INVESTIGATIONS OF HISTONE H1 GENE-SPECIFIC PROMOTER ELEMENTS

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SUMMARY

The work presented in this thesis reports investigations of the histone H1 gene-specific promoter element, 5' AAACACA 3', also referred to as the "H1-box" or "AC-box" (Coles and Wells, 1985). The first approach was to isolate and purify a protein factor, H1-SF, from 9 day chicken embryos. This protein had been previously identified in nuclear extracts from AEV ts34 chicken erythroblasts, and had been demonstrated to interact specifically with the AC-box (Dalton and Wells, 1988a).

In mobility-shift assays using a 40 bp oligonucleotide probe (containing the AC-box) derived from the promoter of the chicken histone H1 gene, 01 H1, a candidate AC-box-binding factor, 40-MS-e, was identified in crude nuclear extracts from ts34 cells. Further characterisation revealed anomalies between some properties of this factor and those of H1-SF. In addition, a specific AC-box-binding factor could not be identified in crude chicken embryo extracts. However, a second AC-box-binding factor, 14-MS-b, was identified in chicken embryo extracts using a 14 bp oligonucleotide probe containing only the AC-box.

14-MS-b was purified from chicken embryo extracts on a large scale, using Heparin Sepharose-, Sephacryl S-300-, and finally DNA-affinity chromatography using concatemers of the 14-mer as the ligand. Analysis of DNA affinity-purified extracts on SDS-polyacrylamide gels revealed two enriched proteins of ~86 kD and ~70 kD. To determine whether either of these proteins was 14-MS-b, the MW of 14-MS-b was estimated by renaturation from SDS-polyacrylamide gels. Surprisingly, the renatured 14-MS-b was no greater than 30 kD: much smaller than either of the enriched proteins, or the estimated mass of H1-SF (90 kD - S. Dalton, unpublished results). Thus it was unlikely that 14-MS-b was analogous to H1-SF.

Another factor, 40-MS-1, was identified in mobility shift assays of DNA affinity-purified extracts using the 40 bp oligonucleotide probe. This factor was also renatured from SDS-polyacrylamide gels, but it too was much smaller (42-46 kD) than the estimated molecular mass of H1-SF. Further investigation of the 86 kD and 70 kD proteins indicated that they were non specific DNA binding proteins.

A final series of mobility-shift assays was conducted using four 25 bp oligonucleotides carrying either wild type or mutated AC-boxes derived from the promoters of the chicken histone 01 H1 and 02 H1 genes. In crude nuclear extracts from ts34 cells, a factor (25-MS-1) which specifically bound the wild type 02 H1 oligonucleotide was identified. 25-MS-1 was also detected in extracts from three other chicken cell lines. However, this factor was not detected in 9 day chicken embryo extracts. In addition, no AC-box-specific binding activity analogous to 25-MS-1 was detected with the wild type 01 H1 oligonucleotide.

It had been shown that deletion or mutation of the AC-box both greatly decreased the steady state levels of H1-mRNA and eliminated cell cycle regulation of H1 gene transcription in stably transfected HeLa cell lines (Dalton and Wells, 1988a). To examine the effect of the AC-box on a heterologous promoter, a series of constructs were made in which tandem repeats of the 25 bp AC-box oligonucleotides were cloned immediately 5' to the Herpes Simplex Virus thymidine kinase (HSVtk) promoter of the expression vector pBLCAT.2 (Luckow and Schütz, 1987). It was found that these oligonucleotides, regardless of copy number or orientation, had no significant effect on transcription from the HSVtk promoter in transiently transfected ts34 cells.

Concurrent with the transfection experiments, a computer alignment of the H1 promoters from several species revealed a distal promoter element which was highly conserved in sequence and position. This element (TG-box) was a near perfect inverted repeat of the AC-box, and the spacing between these elements was also well conserved. CAT reporter vectors were constructed which contained fragments of the 02 H1 and 01 H1 promoters. Deletion of a 29 bp region which included the TG-box resulted in a 50% decrease in expression of the 02 H1 promoter in transiently transfected ts34 cells. However, an H1 promoter fragment containing the TG- and AC-boxes, but no proximal H1 promoter elements, had no significant effect on expression of the HSVtk promoter. These data are discussed in relation to the possible role of the TG- and AC-boxes in the regulation of histone H1 gene expression.