THE ISOLATION AND CHARACTERIZATION
OF CHICKEN HISTONE GENES

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by

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SUMMARY

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1. The work in this thesis is directed towards an understanding of the structure and the control of eukaryote genes. In particular, it describes aspects of the chicken histone gene system.

   The histone gene system although complex, offers several relevant pursuits in the study of gene expression. The genes are reiterated, and both protein and DNA sequences have been shown to be highly conserved. The different histone types are coordinately expressed. The existence of cell-cycle and developmentally regulated variant sub-types, argues for nucleosomal heterogeneity and a role for histones in differential chromatin structure and perhaps control of gene expression. Questions relating to co-ordinate gene expression, gene evolution and the influence of chromatin architecture on gene control may therefore be addressed through a detailed analysis of histone genes.

2. A cDNA probe was prepared from mRNA derived from 5-day chick embryos and assessed for its usefulness as a vertebrate histone gene probe. This probe was used to screen a chicken recombinant genomic DNA library. Positive plaques were screened with a "negative" probe containing globin, ribosomal and 4S RNA sequences to eliminate recombinants selected with known contaminants of the cDNA probe. After plaque purification, there were four possible histone gene containing clones.
3. One recombinant (λ7.4; λCH-01) was chosen for further analysis. This clone was shown to contain histone genes on the basis of three criteria: used as probe, λCH-01 could detect histone genes in total sea urchin DNA; λCH-01 cross-hybridized with a sea urchin histone gene recombinant, λ55; shotgun DNA sequencing revealed histone H2A gene sequences within a coding domain of λCH-01.

4. The organization of the genes within λCH-01 was determined using homologous and cross-species gene-specific DNA probes. Genes detected with embryo cDNA which did not hybridize with the gene-specific probes available were identified by DNA sequencing. The overall arrangement of histone genes within λCH-01 was distinctly disordered.

5. Another recombinant (λ1-6; λCH-02) was also shown to contain histone genes and the gene arrangement was determined. A disordered situation was also found for this clone.

6. Southern blotting analysis was performed to confirm the observations of "disordered arrangement" of chicken histone genes. No evidence for a conserved repeating cluster of histone genes was obtained from Southern blots of chicken DNA.

7. To examine the microstructure of chicken histone genes, two H2B genes were completely sequenced. The two genes coded for the same H2B protein sub-type yet were divergent.
in nucleic acid sequences outside the protein coding portion. The implication to the evolution of histone genes is discussed.

A sequence of 9 bps was found in the 5′-region of both H2B genes which is conserved in all histone H2B genes that have been sequenced. This sequence is therefore implicated in the control of transcription of these H2B genes.

Other flanking sequences common to histone and other eukaryote genes were recognized, and their presence is discussed.

8. Based on the observations made on chicken histone genes, a proposal is put forward to explain the evolution of histone genes from a highly ordered to a considerably disordered state.
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