Section 4.1.2. Determination of the determinants of operator DNA involved in the interaction with L1 repressor

1. Four ‘G’ bases in the top strand and one ‘G’ base in the bottom strand of the 13 bp operator DNA exhibited interaction with L1 repressor.
2. Operator DNA underwent some conformational changes due to repressor binding.
3. Three regions in the top strand and two regions in the bottom strand of the 13 bp operator interacted with L1 repressor.
4. Helical representation of the operator DNA showed that all but one protected ‘G’ residues are present in the two adjacent major grooves. The interacting regions are present in one face of the operator DNA helix.
5. In addition to the interacting ‘G’ bases, other bases of the 13 bp operator DNA were also found to contribute to repressor binding.
6. MBP-CI retained the operator DNA binding activity and formed hybrid dimer with His-CI in solution.
7. CI binding stoichiometry was found to be one indicating that one repressor monomer binds to an operator DNA.
8. L1 repressor induced weak bending in the operator with the bending angle of 30 ± 3.63°.
9. In L1 lysogen, number of L1 repressor monomers per operator site was found to be 44 ± 2.8, which is nearly similar to that of λ repressor system.
Section 4.1.3. Mutations affecting the DNA binding activity, structure and stability of L1 repressor

1. Five mutant L1 repressor proteins (each carrying a point mutation) were generated and purified to homogeneity.

2. W50G and W69C mutant repressors did not show operator DNA binding activity. Compared to the wild-type L1 repressor, P131L mutant repressor yielded weak DNA binding activity at 32°C. The DNA binding activity of E39Q mutant appeared to be marginally higher than that of the wild-type repressor at 32°C. Operator DNA binding activity of E36K was not altered significantly at 32°C.

3. Two domain-structure of L1 repressor was severely affected at 42°C due to the mutations at the 69th and 131st positions of this transcriptional regulator. Mutation at 39th position affected the two domain-structure of L1 repressor partially at 42°C.

4. W69C and P131L mutant repressors were aggregated at about 100 fold higher efficiency than the wild-type or other mutant repressors at 42°C.

5. P131L mutant repressor possesses very weak dimerization ability in solution at 32°C compared to the wild-type or other mutant repressors.

6. E36K mutant repressor appeared to possess the elevated amount of α-helix.

7. Tryptophan residues at positions 50 and 69 were found crucial for preserving the tertiary structure of L1 repressor.

8. The 69th Tryptophan in L1 repressor was suggested to be exposed to surface of this macromolecule.

9. Pro131 residue was found to be evolutionarily conserved, whereas, Trp50 and Trp69 may be replaced by other aromatic amino acid residues.