CHICKEN HISTONE H1 GENES

A thesis submitted to the University of Adelaide

for the degree of Doctor of Philosophy

by

Leeanne Susan Coles, B.Sc. (Hons.)

Department of Biochemistry,

University of Adelaide,

Adelaide, South Australia.

January, 1986.

CONTENTS

SUMMARY

This thesis describes the isolation and analysis of the family of genes coding for H1 histone variants in the chicken.

The first part of this thesis describes the construction of a 5-day chicken embryo cDNA library, and the isolation, from this library, of a recombinant containing H1 coding sequences. The nature of the recombinant was determined by DNA sequencing.

cDNA recombinants containing core histone sequences were also isolated. One of these, contained sequences coding for an extremely variant H2A protein which probably represents the replication-independent variant H2A.Z.

The insert of the H1 cDNA recombinant was used to screen a chicken genomal library. H1 positive isolates containing previously uncharacterized DNA were identified and further characterized by restriction enzyme mapping and hybridization analysis. From this work and that performed by other members of this laboratory a total of six H1 genes were located. All H1 genes were found to be clustered with core histone genes.

Subsequent Southern analysis of chicken genomal DNA suggested that the six located H1 genes represented the full complement of H1 genes, homologous to the H1 cDNA probe, in the chicken genome.

Sequence analysis of the chicken H1 genes revealed that each gene coded for a different H1 protein. This is consistent with estimates of H1 variant numbers in chicken tissues. The coded proteins are quite distinct in primary sequence from the H1-related chicken H5 protein.

The chicken H1 genes, as for most histone genes analyzed, contain conserved histone gene-specific 3' sequence elements and have no introns.

Analysis of the chicken H1 mRNAs also revealed features common to other histone genes, viz., H1 mRNAs were found, by Northern analysis, to be non-polyadenylated and mRNA 3' termini were found, by S1 analysis, to map to a predicted conserved sequence. The H1 genes identified here appeared to be expressed in both embryo and adult tissue.

The chicken H1 gene sequences were analyzed in order to identify sequence elements that could potentially be involved in the various aspects of expression of these genes.

Comparison of the chicken H1 genes to H1 genes from other species resulted in the identification of conserved motifs in both 5' and 3' non-coding regions. Of particular note, a 7 base-pair sequence, 5' AAACACA 3', specific to H1 genes, was located in 5' non-coding regions. This is the first report of an H1 gene-specific sequence element. A role for this sequence in the cell cycle regulation of H1 gene expression is proposed. A G-rich promoter element, found to be required for efficient transcription, was also located. This element is also found in other histone and non-histone genes.

In addition, differences in non-coding regions between the chicken H1 genes are pointed out. Such differences could play a role in differential H1 gene expression.

Finally, H1 genes were compared to the tissue-specifically expressed chicken H5 gene. The H5 gene, in particular, was found to lack the H1 gene-specific 5' element discussed above, but, it does retain the remnants of certain conserved 3' elements. The consequences of these findings with regard to expression and evolution of the H5 gene are discussed.