Chapter 9
Summary & Conclusions
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The present study was aimed at isolation and characterization of novel cis-regulatory DNA elements and their cognate factors from chick embryonic heart. Our laboratory has developed a methodology for isolation of a large repertoire of DNA elements targeted by sequence specific binding proteins. The library was characterized by

1. Sequencing of about two hundred candidate binding sites.
2. TRANSFAC analysis of those sequences showed the presence of a number of well characterized transcription factor binding sites as well as a large number of novel sequences.
3. DNase I foot printing assay was performed and about fourteen exact binding sequences were identified.
4. A large number of sequences similar to those identified by foot-printing analysis were identified by sequence comparison and then their relatedness was conformed by gel mobility shift competition analysis.
5. An in silico protocol was also developed for predicting sequences targeted by the cognate proteins.
6. The database of about 2500 eukaryotic promoters were searched for the occurrence of a number of binding sites (identified in-silico and experimentally and the significance to their occurrence was established by co-occurrence with other known transcription factor binding sites.
7. A 72-hours chick heart cDNA expression library was screened with two candidate binding sites (H2.43 and H2.54) and five novel cDNAs (c43.8, c43.11, c54.11, c54.6 and c54.12) were isolated as candidates for the cognate binding proteins.
8. Blast and BLAT blast analysis of corresponding nucleotide sequences showed that clone 43.8 had partial homology with homeodomain protein six, clone 43.11 had partial homology to an uncharacterized 30 KDa protein from human and clone 54.6 had homology to a hypothetical zinc finger protein from mouse. Clone 54.11 was a variant of RNA binding protein (vigilin), and clone 54.12 did not show any homology to known cDNAs.
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9. Northern analysis was performed with all those cDNAs and the tissue distribution and transcript size for each were analyzed.

10. Clone c54.12 was expressed in E. coli and its DNA binding activity was confirmed.

11. Clone c54.12 was also expressed as GFP fusion proteins and its nuclear localization was established.