TRANSCRIPTIONAL REGULATION OF HISTONE GENE EXPRESSION

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CONTENTS

SUMMARY

The work presented and discussed in this thesis is primarily concerned with the periodic expression of chicken histone genes during S-phase of the cell-cycle and, in particular, their transcriptional activation.

1. Northern blot analysis revealed that regulation of steady-state mRNAs for the major core and H1 chicken histone genes is closely coupled to DNA replication.

2. Pulse-labelling experiments indicate that periodic transcriptional activation of these genes during S-phase plays a major role in this regard.

3. In contrast, transcription of two genes encoding the variant histones, H5 and $H2A_F$, is uncoupled from DNA synthesis and accumulation of their respective mRNAs is not S-phase controlled.

4. The 5' *cis*-regulatory sequences required for transcriptional activation of a chicken histone H1 gene during S-phase of the cell-cycle have been identified. Specifically, a conserved heptanucleotide element (H1-box, 5'-AAACACA-3'), ubiquitous to all H1 genes, is essential for correct cell-cycle regulation. Introduction of point mutations or deletion of this element by site-directed mutagenesis reduces levels of gene-specific transcripts by 10-20 fold *in vivo*. Run-on transcription assays in isolated nuclei indicate that this reflects a loss of S-phase transcriptional control.

5. Similar to viral and cellular enhancers, the H1-box functions bi-directionally and from an upstream position in the H1-promoter.

6. Site-directed deletion and substitution mutagenesis was also performed to characterize the putative regulatory role for an H2B gene-specific motif (H2B-box, 5'-CTGATTTGCATA$^{G}_{C}$-3'). Deletion or disruption of this element decreased H2B steady-state mRNA levels by 10-20 fold <u>in vivo</u>. A concomitant decrease in transcripts generated from a paired and divergently arranged H2A gene was also detected. Pulse-labelling experiments indicate that while a decrease in absolute levels of H2B transcription accounts for this, a clear pattern of S-phase control was retained.

7. A sequence-specific cellular factor (H1-SF) has been partially purified from nuclear extracts and shown to interact selectively with the H1-specific motif as assayed by gel-mobility and DNAase protection assays.

8. Levels of this nuclear factor fluctuate in parallel with elevated and depressed rates of H1 gene transcription, an observation consistent with it being a <u>trans</u>-regulator of H1 gene expression during the cell-cycle. However, levels of a factor which specifically binds to the H2B-box are essentially invariant at corresponding stages.

9. The association between histone genes and the nuclear matrix (NM) during periods of high (S-phase) and low (non-S-phase) transcriptional activity has been investigated with synchronized cells. By DNAase I and restriction enzyme analysis, these studies

reveal that both core and linker histone genes (represented by H2A and H1 genes, respectively) are attached to the NM independent of their transcriptional activity during the cell-cycle.

10. The histone H5 gene, transcribed exclusively in cells of the erythroid lineage, is nuclear matrix-associated in AEV ts34 (early erythroid) cells but not in a T-lymphoid cell-line. DNA sequences necessary for NM-attachment of the H5 gene in AEV ts34 cells lie within a 780 bp region spanning 5' non-coding and coding sequences.